Part VI

Biomass and Energy
Chapter 23
Ethanol Production from Maize

Stefan Schwietzke, Youngmi Kim, Eduardo Ximenes, Nathan Mosier, and Michael Ladisch

Abstract The production of fuel ethanol from corn grain is widely carried out in the US, with total current production at 7 billion gallons. This may soon reach 10 billion gallons or more. This chapter addresses the potential of fuel ethanol as an additional source of product based on utilization of the cellulosic (non-food) portions of maize, and in particular the pericarp, cobs, stalks and leaves of the corn plant. An analysis of the composition of corn, and possible processing schemes that transform the cellulosic portions to ethanol are addressed. Technologies for the bioprocessing of cellulose to ethanol, as well as the impact of cellulose utilization on supplementing corn ethanol, are presented.

Stefan Schwietzke
Laboratory of Renewable Resources Engineering, Purdue University, Potter Engineering Center, 500 Central Drive, West Lafayette, IN 47907, USA

Youngmi Kim, Eduardo Ximenes
Department of Agricultural and Biological Engineering, Purdue University, 225 South University Street, West Lafayette, IN 47907, USA

Nathan Mosier
Laboratory of Renewable Resources Engineering, Purdue University, Potter Engineering Center, 500 Central Drive, West Lafayette, IN 47907, USA

Department of Agricultural and Biological Engineering, Purdue University, 225 South University Street, West Lafayette, IN 47907, USA

Michael Ladisch
Laboratory of Renewable Resources Engineering, Purdue University, Potter Engineering Center, 500 Central Drive, West Lafayette, IN 47907, USA

Department of Agricultural and Biological Engineering, Purdue University, 225 South University Street, West Lafayette, IN 47907, USA

Weldon School of Biomedical Engineering, Purdue University, 206 S. Intramural Drive, West Lafayette, IN 47907, USA
e-mail: ladisch@purdue.edu
23.1 Introduction

Ethanol is commonly derived from biological feedstocks utilizing fermentation processes. During these processes, monosaccharides are fermented to ethanol by yeast or bacteria. There are a variety of carbohydrate-containing feedstocks that yield monosaccharides for fermentation, such as corn grain, sugarcane, wheat, sugar beet and other biomass. Brazil produces similar volumes of ethanol as the USA, using sugarcane as the main feedstock, which requires less processing than corn since the sugar is present in a soluble form, rather than as a structural polysaccharide.

The USA and Brazil are the world’s largest producers of fuel ethanol, with outputs of 4.9 and 4.5 billion gallons, respectively, in 2006. US production reached 4.65 billion gallons by September, 2007, while demand was 4.85 billion gallons. The demand for fuel ethanol in the USA more than doubled between 2000 and 2004 and has increased to 7 billion gallons in response to the Energy Act of 2005. In comparison, Brazil used about 4 billion gallons of ethanol in 2006 and produced sufficient quantities of ethanol from sugarcane to satisfy its own demand and export 0.43 billion gallons to the USA. In Brazil, the price of ethanol is tied to the price of gasoline (Energy Information Administration 2007; Renewable Fuels Association 2007). Ethanol production in the USA in 2006 and 2007 increased significantly as new plants started up and increased production by an estimated 2 billion gallons to levels now approaching 7 billion gallons (Renewable Fuels Association 2007).

23.2 Maize as a Feedstock for Ethanol Production

In the USA, maize is a key candidate for ethanol production. It yields corn grain which is converted to ethanol. The potential for ethanol from maize lies not only in converting the grain to ethanol, but also in applying cellulose conversion technology to the pericarp that covers the grain. Cellulose conversion technology, consisting of pretreatment and hydrolysis, offers the prospect of extending conversion to other parts of the corn plant, such as corn stover (cobs, stalks, and leaves). Both corn grain and corn stover are discussed in this chapter. Significant increases in the ethanol yield per acre of maize harvested is possible if biomass from the maize residue is utilized for ethanol production. A quantitative analysis of mass balance has been carried out to address this issue.

The corn cob, stalks, and leaves can be converted to fermentable sugars with cellulose processing technology that consists of pretreatment, hydrolysis, and fermentation using yeast or other microorganisms. In contrast to grain-based feedstocks, cellulose-based ethanol production requires microorganisms that are capable of producing ethanol from both glucose and xylose.

Corn grain contains high amounts of starch, which is readily convertible to monosaccharides upon pretreatment (i.e., cooking in water) and hydrolysis. Glucan is also present in the cob, the stalk, and the leaves, but in a different form, i.e., cellulose, and at lower amounts compared to corn grain. The other major structural
Table 23.1 Compositions of corn grain, corn cob, and corn stover in percentage of total. The lower portion of the table shows the sugar and ethanol yields from these components. \( n/m \) indicates not measured.

<table>
<thead>
<tr>
<th>Type of material</th>
<th>Grain(^a)</th>
<th>Cob(^b)</th>
<th>Stover(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>71.7</td>
<td>n/m</td>
<td>n/m</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2.4</td>
<td>42.0</td>
<td>36.0</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>5.5</td>
<td>33.0</td>
<td>26.0</td>
</tr>
<tr>
<td>Protein</td>
<td>10.3</td>
<td>n/m</td>
<td>5.0</td>
</tr>
<tr>
<td>Oil</td>
<td>4.3</td>
<td>n/m</td>
<td>n/m</td>
</tr>
<tr>
<td>Lignin</td>
<td>0.2</td>
<td>18.0</td>
<td>19.0</td>
</tr>
<tr>
<td>Ash</td>
<td>1.4</td>
<td>1.5</td>
<td>12.0</td>
</tr>
<tr>
<td>Other</td>
<td>4.2</td>
<td>5.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Maximum yield of monosaccharides (lb/ton, 100% efficiency)</td>
<td>1778</td>
<td>1684</td>
<td>1392</td>
</tr>
<tr>
<td>Calculated best case ethanol yield (gal/ton, 100% efficiency)</td>
<td>135</td>
<td>128</td>
<td>105</td>
</tr>
<tr>
<td>Dry weight (%)(^d)</td>
<td>52.4</td>
<td>(9.5)</td>
<td>47.6</td>
</tr>
<tr>
<td>Dry weight (kg/acre)(^e)</td>
<td>4000</td>
<td>(725)</td>
<td>3630</td>
</tr>
</tbody>
</table>

\(^a\)Gulati et al. (1996)
\(^b\)Corn cob composition was measured at LORRE
\(^c\)US DOE (2007)
\(^d\)Pordesimo et al. (2005). Reference data are based on maize harvest at 151 days after planting
\(^e\)Absolute weight for corn grain is based on corn grain data provided by the USDA National Agricultural Statistics Service (2005) which were used for calculation of ethanol yields. Absolute weights for corn cob and corn stover are derived from the given weight percentages based on the absolute weight of corn grain.

Polysaccharide is hemicellulose, which is predominantly xylan. The conversion of the xylan fraction and the ability to ferment it to ethanol is therefore important to improve the efficiency and the economics of ethanol production from maize.

The main components of maize are corn grain and corn stover (corn cob, stalk, and leaves). The ratio of grain:stover is about 1:1, with the grain accounting for slightly more weight than stover. The corn cob represents approximately 20% of the weight of corn stover. Corn grain includes the starchy endosperm, germ, and corn hull. Corn stover contains principally cellulose and hemicellulose, which are the main sources of fermentable sugars for ethanol production. The corn cob contains more cellulose and hemicellulose than stalks and leaves. This enables a higher potential yield of ethanol. The compositions of the three major components of maize are summarized in Table 23.1. Compositional analysis of cob is based on an analysis carried out in LORRE (Laboratory of Renewable Resources Engineering – Purdue University) for cobs obtained in the Delphi, Indiana, area. The data in the last three rows give the mass composition for grain, cob, and stover.

Starch is a type of glucan that is readily convertible to glucose. Cellulose and hemicellulose are also sugar polymers, and the cellulose is more difficult to convert into fermentable sugars than starch due to its crystalline structure and close association with lignin and hemicellulose. The efficiency of starch hydrolysis is a reflection of the long experience in industrial processing of starch, while the well-known recalcitrance of the cellulose itself forms an impediment to hydrolysis. Furthermore,
Fig. 23.1 The seven major unit operations of a biorefinery. Biorefinery as represented here is viewed as being energy self-sufficient through combustion or gasification of residual lignocellulose. If only corn grain is processed, the remaining solids that are high in protein are recovered as co-products and sold as animal feed, which has a higher value than use as a boiler fuel. CO2 is recycled into plant matter through production agriculture. (Adapted from Eggeman and Elander (2005))

a large portion of the hemicellulose (xylan and arabinan) consists of 5-carbon sugars which cannot be fermented by natural yeast. As a result, only the starch part of the maize is used for ethanol conversion in current industrial processes. Since starch makes up less than half of the weight of the entire plant, only about 40–50% of the theoretical yield of ethanol of a maize plant is obtained from starch. A higher yield of ethanol for every acre of harvested corn can be achieved if the cellulose and hemicellulose fractions in corn stalks, cobs, and leaves are used to produce ethanol in addition to starch.

Processing technology consisting of pretreatment and hydrolysis is necessary to make use of the glucan, xylan, and arabinan portions of the plant. Enzymes are required for the conversion of cellulose and hemicellulose into monomeric sugars, and recombinant yeast or bacteria are needed for subsequent fermentation of both 5-carbon and 6-carbon sugars into ethanol. Figure 23.1 is a schematic representation of the key steps in the sequence of pretreatment, hydrolysis, and fermentation that make up a biorefinery for converting both starch and lignocelluloses to ethanol.

23.3 Ethanol Production from Corn Grain

Ethanol is produced when yeast ferments 6-carbon sugars (mainly glucose) via the glycolytic pathway. In the USA, starch from maize is used as a feedstock for hydrolysis, where the glucan is converted to glucose by enzyme hydrolysis and fermentation of the glucose to ethanol by yeast. The mash is fermented using natural yeast and bacteria in a process that takes up to 40 hours. The fermented mash is separated into ethanol and residues (for feed production) via distillation. A simplified process scheme for ethanol production from starch is shown in Fig. 23.2. Hydrolysis is carried out using amylase and glucoamylase, i.e., starch and maltose
Fig. 23.2 Process flow diagram for ethanol production from starch

hydrolyzing enzymes. The substrate, in this case, is starch that has been gelatinized (i.e., pretreated) by cooking it in water.

Either wet or dry milling processes may be used for ethanol production from corn starch. A dry-grind process is simpler and typically will require less capital than wet milling. The capital costs for large-scale dry milling plants have ranged from about $1.50 to $2.20 per annual gallon of capacity (2006). A dry-grind process entails grinding the corn into a fine powder, which is then cooked, hydrolyzed, and fermented. In a wet-milling plant, the number of co-products is higher and more flexible, with processing consisting of steeping and separation of the corn kernel into germ, starch, and other components. Currently, most new US fuel ethanol production facilities utilize dry-grind processes. The expansion in the industry in 2005 to 2007 was attained through construction of new or an increase in existing dry-grind facilities. The key steps involved in both technologies are shown in Fig. 23.3 (wet milling) and Fig. 23.4 (dry milling).

23.3.1 Wet Milling

The wet milling process fully fractionates the corn grain into carbohydrates, lipids, and protein. These can be efficiently recovered and purified for the production of value-added products. When the starch is converted to fuel ethanol, the processing steps of saccharification, fermentation, and recovery are similar to those in a dry-grind operation.

The first step in the wet milling process is steeping, where the corn kernel is placed in an aqueous solution of 0.1–0.2% SO$_2$ and allowed to cook at 48–52°C for 30–50 hours. This facilitates downstream fractionation by hydrolyzing disulfide bonds in proteins so that they are more soluble. The corn is then ground in its wet state and oil, fiber, and gluten are separated from the starch for further processing into value-added co-products. During saccharification, enzymes break down the starch into glucose. In the fermentation step, yeast grown in seed tanks is added to
the corn mash to ferment the simple sugars (glucose) to ethanol. Finally, ethanol is separated from the water by means of distillation and dehydration.

Corn fiber contains cellulose and hemicellulose (Table 23.2) which cannot be used for producing ethanol in wet mill facilities, since these facilities do not...
Table 23.2 Compositions of corn fiber, DG, and DDGS in percentage of dry matter. \( n/m \) indicates not measured

<table>
<thead>
<tr>
<th>Type of material</th>
<th>Corn fiber(^a)</th>
<th>DG(^b)</th>
<th>DDGS(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucan (cellulose and starch)</td>
<td>38.0</td>
<td>18.5</td>
<td>21.2</td>
</tr>
<tr>
<td>Xylan (hemicellulose)</td>
<td>28.0</td>
<td>20.4</td>
<td>13.5</td>
</tr>
<tr>
<td>Protein</td>
<td>12.0</td>
<td>37.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Oil</td>
<td>n/m</td>
<td>10.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Lignin</td>
<td>8.0</td>
<td>n/m</td>
<td>n/m</td>
</tr>
<tr>
<td>Ash</td>
<td>0.4</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Other</td>
<td>13.6</td>
<td>12.1</td>
<td>23.3</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Weight (kg/acre)(^c)</td>
<td>1135</td>
<td>n/m</td>
<td>1510</td>
</tr>
</tbody>
</table>

\(^a\) Mosier et al. (2005b)  
\(^b\) Kim et al. (2008b)  
\(^c\) Graboski (2002). Corn grain yield per acre is based on corn grain data provided by the USDA National Agricultural Statistics Service (2005) which were used for calculation of ethanol yields in Table 23.3

Currently incorporate cellulose conversion technology. Fiber is a potential feedstock for additional ethanol production if lignocellulosic conversion technologies are applied. Each acre of harvested corn that is processed by wet milling yields approximately 1135 kg of fiber (Graboski 2002).

### 23.3.2 Dry Milling

Dry milling technology produces high ethanol yields at lower capital investment than wet milling. However, the only major co-products, other than \( \text{CO}_2 \), are the fermentation residuals which are sold as animal feed. These products are commonly known as distillers’ grains (DG) and dried distillers’ grains with solubles (DDGS). Compositions of DG and DDGS are given in Table 23.2.

In a dry mill, cleaned corn is first ground in hammer mills, which breaks the tough outer coating of the seed and grinds the corn into a fine powder. During the liquefaction process, water and enzymes are added to the ground corn in order to create a slurry. The gelatinized starch feedstock is easier to hydrolyze into monomeric sugars than uncooked corn, although processes that avoid the cooking step are being considered for ethanol plants. Saccharification and fermentation are similar to the processes performed in a wet mill. Ethanol is obtained from the water slurry via a number of complex steps including distillation and dehydration. A co-product of the dry milling process, heavy stillage, leaves the bottom of the first distillation column. The heavy stillage is centrifuged to remove the majority of the solids. The thin stillage is partly recycled to the liquefaction step. The centrifuged solids are referred to as wet cake or wet distiller’s grains (35–40% solids). These are further dried to give DDGS.
23.3.3 Enzymes

Starch processing is a mature technology that makes use of enzymes for liquefaction and saccharification. These produce a relatively clean glucose stream which can be further fermented to ethanol by yeast (*Saccharomyces*). In the liquefaction step, a thermostable α-amylase is added before the heat treatment (105–110°C) for 5–7 min. Taking into consideration that the starch-slurry is then flash-cooled to 95°C and kept at that temperature for 60–120 min to complete the enzymatic liquefaction, a highly thermostable enzyme is required that will be active during the entire procedure. In addition to the originally used enzymes from *Bacillus stearothermophilus* or *B. licheniformis*, there are several other enzyme preparations available and marketed for this purpose (for example, Valley “Ultra-thin™” from Valley Research/Diversa, Multifect AA 21L® from Genencor, and Termamyl® and Liquozyme® from Novozymes). Ideally, the enzyme should be active and stable at a low pH (~4.5) and not demand calcium for stability (Van der Veen et al. 2006; Turner et al. 2007). Some engineered enzymes have been reported to fulfill these desired properties. Genetic engineering has been extensively used for cloning α- and gluco-amylase genes from different microbial sources, in order to express enzymes with such desirable properties in appropriate hosts. The development of low-pH α-amylases that simplify starch processing and reduce chemical costs as well as improve ethanol production (Gray et al. 2006) is an example. Another example is that of a glucoamylase from a thermoacidophilic microorganism (*Thermoplasma acidophilum*) which has been cloned and successfully expressed in *Escherichia coli*; the amylase activity of the recombinant enzyme is reported to be maximal at 75°C and pH 5.0 (Dock et al. 2008).

The saccharification step involves hydrolysis of remaining oligosaccharides into either maltose syrup (using β-amylase) or glucose/glucose syrups (using glucoamylase) (Pandey 1995). The process conditions (pH 4.2–4.5 and 60°C) are consistent with the range over which *Aspergillus niger* glucoamylase is stable. However, the liquefied corn slurry must be cooled down after liquefaction and the pH must be adjusted in order to achieve the best conditions for the glucoamylase action. Perhaps less costly would be to utilize an enzyme that is active in the same pH and temperature range as the liquefaction enzymes. It is also possible to increase the efficiency in saccharification by use of a debranching enzyme. Pullulanases have been added to the process for this purpose (Turner et al. 2007).

23.3.4 DDGS

With increasing dry mill capacities, the use of DDGS as a feed may be limited (Belyea et al. 2004). DDGS contain large amounts of fiber that contain cellulose and hemicellulose as well as protein, some starch, and oil (Table 23.2). Integrating lignocellulosic conversion technologies in dry-grind facilities might further increase the value of DDGS by lowering its fiber content and increasing its relative
protein content, while increasing the overall ethanol yield per bushel (Mosier et al. 2005b; Kim et al. 2008c). In a dry grind mill, each acre of harvested corn yields approximately 1510 kg of DDGS, which can be used for ethanol conversion (Graboski 2002).

Previous studies (Mosier et al. 2005b; Kim et al. 2008a) have shown that the fiber-containing co-products of dry milling and wet milling processes, such as DDGS and corn fiber, are easily hydrolyzed upon cooking in liquid hot water and addition of cellulase enzymes. Processing of the co-products, such as DDGS and corn fiber, of dry or wet milling processes for additional fermentable sugars and ethanol is achieved by releasing fermentable sugars from glucans and other polymeric sugars left in these co-products. Due to the recalcitrant nature of the cellulose, these materials need pretreatment prior to saccharification.

Kim et al. (2008c) have described several dry mill process alternatives in which the DDGS is recycled and hydrolyzed to produce more ethanol. The modified dry grind process involves recycling the sugar-rich liquid stream in the process, which, in turn, results in accumulation of various fermentation inhibitors. A simulated material balance model by Kim et al. (2008c) shows that two- to five-times higher concentrations of by-products and inhibitory components during the fermentation step of the modified processes may accumulate due to water recycling as compared to the conventional dry grind process. Nonetheless, ready fermentation of the sugars to ethanol is still achieved. The water balance for the modified dry grind processes also shows that the proposed processes require less fresh water input for the liquefaction than a conventional dry grind process. The water input due to additional unit operations required to extract and ferment sugars from the by-products can be compensated for by the water saved for the liquefaction in the modified process.

Processing the co-products for enhanced ethanol yield in the dry milling or wet milling process also affects the compositions of the final co-product exiting the distillation column. It contains a higher amount of protein per total mass as the polysaccharides are extracted to produce more ethanol, resulting in a reduced amount of co-product at the end of the process. The total wet solids sent to the drier are decreased, thus decreasing the drier load.

### 23.4 Ethanol Production from Corn Cob and Corn Stover

The agricultural residues from maize production are potential sources of sugar for ethanol production, in addition to starch and by-products. When maize is harvested in the field, the corn grain is separated from the cobs, stalks, and leaves. While the grain is transported for storing and processing, the stover is currently not widely collected. However, this biomass could be used for lignocellulosic ethanol production. Corn stover includes stalks, leaves, and corn cobs. Unlike the corn grains, of which the major component is starch, the main components of corn stover are cellulose, hemicellulose, and lignin (Table 23.1). Corn stover production is 75 million dry tons per year in the USA, which represents about 38% of all biomass from agricultural
lands (US DOE 2005). However, the return of some of this material to the soil is also important for maintaining the organic matter content and fertility of the soil (Blanco and Lal 2007; Kim and Dale 2005; Mann et al. 2002).

Lignocellulosic material is characterized by its strength and complexity due to a network formed between hemicellulose and cellulose in close association with lignin. A number of processing steps is required to overcome this complex structure to make it suitable for fermentation. The first step in producing cellulosic ethanol is biomass handling where the size of the lignocellulose is reduced to make handling easier and ethanol production more efficient. During pretreatment, cellulose structure is disrupted, the lignin seal is broken, and the hemicellulose is partially removed. This increases the specific surface area that is accessible to enzymes. Pretreatment is one of the many steps in the cellulose-to-ethanol process, but represents a currently critical step for hydrolysis. An effective pretreatment is performed at conditions that avoid degradation of pentose from hemicellulose, or glucose from cellulose, and limit formation of degradation products that inhibit the growth of fermentative microorganisms. Pretreatments should also limit energy, chemical, and/or enzyme usage in order to limit the cost of the pretreatment process itself (Mosier et al. 2005a). The effect of pretreatment on the structure of lignocellulose is depicted in Fig. 23.5.

After pretreatment, the cellulose and hemicellulose chains are more accessible to enzymes in the form of polymers and oligomers. Hydrolysis by enzymes breaks the chains into monomers. Enzymatic hydrolysis has the potential to make ethanol, derived from cellulose biomass, competitive when compared to other liquid fuels on a large scale (Wyman 1999). The monomers are then fermented by natural yeast and by genetically engineered bacteria or yeast. *Saccharomyces cerevisiae* (yeast) is currently used to produce ethanol for corn. *Saccharomyces* yeasts are currently applied in large-scale corn-to-ethanol or sugarcane-to-ethanol industry.

Ethanol production from lignocellulose requires not only fermentation of glucose but also fermentation of pentose sugars. Wild-type *Saccharomyces* is not able to ferment pentose. Utilization of genetically modified yeasts, specifically engineered for

Fig. 23.5 Pretreatment effect on lignocellulosic material. (Reproduced from Mosier et al. (2005a))

![Lignin, Cellulose, Hemicellulose, Amorphous Region, Crystalline Region](image)
the purpose of co-fermenting xylose and glucose, address this need (Ho et al. 2000). The result of fermentation is a mixture of water, ethanol, and residues, with CO₂ being formed and removed as a gas from the fermentation.

Starch is a storage compound consisting of glucose linked via α-1,4 and α-1,6 glucosidic linkages (amylose and amylpectin). It is present in plants as an energy source. Cellulose, on the other hand, is a structural cell wall component with a function to provide rigidity to the plant. It is a highly crystalline and compact substrate, composed of glucose linked via β-1,4 glycosidic bonds, which makes it resistant to enzymatic hydrolysis (Gray et al. 2006). In this sense, it is not surprising that lignocellulosic substrates are more resistant to biological attack than starch. It is estimated, on a protein weight basis, that 40–100 times more enzyme is required to degrade cellulose to glucose than is used to hydrolyze starch in order to produce equivalent amounts of ethanol. Since the cost of enzyme production is not substantially different (Merino and Cherry 2007), the overall cost of enzyme use is higher for lignocellulosic ethanol conversion.

23.4.1 Cellulolytic Microorganisms

Several organisms exist in nature as complex consortia (fungi, bacteria, and protozoa) that work synergistically to deconstruct the plant cell wall. They rely on biomass degradation for their survival. Although all of these organisms can be considered as potential sources of biomass-degrading enzymes, fungi are predominantly used for the enzymatic cellulose hydrolysis to glucose, e.g., Trichoderma, Penicillium, and Aspergillus species (Galbe and Zacchi 2002). These microorganisms produce (at high productivity) a complex mix of enzymes with high catalytic efficiency in hydrolyzing cellulose to soluble sugars and glucose. These features are required for low-cost enzyme supply. Additionally, enzymes from fungi are generally secreted in the growth medium, facilitating the separation of active enzymes in a liquid form suitable for using in a hydrolysis reactor (Merino and Cherry 2007).

The saprophytic mesophilic fungus Trichoderma reesei is currently used for the commercial manufacturing of cellulase and hemicellulase products and has been proposed as the most promising organism for production of enzymes for lignocellulose conversion to fermentable sugars. In addition to a long history of safe commercial use, this fungus secretes significant quantities of enzymes and has well-developed genetic systems (Potera 2006; Li et al. 2007).

23.4.2 Cellulolytic Enzymes

The cellulolytic system produced by T. reesei is formed by exoglucanases (two cellobiohydrolases, CBHI and II), a number of endoglucanases (EG), and β-glucosidases. CBH I and CBH II constitute approximately 60% and 20%,
respectively, of the protein secreted by this fungus, and they have a critical role in the efficient hydrolysis of cellulose (Hazell et al. 2000). EG randomly attack the cellulose chain, creating new reducing and non-reducing ends for the celllobiohydrolases. A third type of enzyme, β-glucosidase, which hydrolyzes celllobiose and some other short-chain cellodextrins into glucose, is also necessary. In the absence of β-glucosidase, end-product inhibition from celllobiose will occur for the other enzymes (Hahn-Haegerdal et al. 2006). β-glucosidase can also be inhibited by the accumulation of glucose. The sensitivity of the enzyme to inhibition by a specific end-product is reflected by experimentally measured inhibition constant (Ki). When a β-glucosidase (Ki 3.6 mM, competitively inhibited by glucose) from a different microorganism source (anaerobic fungus Orpinomyces strain PC-2) was used in combination with T. reesei cellulases, the conversion of cellulosic materials into glucose by T. reesei enzymes was significantly enhanced, demonstrating the potential of this specific enzyme for use in biofuel and feedstock chemical production (Li et al. 2007).

The maximum cellulase activity of most fungal cellulases and β-glucosidases is generally at 50°C and a pH of 4.0–5.0. Optimal conditions may vary with the hydrolysis time and are also dependent on the source of the enzymes (Hahn-Haegerdal et al. 2006). It is well known that efficient cellulose hydrolysis requires a complex enzyme mix. In order to decrease the amount of enzymes required for this purpose it is important that enzymes with superior specific activities be identified, or additional enzymes be added, reducing the total enzyme loading. It is also important to determine the synergism among the enzymes. A significant contribution to the degradation of lignocellulosic substrates can also be seen by the presence in certain hydrolytic enzymes of carbohydrate-binding modules connected by linkers to the catalytic modules.

Hydrolysis performed separately from fermentation is known as separate hydrolysis and fermentation (SHF). Cellulose hydrolysis carried out in the presence of the fermentative organism is referred to as simultaneous saccharification and fermentation (SSF). Simultaneous saccharification of both cellulose (to glucose) and hemicellulose (to pentose) and co-fermentation of both glucose and pentose (SSCF) is realized by combining enzymes with genetically engineered microbes (Mosier et al. 2005a). The ethanol fermentation of glucose, mannose, and galactose by baker’s yeast, Saccharomyces cerevisiae, is well established on a large scale. For the conversion of pentose sugars (xylose and arabinose) to ethanol, most of the work has been focused so far on xylose (Hahn-Hagerdal et al. 2006). Pentose-fermenting Escherichia coli (Ingram et al. 1987) and Klebsiella oxytoca (Burchhardt and Ingram 1992) have been generated by introducing ethanologenic genes from Zy- momonas mobilis. The use of genetically modified yeasts specifically engineered for the purpose of co-fermenting xylose and glucose has also been described (Ho et al. 2000).

Ethanol is recovered from the fermentation broth by distillation (Ladisch et al. 1984). 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 co-products (Wyman 1994). CO₂ is recycled into plant matter through production agriculture.

Figure 23.6 shows a process sequence for ethanol production from lignocellulose. The key steps are preparation (size reduction) of biomass, pretreatment to soften up and disrupt the structure of the cellulose (Fig. 23.5), hydrolysis to break the cellulose down into sugars, and then fermentation of the sugars to ethanol. Product separation is the final step and consists of distillation followed by drying using either molecular sieve or a corn-based adsorbent (Ladisch et al. 1984; Gulati et al. 1996).

23.5 Comparison of Ethanol Yields for Conversion of Starch, By-products, and Corn Stover

It is widely recognized that feedstock costs are a critical factor in the production of fuel ethanol. One way of reducing these costs is to increase the feedstock yields per acre that are harvested. Today, only the starch from maize is used for ethanol conversion. The yield would be increased if a larger portion of the maize plant were to serve as a feedstock. Lignocellulosic ethanol technology is the key to enable the conversion of plant matter other than starch into renewable fuel.

A comparison of four different best case scenarios is given in Table 23.3, where compositions are as shown in Table 23.4. In the base scenario, ethanol is produced using only the starch portion of the plant. Scenario 2 includes ethanol production from both starch and by-products (fiber, DG, and DDGS). In scenarios 3a and 3b, ethanol is derived from starch, by-products, and corn stover. The two different scenarios including corn stover account for different harvesting techniques. In scenario 3a (normal cut), the maize is cut at 40 cm above the ground. This has been evaluated as the optimal height for harvesting (Hoskinson et al. 2007). In the study by Hoskinson et al., different harvest scenarios have been assessed in terms of sustainability, economics, nutrient value, and suitability for ethanol production. In scenario 3b (low cut), a larger portion of the corn stover is collected when compared to scenario 3a (10 cm cutting height).
Table 23.3: Theoretical ethanol production using starch, cellulose, and hemicellulose as feedstocks, reported in dry kilograms per acre

<table>
<thead>
<tr>
<th>Component</th>
<th>Base scenario (starch)</th>
<th>Scenario 2 (starch and by-products)</th>
<th>Scenario 3a “normal cut”a (starch, by-products, and corn stover)</th>
<th>Scenario 3b “low cut”b (starch, by-products, and corn stover)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feedstock</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn grain</td>
<td>4000</td>
<td>4000</td>
<td>4000</td>
<td>4000</td>
</tr>
<tr>
<td>Corn stover</td>
<td>0</td>
<td>0</td>
<td>2060</td>
<td>2703</td>
</tr>
<tr>
<td><strong>Sugar</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucan from starch</td>
<td>2868</td>
<td>2868</td>
<td>2868</td>
<td>2868</td>
</tr>
<tr>
<td>Glucan from cellulose</td>
<td>0</td>
<td>96</td>
<td>96 + 750</td>
<td>96 + 984</td>
</tr>
<tr>
<td>Xylan</td>
<td>0</td>
<td>220</td>
<td>220 + 505</td>
<td>220 + 662</td>
</tr>
<tr>
<td><strong>Ethanol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol from starch</td>
<td>1628</td>
<td>1628</td>
<td>1628</td>
<td>1628</td>
</tr>
<tr>
<td>Ethanol from cellulose</td>
<td>0</td>
<td>55</td>
<td>55 + 426</td>
<td>55 + 559</td>
</tr>
<tr>
<td>Ethanol from hemicellulose</td>
<td>0</td>
<td>128</td>
<td>128 + 293</td>
<td>128 + 384</td>
</tr>
<tr>
<td>Total ethanol (kg)</td>
<td>1628</td>
<td>1811</td>
<td>2530</td>
<td>2754</td>
</tr>
</tbody>
</table>

aNormal cut is 40 cm above the ground
bLow cut is 10 cm above the ground

The following assumptions were made for estimating the total ethanol yield in Table 23.3. Calculations are based on maximum theoretical ethanol yield per acre of maize using current practices (starch ethanol). This means that all available sugar polymers (according to the different scenarios) are converted into monomeric sugars and that all sugars are fermented to ethanol without losses. The data of the compositional analyses of corn grain and corn stover for calculation of the sugar yields are shown in Table 23.4. The data in the last two columns represent the compositions of

Table 23.4: Compositions of corn grain (scenarios 1 and 2) and corn stover (scenarios 3a and 3b). n/m indicates not measured

<table>
<thead>
<tr>
<th>Type of material</th>
<th>Corn graina</th>
<th>Corn stover “normal cut”b</th>
<th>Corn stover “low cut”b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>71.7</td>
<td>n/m</td>
<td>n/m</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2.4</td>
<td>36.4</td>
<td>38.2</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>5.5</td>
<td>24.5</td>
<td>23.4</td>
</tr>
<tr>
<td>Protein</td>
<td>10.3</td>
<td>4.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Oil</td>
<td>4.3</td>
<td>n/m</td>
<td>n/m</td>
</tr>
<tr>
<td>Lignin</td>
<td>0.2</td>
<td>12.7</td>
<td>15.5</td>
</tr>
<tr>
<td>Ash</td>
<td>1.4</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Other</td>
<td>4.2</td>
<td>19.8</td>
<td>15.7</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

aGulati et al. (1996)
bHoskinson et al. (2007). Normal cut is 40 cm above the ground; low cut is 10 cm above the ground
corn stover that incorporate the compositional differences between corn cobs, corn stalks, and leaves.

Data for both corn grain and corn stover yields have been adopted from Hoskinson et al. (2007), which are representative values for Story County, Iowa, and as reported by the National Agricultural Statistics Service (NASS) (USDA National Agricultural Statistics Service 2005). The average corn grain yield per acre and per year in the USA is assumed to be 150 bushels (equal to 3225 kg) (USDA 2006), which is lower than the data provided by NASS (4000 kg). However, both sources give the same weight ratio of 1:1 for corn grain yield to corn stover yield, which is an important parameter in differences in ethanol yield when comparing the four scenarios.

In scenario 2, glucan and xylan yields from cellulose and hemicellulose, respectively, are derived from the corn hull. In scenario 3, there are additional glucan and xylan yields when compared to scenario 3 which account for the sugars available from the corn stover. A number of formulae were used to calculate the ethanol yields for the three scenarios. These encompass hydrolysis of glucan to glucose and xylan to xylose (Eqs. 23.1 and 23.2), and fermentation of glucose and xylose to ethanol (Eqs. 23.3 and 23.4):

\[
\text{Glucose (kg)} = \text{Glucan (kg)} \times \frac{180}{162} \quad (23.1)
\]
\[
\text{Xylose (kg)} = \text{Xylan (kg)} \times \frac{150}{132} \quad (23.2)
\]
\[
\text{EtOH (kg)} = \text{Glucose (kg)} \times 0.511 \quad (23.3)
\]
\[
\text{EtOH (kg)} = \text{Xylose (kg)} \times 0.511 \quad (23.4)
\]

The results are summarized in Table 23.3. In the base scenario, 1628 kg of ethanol is produced for each acre of harvested corn when only starch is used as a feedstock. The ethanol yield is 11.2% (183 kg) higher compared to the base scenario when co-products are converted into monomeric sugars and subsequently fermented. The increase in ethanol yield is due to the additional sugar yield which originates from both cellulose and hemicellulose in the hull of the corn kernel. Kim et al. (2008c) report a 14% increase in ethanol yield when by-products are used for additional ethanol production. The 2.8% difference can be explained by the different modeling approaches. While this book chapter separates ethanol yields from starch and lignocellulose, Kim et al. distinguish between starch ethanol production and by-product ethanol production. In the latter case, ethanol produced from by-products includes ethanol from the unutilized residual starch in the by-products. Therefore, the number in Kim et al. (2008c) is slightly higher.

A 55% (902-kg) higher ethanol yield (compared to base scenario) per acre is possible when the corn stover is also harvested and processed (scenario 3a). If agricultural practices were improved so that the maize could be low cut (scenario 3b), the total ethanol yield would rise to 2754 kg of ethanol. This equals an increase of 69% compared to the base scenario. However, as mentioned previously, continuous complete removal of the stover is probably not desirable due to
adverse effects on the soil and hence only about half of the available stover will be harvested.

### 23.6 Conclusion

The potential of maize to provide feedstock for alcohol production in excess of 15 billion gallons of ethanol per year in the USA will depend on utilization of cellulose and hemicellulose portions of the plant. Consequently, cellulose conversion will play a role in increasing yields. This chapter has discussed both the challenges and potential of converting the pericarp, stalks, cobs, and leaves to obtain additional fermentable sugars and ethanol. If sustainable, cost-effective, and environmentally compatible agricultural practices are developed and coupled to cellulose conversion technology, maize has the potential to provide 20–24 billion gallons of ethanol per year through a combination of starch processing and cellulose conversion.

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**References**


