CHAPTER

7

Biochemical Processing of Carbohydrate-Rich Biomass

7.1 Introduction

A variety of processes have been developed for converting biomass into chemicals and fuels. These can be generally classified as biochemical or thermochemical processing of biomass. Biochemical processing utilizes enzymes and microorganisms to convert carbohydrate-rich biomass into desired products, while thermochemical processing uses heat and catalysts to convert lignocellulosic biomass into products. Sometimes biochemical and thermochemical processes are combined into a hybrid processing scheme. This chapter covers biochemical processing of carbohydrate-rich biomass, while Chapter 8 considers thermochemical processing and hybrid processing of lignocellulosic biomass. Lipid-rich feedstocks, which are processed in ways quite distinct from carbohydrate-rich and lignocellulosic biomass, are covered in Chapter 9.

Biochemical processes considered for the conversion of carbohydrate-rich biomass into fuels and chemicals include fermentation of sugars and starches; acid and enzymatic hydrolysis of lignocellulose to simple sugars followed by subsequent fermentation; and consolidated bioprocessing (CBP) of lignocellulose.

7.2 Fermentation of Sugars and Starches

Fermentation is a biological process in which enzymes produced by microorganisms catalyze energy-releasing reactions that break down complex organic substrates. Anaerobic conditions, characterized by the exclusion of oxygen, are most common, but aerobic processes, in which oxygen is present, are also possible. The French chemist Louis Pasteur established the field of microbiology during his efforts to help the brewery industry improve its product. He introduced the word

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fermentation from the Latin *fermentare* (to boil) to describe microbial activity that releases gas as foam.

Fermentation can produce a wide variety of chemicals although most of them are organic acids and alcohols. Most microorganisms used in commercial fermentations require six-carbon sugars (hexoses) or disaccharides as substrates although the microbial world contains organisms that can breakdown virtually any organic compound. Recent technological advances have made possible the fermentation of five-carbon sugars (pentoses).

Several factors limit the use of fermentation technology in the production of chemicals. Production rates by microorganisms in aqueous media of low solids volume are inherently low. The microorganisms are both sensitive to inhibitors and operating conditions, especially temperature and pH. Most fermentations require aseptic conditions, which can be difficult to achieve in large-scale operations. Recovery of water-soluble products from dilute solutions can be expensive. Effluent usually contains high biochemical oxygen demand (BOD), which requires wastewater treatment before discharge.

A major emphasis in the fermentation industry is the production of ethanol, which is marketed to both fuel and beverage industries. Table 7.1 lists ethanol fermentation yields for a variety of carbohydrate feedstocks. The maximum theoretical yield of ethanol is 0.51 (mass ethanol/mass carbohydrate, corresponding to 51% of the carbohydrate converted to ethanol on a mass basis), the balance being carbon dioxide. Typically, about 5–12 wt% of the carbohydrate is converted to cells; thus, not more than 47 wt% of the fermented carbohydrate is converted to

Table 7.1 Ethanol yields from various biorenewable resources

Feedstock	Yield (L/ton)
Apples	64
Barley	330
Cellulose	259
Corn	355–370
Grapes	63
Jerusalem artichoke	83
Molasses	280–288
Oats	265
Potatoes	96
Rice (rough)	332
Rye	329
Sorghum (sweet)	44–86
Sugar beets	88
Sugarcane	160–187
Sweet potatoes	125–143
Wheat	355

Source: Klass, D.L. (1998) *Biomass for Renewable Energy, Fuels, and Chemicals*. Academic Press.

ethanol. Of course, other products can also be produced by fermentation, which are described later in this chapter.

The following sections explore the technologies by which carbohydrates, including sugar, starch, and lignocellulose, are converted to coproducts. Regardless of the carbohydrate, the following processes are involved: release of simple sugars from the carbohydrate, fermentation to organic acids or alcohols, distillation of the fermentation broth to recover the fermentation products, and utilization of coproducts. Differences among the carbohydrates are how simple sugars are released and how coproducts are utilized.

7.2.1 Sugar Crops

Traditional sugar crops used for fermentation include apples, grapes, other fruits, sugarcane, sugar beets, and sweet sorghum. Molasses, which is the residual syrup remaining from crystallization of sugar from sugarcane and sugar beets, is also a common feedstock. Even pulp and paper mill sludges contain as much as 40–50 wt% glucose. These sugars can be directly fermented by the yeast *Saccharomyces cerevisiae*, which contains enzymes that hydrolyze disaccharides to simple sugars and catalyze the fermentation of four hexoses: glucose, mannose, fructose, and galactose.

The most important sugar crop for production of biofuels is sugarcane. Traditionally, the leaves and tops of the sugarcane plant are burned or left in the field, while the stalk is transported to a sugar mill. As shown in Figure 7.1, the conversion of sugarcane into ethanol consists of four major processes: milling the cane, filtration/evaporation of the syrup, fermentation of the sugar, and distillation of the ethanol. Milling is designed to separate sugar, making up 20–30 wt% of the cane, from the fibrous part of the plant. The cane is chopped and shredded with revolving blades, and washed with hot water to produce a sugar-rich juice and a fibrous residue, known as bagasse. Bagasse can be used as boiler fuel, containing one-third of the total energy found in sugarcane, and is used by Brazilian ethanol plants as their primary source of electricity as well as heat for evaporation and distillation processes in the plant. After the bagasse has been separated, the juice is filtered multiple times, pasteurized, and evaporated until a mixture of crystalline sugar and molasses remains. The sugar content of the juice is adjusted to less than 22 wt% of sugar to avoid inhibition of yeast activity. Nutrients such as ammonium sulfate are added as needed to make up deficiencies in the original feedstock. The broth is allowed to ferment for up to 12 hours, producing a liquid similar to wine with an alcohol content of up to 10%. Finally, this liquid is distilled to produce 100% ethanol.

The cost of building a modern sugarcane ethanol plant capable of producing 50 million gallons per year was $150 million in 2008 with half of the processed sugarcane being used to produce crystalline sugar and the other half to produce

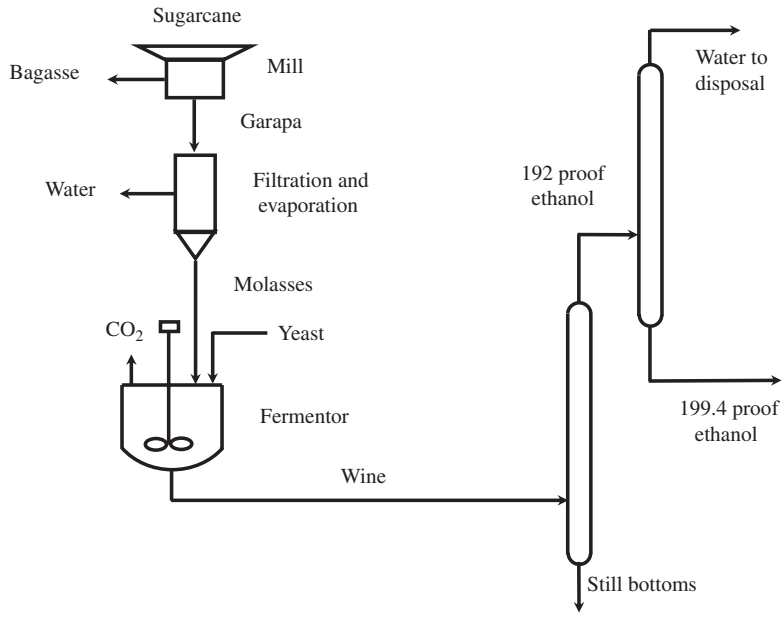

FIG. 7.1 Sugarcane processing to sugar and ethanol.

ethanol. These plants produce roughly 20 gallons of ethanol per ton of sugarcane processed. The cost of production largely depends on the price of sugarcane, which averaged $10.40 per ton in 2005, or $0.54/gallon. After including other expenses like invest and transportation costs, the ultimate cost of production varies from $0.63 to $0.76 per gallon of ethanol, which is 20–80% less expensive than grain ethanol. Because a gallon of ethanol contains only 67% of the energy that a gallon of gasoline contains, this cost is more accurately portrayed as $0.95–$1.13 per gallon of gasoline equivalent, making it competitive with gasoline at $40–$50 per barrel prices.

The ability to use bagasse, the plant fibers that remain after syrup has been pressed from sugarcane, as boiler fuel makes ethanol from sugarcane relatively fossil-fuel efficient. Whereas the net energy balance—the ratio of the energy in a unit of ethanol divided by the fossil fuel energy required to produce it—for corn-based ethanol is 1.3, the balance for sugarcane-based ethanol ranges between 8.2 and 10, due in large part to the energy self-sufficiency of many Brazilian ethanol plants. This use of bagasse also contributes to roughly a 90% decrease in greenhouse gas emissions resulting from using ethanol instead of gasoline.

Brazil has built a transportation sector that relies heavily upon ethanol fermented from sugar crops. However, in the United States, sugar subsidies make it too expensive for production of ethanol.

7.2.2 Starch and Inulin Crops

Starch is a polymer that accumulates as granules in many kinds of plant cells where they serve as a storage carbohydrate. Mechanical grinding readily liberates starch granules. The hydrogen bonds between the basic units of maltose in this polymer are easily penetrated by water, making depolymerization and solubilization relatively easy.

Hydrolysis, the process by which water splits a larger reactant molecule into two smaller product molecules, is readily accomplished for starch. Acid-catalyzed hydrolysis in "starch cookers" at temperatures of 150–200°C proceeds to completion in seconds to minutes. In recent years, enzymatic hydrolysis has supplanted acid hydrolysis due to higher selectivity.

Starch is a glucose polymer with two main components: amylose, a linear polymer of glucose with α-1,4 linkages, and amylopectin, a branched chain including α-1,6 linkages at the branch points. Thus, enzymatic saccharification of starch requires two enzymes. The enzyme amylase hydrolyzes starch to maltose in a process known as liquefaction. The enzyme maltase hydrolyzes maltose into glucose in a process known as saccharification. The consumption of either acid or enzymes for starch hydrolysis is less than 1:100 by weight, making the cost of hydrolysis only a small part of the cost of starch fermentation.

Inulin, like starch, is a storage carbohydrate, but its basic unit is fructose rather than glucose. It is commonly found in tuber crops such as dahlias and Jerusalem artichokes. It is also easily depolymerized and solubilized by both acid- and enzyme-catalyzed hydrolysis. Although potentially an important fermentation feedstock, reversion of fructose to undesired oligofructosans and the lack of widespread cultivation of inulin crops have limited their use.

Cereal grains, such as corn, wheat, and barley, are the most widely used sources of starch for fermentation. The cell walls of grains must be disrupted to expose starch polymers before they can be hydrolyzed to fermentable sugars (i.e., monosaccharides and disaccharides). Grain starch consists of 10–20 wt% amylose and 80–90 wt% amylopectin, both of which yield glucose or maltose on hydrolysis. Although the amylose is water soluble, the amylopectin is insoluble, requiring a "cooking" operation to solubilize it prior to hydrolysis.

Cereal grains also contain other components, such as protein, oil, and fiber, which may be of sufficient value to recover along with the starch. For example, gluten, a mixture of plant proteins occurring in cereal grains, chiefly wheat and corn, is of value as an adhesive and animal feed. If these components are to be separately recovered, extensive pretreatment, known as wet milling, is required before the starch is hydrolyzed and fermented. Under some circumstances, separation of plant components is not economically justified; simpler dry milling is employed to release starch polymers and the whole grain is fermented. Of the 50.5 GL (13.3 billion gal) of fuel ethanol annually produced from corn starch in the United

States, more than 80% comes from dry milling plants, while the remaining 20% is the result of wet milling.

The pretreatments of dry milling and wet milling are distinct in their operations, as detailed in the following paragraphs. The subsequent processes of hydrolysis, fermentation, and distillation, however, are very similar for the two kinds of corn milling plants. Either relatively pure starch from wet milling or a starch-rich "mash" from dry milling is treated with the enzyme amylase and heated to around 93°C to partially hydrolyze the starch in a process called liquefaction to form a mixture of oligosaccharides and polysaccharides known as dextrin. The mash of dextrin is cooled to between 60°C and 70°C, the pH adjusted to 3–5, and the enzyme glucoamylase is added for the final step of hydrolysis, known as saccharification, which yields glucose.

The capital investment for dry milling is less than that for a comparably sized wet milling plant. However, the higher value of its by-products, greater product flexibility, and simpler ethanol production can make a wet milling plant a more profitable investment.

7.2.3 Dry Milling of Corn

Dry milling is essentially a simple grinding procedure. When employed in the food industry, a series of grinding and screening steps are employed. First, the corn is cleaned and then conditioned for 24 hours to increase moisture content to 15–20 wt%. The kernels then pass through roller mills or crushers to separate the kernel into germ, endosperm, and fibrous hull by gravity. The germ contains 18–25 wt% oil, which can be recovered by solvent extraction. The starchy particles of the endosperm can be further ground and sieved to three products distinguished by their particle size: flour, cornmeal, and grits. About 75 wt% of the kernel becomes flour, cornmeal, or grits, 5 wt% is recovered as oil, and 11 wt% is used as fibrous feed. The starchy components can be used in food products or saccharified for fermentation.

For production of fuel ethanol by dry milling, illustrated in Figure 7.2, separation into various products is not necessary. Kernels are ground in a roller mill to grain meal consistency to expose the starch. The meal is slurried with water to form a mash, which is fermented to ethanol. The fibrous residue remaining upon completion of fermentation is recovered from the base of the beer stripping column, mixed with yeast and other unfermented residues, and dried to a coproduct known as distillers' dried grains and solubles (DDGS). This coproduct, containing about 25 wt% protein and residual oil, is a valuable feed for cattle. Profitability of a corn-to-ethanol plant is strongly tied to the successful marketing of DDGS.

A typical dry milling plant will produce about 10.6 L (2.8 gal) of ethanol per bushel of corn processed. Yields of coproducts per bushel of corn are about 7.1 kg (15.7 lb) of DDGS and 7.5 kg (16.5 lb) of carbon dioxide evolved from

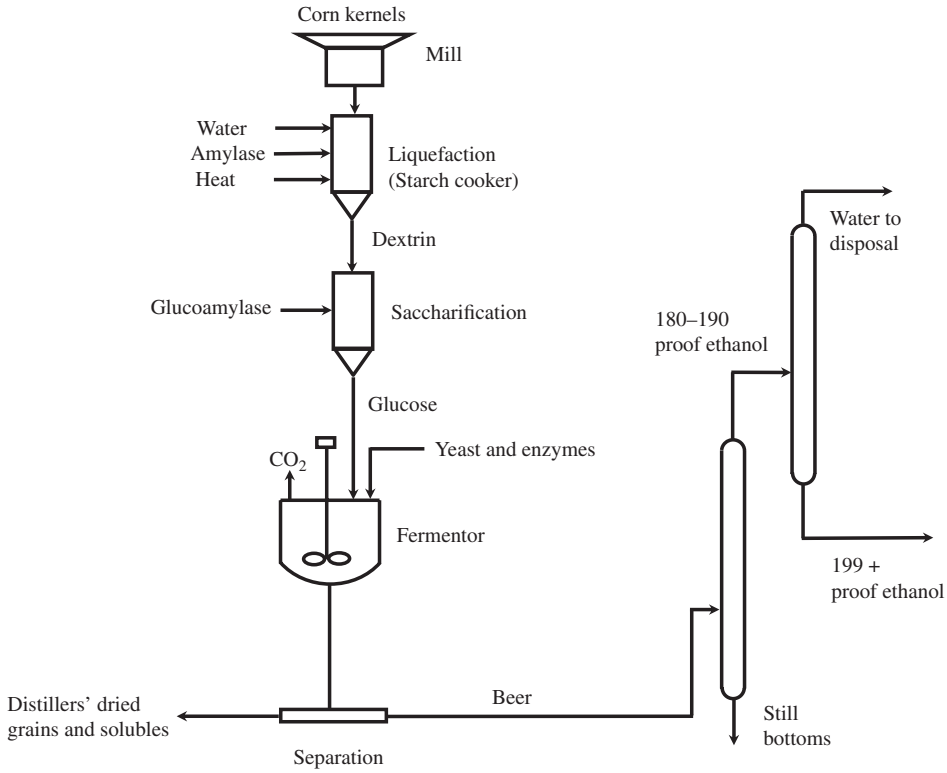

FIG. 7.2 Dry milling of corn.

fermentation, the latter of which can be sold to the carbonated beverage industry. As a rule of thumb, the three products are produced in approximately equal weight per bushel, with each accounting for approximately one-third of the initial weight of the corn.

7.2.4 Wet Milling of Corn

Wet milling has the advantage that it separates plant components into carbohydrate (starch), lipids (corn oil), a protein-rich material (gluten), and fiber (hulls). This gives a company access to higher value markets as well as provides flexibility in the use of starch as a food product or in the production of fuel ethanol.

The wet milling process is illustrated in Figure 7.3. The corn is cleaned and then conveyed into steep tanks where it is soaked in a dilute solution of sulfur dioxide for 24–36 hours, which swells and softens the corn kernels. Some of the protein and other compounds are dissolved in the resulting corn steep liquor, which represents an inexpensive source of nitrogen and vitamins.

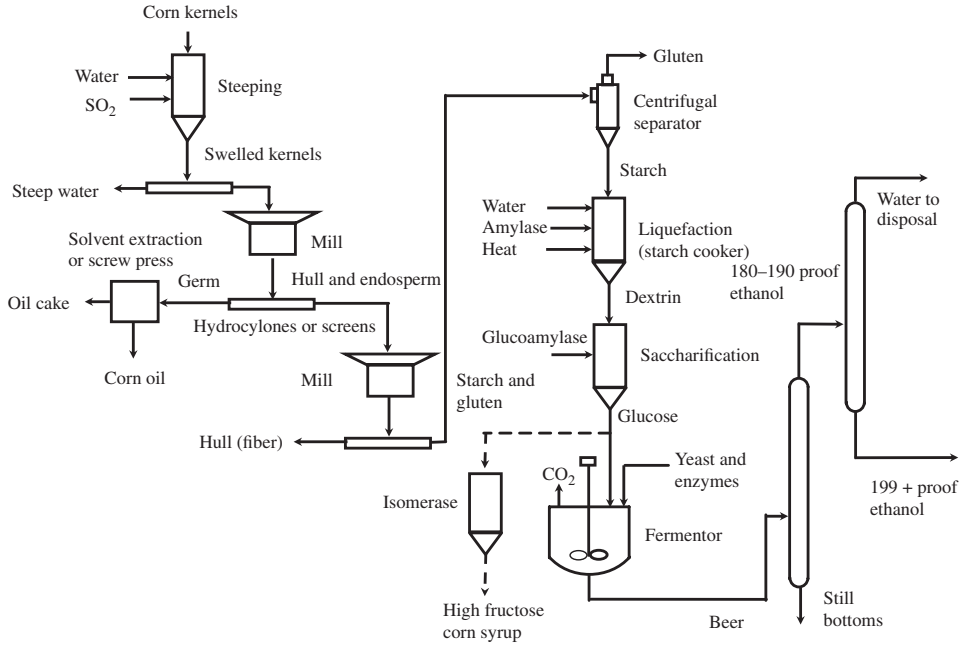

FIG. 7.3 Wet milling of corn.

After separating the corn from the steep liquor, the wet kernels are coarsely ground to release the hull and germ from the endosperm. Hydrocyclones or screens separate the germ from the rest of the components. After drying, oil is extracted from the germ by either solvents or a screw press, leaving a residual oil cake. The hull and endosperm pass through rotating disc mills that grind the endosperm into fine fractions of starch and gluten, while the hull yields coarser fiber particles, which can be screened out from the finer fractions. Centrifugal separators separate the lighter gluten from the starch.

The starch can be used directly as a food product or for industrial manufacturing processes, especially papermaking. The starch can also be converted to monosaccharides for the production of food or fuel, depending on relative market demand. Saccharification by amylase enzymes yields corn syrup, a glucose solution that can be directly fermented to fuel ethanol. Alternatively, treated with isomerase enzymes, the glucose is partially converted to fructose to yield a liquid sweetener known as high fructose corn syrup (HFCS). In plants that can alternate between fuel ethanol and HFCS production, relatively more ethanol is produced in the winter, while relatively more HFCS is produced in the summer.

The gluten product, known as corn gluten meal, contains 60% protein and is used primarily as poultry feed. The fiber from the hulls is combined with

other byproducts, such as the oil cake, steep water solubles, and excess yeast from stillage, dried and sold as corn gluten feed. Containing 21% or more of protein, it is primarily used as feed for dairy cattle.

A typical wet milling plant will produce about 10.6 L (2.8 gal) of ethanol per bushel of corn processed. Yields of other coproducts per bushel of corn are 0.7 kg (1.7 lb) of corn oil, 1.4 kg (3 lb) of corn gluten meal (60% protein), 5.9 kg (13 lb) of corn gluten feed (21% protein), and 7.7 kg (17 lb) of carbon dioxide. Like dry milling, the three products of ethanol, feed, and carbon dioxide are produced in approximately equal weight per bushel, with each accounting for approximately one-third of the initial weight of the corn.

7.2.5 Fermentations to Products Other Than Ethanol

Only a small fraction of commercial production of organic compounds comes from microbial processes. With the exception of ethanol, most fermentation products are specialty chemicals including carboxylic acids, amino acids, antibiotics, industrial and food enzymes, and pharmaceuticals. Examples of organic chemicals produced from fermentation are listed in Table 7.2.

Although many biorenewable resources are cheaper than petroleum, virtually all commodity organic chemicals are produced from petroleum, because processing costs are generally much cheaper for the conversion of hydrocarbons to these products. Only a few fermentation products can be considered as commodity chemicals (worldwide production exceeding 50 000 tons per year): ethanol, monosodium glutamate, citric acid, lysine, and gluconic acid. Ethanol leads this group (15 million tons per year) by virtue of various tax incentives for its manufacture from renewable resources. New developments in fractionation of lignocellulose to simple sugars and environmental considerations may ultimately increase the production of commodity chemicals from biorenewable resources.

Only a relative small number of organic compounds are produced as the primary products of anaerobic fermentation. These include a few simple alcohols and acids, a single ketone (acetone), and two gases of interest as fuel: methane and hydrogen. All of these products are derived from the single intermediate, pyruvate. The primary substrates for most of these fermentations are monosaccharides or oligosaccharides consisting of two or three monosaccharides. Because transport of nutrients and metabolic products through the cell walls of microorganisms are limited to low-molecular-weight compounds, the higher oligosaccharides and polysaccharides must be depolymerized outside the organisms to low-molecular-weight sugars before they can be metabolized. Microorganisms accomplish this by secreting enzymes such as amylase or cellulase to break down starch and cellulose, respectively, to sugars that can be transported into the cell. The following paragraphs describe how various commodity chemicals could be produced through fermentation.

Table 7.2 Examples of chemical products from fermentation of carbohydrates

Chemical	Substrate(s)	Microorganism(s)
Acetic acid	Various sugars	*Acetobacter aceti* *Clostridium thermoaceticum* *Pachysolen tannophilus*
Acetone	Various sugars	*Clostridium* sp.
2,3-Butanediol	Various sugars and acids	*Aerobacter aerogenes* *Bacillus polymyxa* *Klebsiella oxytoca* *Klebsiella pneumoniae*
n-Butanol	Various sugars and organics	*Clostridium* sp.
Butyraldehyde	Glucose	*Clostridium acetobutylicum*
Butyric acid	Various sugars	*Clostridium* sp.
Citric acid	Various sugars	*Aspergillus niger* *Saccharomycopsis lipolytica*
Ethanol	Various sugars	*Kluyveromyces* sp. *Candida utilis* *Saccharomyces cerevisiae* *Zymomonas mobilis*
C12–C20 fatty acids	Sucrose	Bacteria, mold, yeast
Gluconic acid	Glucose	*Aspergillus niger* *Gluconobacter suboxydans*
Isopropyl alcohol	Various sugars	*Clostridium* sp.
Itaconic acid	Glucose, sucrose	*Aspergillus itaconicus* *Ustilago zeae* *Aspergillus terreus*
Linoleic acid	Glucose, lactose	*Candida curvata*
Linolenic acid	Various sugars	*Mortierella ramammiana*
Oleic acid	Glucose, lactose	*Candida curvata*
Palmitic acid	Glucose, lactose	*Candida curvata*
Propanediol	Algal biomass, glucose	*Clostridium pasteurianum* *C. thermosaccharolyticum*
n-Propanol	Glucose	*Clostridium fallax*
Propionic acid	Various sugars	*Clostridium* sp. *Propionibacterium shermanii*
Pyruvic acid	Glucose	*Pseudomonas aeruginosa*
Sorbitol	Sucrose	*Zymomonas* sp.
Stearic acid	Glucose, lactose	*Candida curvata*
Succinic acid	Various sugars	Many species

Source: Leeper, S.A., Ward, T.E., and Andrews, G.F. (1991) Production of organic chemicals via bioconversion: a review of the potential. Report EGG-BG-9033. Idaho Falls, ID: Idaho National Engineering Laboratory.

Butanol

Butanol can be produced biologically through so-called acetone–butanol–ethanol (ABE) fermentation. Certain strains of *Clostridia* bacteria, particularly *Clostridium acetobutylicum* and *Clostridium beijerinckii* are able to ferment soluble C6 and C5 sugars in two distinct phases. The first phase of acidogenesis produces carboxylic acids such as lactic, acetic, and butyric acids and carbon dioxide and hydrogen. In the second phase, solventogenesis converts these acids to solvents including

acetone, butanol, and ethanol. Typical product ratios of acetone, butanol, and ethanol are 3:6:1.

ABE fermentations were used commercially during World War I for production of acetone and in the 1940s and 1950s to produce biobutanol although it was eventually superseded by cheaper petroleum-derived butanol. In batch fermentations, yields are limited by *Clostridia*'s susceptibility to butanol toxicity, which resulted in high cost of butanol recovery from the dilute product solutions. Reactor productivity of butanol is typically limited to 0.5 g/L/h and concentrations of only 20 g/L.

A number of techniques have been recently developed to improve the prospects for butanol fermentation, including introduction of hyper-butanol-producing strains, cell immobilization, continuous fermentation, alternative product recovery techniques. As a result, reactor productivity has increased a factor of 10–30 and concentrations increased a factor of three or more. Fermentation substrates other than sugar are possible. *Clostridium pasteurianum* can convert glycerol into butanol at a maximum concentration of 17.0 g/L. *Clostridium carboxidivorans* P7 is able to ferment syngas from the gasification of biomass although producing butanol at relatively low concentrations (2.2 mg/L), which could be improved by enhancing mass transfer between the syngas and the aqueous fermentation broth (e.g., using hollow membrane fibers).

2,3-Butanediol

The heating value of butanediol (27 198 J/g) is close to that of ethanol (29 055 J/g) and methanol (22 081 J/g), making it a potentially attractive fuel. Many strains of microorganisms are able to ferment five- and six-carbon sugars to 2,3-butanediol including *Bacillus polymyxa* and *Klebsiella (Aerobacter) pneumoniae*. Under anaerobic conditions, approximately equimolar amounts of ethanol and 2,3-butanediol are produced by all strains. Limited aeration decreases ethanol production and 2,3-butanediol becomes the major or even sole product. Final product concentration for *B. polymyxa* is only 2–3% w/v, while *K. pneumoniae* can accumulate to 6–8% w/v. Recovery of 2,3-butanediol is difficult for several reasons, including high boiling point, high heat of vaporization, and its hydrophilic nature.

Aliphatic Acids

Aliphatic acids are derived from straight- or branched-chain organic compounds that include alkenes, alkanes, or alkynes. Some short-chain aliphatic acids, such as acetic and propionic acids, can be produced as major products of fermentation. More typically, though, a mixture of acids and alcohols are produced. These are the same acetogenic bacteria involved in anaerobic digestion as described in Chapter 9 except that the methanogenic step is not allowed to proceed. The accumulating organic acids are toxic to the microorganisms that create them. Hydroxides or carbonates of sodium, potassium, magnesium, calcium, or ammonium are added to

the fermentation broth to neutralize organic acids as they are formed. For example, *Clostridium thermoaceticum* and *Clostridium thermoautotropicum* ferment fructose and a few six-carbon sugars to two moles of acetate by decarboxylation of pyruvate, while the third mole is produced by reduction and incorporation of CO_2.

High base consumption is an inherent feature of organic acid fermentation; the resulting salts of aliphatic acids (acetate) must be decomposed and the cations must be recovered if the process is to be cost-effective. One approach is to pyrolyze the resulting acetate at 300°C to form ketones such as 2-propanone, 2-butanone, 2-pentanone, and 3-pentanone. The mixed ketones, which have high energy content, high octane rating, and boiling range compatible with gasoline, have been proposed as transportation fuel. Alternatively, the ketones can be hydrogenated to mixed alcohols (i.e., 2-propanol, 2-butanol, 2-pentanol, and 3-pentanol).

Lactic Acid

Lactic acid, a three-carbon molecule, is used in the production of polylactide (PLA) resin, a biodegradable polymer expected to compete with polyethylene and polystyrene in the synthetic fibers and plastics markets. Lactic acid is currently produced by milling corn, separating the starch, hydrolyzing the starch to glucose, and anaerobically fermenting the glucose to lactic acid with *Bacillus dextrolacticus* or *Lactobacillus delbrueckii*. Esterification with ethanol produces ethyl lactate, which can be polymerized to polylactate resin.

Succinic Acid

Succinic acid is used in producing food and pharmaceutical products, surfactants and detergents, biodegradable solvents and plastics, and ingredients to stimulate animal and plant growth. Although it is a common metabolite formed by plants, animals, and microorganisms, its current commercial production of 15 000 tons per year is from petroleum. However, the recently discovered rumen organism *Actinobacillus succinogenes* produces succinic acid with yields as high as 110 g/L, offering prospects for producing this chemical from biorenewable resources. In contrast to most other commercial fermentations, the process consumes carbon dioxide; integrated with a process like ethanol fermentation, succinic acid production could contribute toward reductions in greenhouse gas emissions.

Optimum yields occur under pH conditions where succinate salt rather than free acid is produced. Thus, recovery entails concentration of the salt, conversion back to free acid, and polishing of the acid to desired purity. Downstream purification can account for 60–70% of the product cost. One recovery approach uses calcium hydroxide to precipitate calcium succinate from the fermentation broth. Acidification of the precipitate yields gypsum. Disadvantages include handling and disposing of large amounts of wet gypsum. Another recovery approach employs simultaneous electrodialysis, acidification, and crystallization. Sodium succinate

and other ionic species are transported across the ion-exchange membranes and separated from concentrated sugars, proteins, and amino acids.

Hydrocarbons

Hydrocarbons can also be synthesized in microbial fermentations. The common yeast *Saccharomyces* can synthesize a variety of straight-chain and branched-chain hydrocarbons containing between 10 and 34 carbon atoms, which is a range suitable for upgrading to transportation fuels. Yields are a respectable 10.2 wt% of dry biomass under anaerobic conditions. The highest reported yield of hydrocarbons in wild-type bacteria is the salt-tolerant bacterium *Vibrio furnissii*, which produces hydrocarbons at 60 wt% of dry biomass. More commonly, hydrocarbon yields are just a few percent weight of dry biomass. Metabolic engineering could improve yields of hydrocarbons by mapping metabolic fluxes associated with their production and identifying enzymes responsible for hydrocarbon synthesis.

For example, terpene synthesis has been promoted in both eukaryotes and prokaryotes through metabolic engineering. Unlike most biomolecules, which incorporate copious quantities of oxygen into their molecular structures, terpenes contain little or no oxygen. Terpenes are constructed from a molecular building block known as isoprene C_5H_8, which is one of a class of compounds characterized by the presence of two pairs of double carbon bonds separated by a single carbon bond (isoprenes). Both terminal carbon atoms can bond with terminal carbon atoms of other isoprenes to form a variety of hydrocarbon compounds with different degrees of saturation (also of interest are closely related terpenoids, which are derived from terpenes by moving or removing methyl groups or adding oxygen atoms). Like other forms of lipids, terpenes must be upgraded to a final fuel product. Although very little oxygen would have to be removed, saturation and structural rearrangement of the terpene molecules is required to obtain suitable gasoline, diesel fuel, or aviation fuel products.

7.3 Conversion of Lignocellulosic Feedstocks to Sugar

Much of the carbohydrate in plant materials is structural polysaccharides, providing shape and strength to the plant. The hydrolysis of polysaccharides in cell walls is more difficult than the hydrolysis of storage polysaccharides such as starch. This structural material, known as lignocellulose, is a composite of cellulose fibers embedded in a cross-linked lignin–hemicellulose matrix. Depolymerization to basic plant components is difficult, because lignocellulose is resistant to both chemical and biological attack.

A variety of physical, chemical, and enzymatic processes have been developed to fractionate lignocellulose into the major plant components of hemicellulose,

cellulose, and lignin. The hemicellulose fraction is readily hydrolyzed to pentoses (five-carbon sugars), but pentoses are difficult to ferment. The cellulose exists as both amorphous and crystalline forms, which hydrolyze to hexoses (six-carbon sugars). Crystalline cellulose is recalcitrant to hydrolysis. However, the resulting hexoses are readily fermented. Distillation can recover the desired products of fermentation. Lignin, which is not susceptible to biological transformation, can be chemically upgraded or, more frequently, simply burned as boiler fuel. The steps of pretreatment, hydrolysis, fermentation, and distillation in the production of biobased products from lignocellulose are described in the following sections.

7.3.1 Pretreatment

Pretreatment is one of the most costly steps in conversion of lignocellulose to sugars, accounting for about 33% of the total processing costs. Pretreatments often produce biological inhibitors, which impact the cost of fermenting the resulting sugars. Accordingly, much attention is directed at developing low cost and effective pretreatments.

An important goal of all pretreatments is to increase the surface area of lignocellulosic material, making the polysaccharides more susceptible to hydrolysis. Thus, comminution, or size reduction, is an integral part of all pretreatments. Primary size reduction employs hammer mills to produce particles that can pass through 3 mm screen openings. The process has relatively modest energy requirements, ranging from 24 000 kJ per dry ton for wheat straw to 200 000 kJ per dry ton for aspen wood. However, energy consumption increases exponentially with decreasing particle size. If the subsequent hydrolysis process requires further improvements in accessibility and susceptibility of polysaccharides, alternatives to finer milling are usually employed.

As described in the next section, three types of hydrolysis have been developed for releasing sugars from lignocellulose: two that employ mineral acids and one based on enzymes. The molecules of mineral acids, which are small compared to the pore volume of plant tissue, diffuse deeply into lignocellulosic material; thus primary size reduction produces sufficient surface area for acid hydrolysis. Additional pretreatment prior to acid hydrolysis, though, is often practiced to improve the yield of pentose sugars, as subsequently explained. Enzymatic hydrolysis always requires pretreatment beyond size reduction. Cellulase enzymes are large proteins with molecular weights ranging from 30 000 to 60 000 and are thought to be ellipsoidal with major and minor dimensions of 30 and 200 Å. Typically, only 20% of the pore volume of plant tissue is accessible to these large molecules. Without additional pretreatment, sugar yields from enzymatic hydrolysis are less than 20% of theoretical, whereas pretreatment can increase yields to 90% and higher.

The mechanisms by which pretreatments improve the digestibility of lignocellulose are not well understood. Pretreatment effectiveness has been correlated

with removal of hemicellulose and lignin. Lignin solubilization is beneficial for subsequent hydrolysis, but may also produce derivatives that inhibit enzyme activity. Some pretreatments are thought to reduce crystallinity of cellulose, which improves reactivity, but this does not appear to be the key for many successfully pretreatments. The large variety of pretreatment processes developed can be broadly classified as biological, alkaline, steam explosion, prehydrolysis, ammonia fiber explosion (AFEX), and treatment with organic solvents.

Biological pretreatments employ microorganisms that produce lignin-degrading enzymes (ligninase). As lignin is decomposed, cellulose and hemicellulose are released from the lignocellulosic matrix. The exploitation of ligninase-producing microorganisms has been little developed and faces several hurdles including long reaction times (measured in weeks) and the fact that many ligninolytic microorganisms also produce cellulases and hemicellulases and grow on the resulting sugars, degrading yields.

Alkali metal hydroxides not only break lignin–hemicellulose bonds but also dissolve lignin and hemicelluloses. At very high concentrations (5–20 wt%) alkaline metal hydroxides also swell cellulose. The degradative and dissolving action of hot sodium hydroxide solutions is the basis of the kraft pulping process described in Chapter 10. However, alkali pulping creates long cellulose fibers, which complicates subsequent biochemical processing, and destroys hemicelluloses, a significant source of fermentable sugars. The process can be adjusted to provide a pretreatment that is compatible with subsequent enzymatic hydrolysis of herbaceous biomass, such as wheat straw and cornstover, and some hardwoods. It has not been achieved for softwoods. A barrier to commercial use of this pretreatment is the significant amount of chemicals consumed in neutralizing the acidic carboxylic groups in biomass.

Steam explosion involves saturation of the pores of plant materials with steam followed by rapid decompression; the explosive expansion of steam reduces the plant material to separated fibers, presumably increasing the accessibility of polysaccharides to subsequent hydrolysis. Early research focused on the mechanical disruption of plant tissues and employed relatively high temperatures (220–270°C) and short residence times (40–90 s). Conditions and equipment for steam explosion are similar to the commercial masonite process developed in the 1930s for the production of fiberboard.

For sugar production from lignocellulose, recent research suggests that more important than mechanical disruption are chemical changes that occur prior to the rapid decompression step, including hydrolysis of hemicellulose and the condensation of lignin into small droplets. The removal of hemicelluloses and concurrent condensation of lignin creates numerous large pores in hardwoods and herbaceous biomass, which allow penetration of cellulase enzymes to cellulose fibers. Researchers discovered that comparable hydrolysis of hemicellulose could be achieved at lower temperatures (190–210°C) by use of longer residence times

(3–15 minutes), while reducing pyrolytic decomposition. Hydrolysis of hemicellulose yields mostly pentoses. Xylan, the most common polysaccharide in hemicelluloses, consists of xylose units. Thus, the dominant pentose in the hydrolysate from hemicellulose is xylose followed by arabinose.

This steaming of biomass for several minutes is called autohydrolysis based on the hypothesis that acidic compounds released from the biomass are responsible for the process. Although this hypothesis has never been proven, the name persists in the literature. The main drawback of autohydrolysis is the relatively low yield of hemicellulosic sugars (about 50%) due to partial hydrolysis and pyrolytic decomposition at high temperatures. Furthermore, the process is not effective with softwoods.

Addition of small amounts of mineral acid, usually sulfuric acid, improves hydrolysis of hemicellulose at reduced temperatures. This process is variously known as prehydrolysis, dilute acid pretreatment, or acid-catalyzed steam explosion. The comminuted biomass is treated with 1 wt% sulfuric acid and incubated at 140°C for 30 minutes or at 160°C for as little as 5–10 minutes to achieve complete hemicellulose removal, which increases enzymatic digestibility of the remaining cellulose to as high as 90%.

Alternatively, sulfur dioxide gas can be added in the amount of 2–3 wt% to moist biomass chips and heated to 150°C for 20 minutes to hydrolyze hemicellulose. The sulfur dioxide rapidly diffuses into biomass pores before it is converted into sulfuric acid, providing superior performance compared to the direct use of an acid catalyst. It is far less corrosive than mineral acids. Steam pretreatment of hardwoods using SO_2 can recover more than 80% of the hemicellulose as monomers, more than 90% of the lignin by alkali washing, while enzymatic hydrolysis of the pretreated biomass can convert 100% of the cellulose to fermentable sugars. The same process can recover 65% of the hemicellulose as monomers, 80% of the lignin by a combination of both alkali and peroxide washing, and complete conversion of the cellulose by enzymatic hydrolysis. Corncobs, which have very high hemicellulose content, are particularly easy to digest. Prehydrolysis at 150°C converts the hemicellulose to xylose after only 5 minutes.

The resulting prehydrolysates contain soluble dextrins and sugars, acetic acid, furfural, and low-molecular weight phenols derived from lignin, and other fermentation inhibitors. The acetic acid is inhibitory to fermentation above a threshold value of 0.5 g/L. Hardwoods such as aspen release as much as 6–10 g/L of acetic acid during prehydrolysis, while softwoods, which have fewer acetate groups in their hemicellulose, produce 2–4 g/L. Acetic acid and other volatile compounds can be readily removed from the pretreated biomass by steam to the required 0.5 g/L level or less. Furfural and other volatile compounds are also stripped out.

Prehydrolysis also yields furfural from xylose, which can be recovered during the flash cooling that occurs during pressure letdown at the discharge of the reactor. Furfural, which is a valuable solvent in oil refining and is also a base for furan

resins, has been commercially produced from a variety of agricultural residues, including corncobs, cornstover, and oat hulls.

A disadvantage of prehydrolysis is the need to neutralize the acidified biomass. Although calcium hydroxide (lime) is a relatively inexpensive base, the resulting calcium sulfate (gypsum) is of low value and represents a waste disposal problem. Also, some sugar decomposition invariably occurs.

Ammonia fiber explosion is similar to steam explosion except that liquid ammonia is employed. Pressures exceeding 12 atm are required for operation at ambient temperature with ammonia loadings in the range of 1.0–2.5 kg per kg dry biomass. The mixture is incubated for several minutes to up to an hour to enable ammonia to penetrate the lignocellulosic matrix. Hydrolysis yields from AFEX-treated agricultural residues are 80–90% of theoretical, which is superior to that achieved by prehydrolysis. Furthermore, no gypsum byproduct is generated. The process has not been successfully applied to either hardwoods or softwoods.

Organic solvents have been used for pretreatments to remove lignin from biomass. Lignin can adsorb significant amounts of cellulase enzymes employed for enzymatic hydrolysis of cellulose. Thus, lignin removal can decrease the requirement for costly enzymes in downstream processing. Selective removal of lignin can be accomplished by the addition of organic solvents (usually lower alcohols) to the acidic or alkaline aqueous solutions of pulping or pretreatment processes.

7.3.2 Hydrolysis

Three basic methods for hydrolyzing structural polysaccharides in plant cell walls to fermentable sugars are available: concentrated acid hydrolysis, dilute acid hydrolysis, and enzymatic hydrolysis. The two acid processes hydrolyze both hemicellulose and cellulose with very little pretreatment beyond comminution of the lignocellulosic material to particles of about 1 mm size. The enzymatic process must be preceded by extensive pretreatment to separate the cellulose, hemicellulose, and lignin fractions.

Concentrated acid hydrolysis is based on the discovery over a century ago that carbohydrates in wood will dissolve in 72% sulfuric acid at room temperature, leaving behind the lignin fraction. For fermentation, the solution of oligosaccharides is diluted to 4% H_2SO_4 and heated at the boiling point for 4 hours or in an autoclave at 120°C for 1 hour to yield monosaccharides. Following neutralization with limestone, the sugar solution can be fermented. Concentrated acid hydrolysis is relatively simple and is attractive for its high sugar yields, which approach 100% of theoretical hexose yields.

Both sulfuric acid and hydrochloric acid have been considered for commercial development of concentrated acid hydrolysis. The low price of sulfuric acid makes it attractive as a hydrolyzing agent. Nevertheless, the large volume of acid required, about equal to the weight of sugars produced, mandates its recovery and reuse. The

recovery of sulfuric acid is complicated by its high boiling point. Electrodialysis and solvent extraction are possible recovery options. Hydrochloric acid is significantly more expensive and corrosive than sulfuric acid, but its higher volatility presents opportunities for recovery by distillation. Evaporation under vacuum yields a high boiling azeotrope (18% HCl, 120°C), which presents difficulties in further recovery. Neutralization of the acid with limestone also generates a large waste stream of gypsum ($CaSO_4$). An ethanol plant would generate over 2 kg of gypsum per liter of ethanol produced, or 40 000 tons of wet gypsum for a 20 million L/year plant.

Dilute acid hydrolysis (about 1% acid by weight) greatly reduces the amount of acid required to hydrolyze lignocellulose. The process is accelerated by operation at elevated temperatures: 100–160°C for hemicellulose and 180–220°C for cellulose. Unfortunately, the high temperatures cause oligosaccharides released from the lignocellulose to decompose, greatly reducing yields of simple sugars to only 55–60% of the theoretical yield. The decomposition products include a large number of microbial toxins such as acetic acid and furfural, which inhibit fermentation of the sugars. The need for corrosion-resistant equipment and low concentrations of sugars from some reactor systems also adversely impact the cost of sugars.

Early efforts in both concentrated and dilute acid hydrolysis of lignocellulose focused on recovery of hexoses, which are easily fermentable. Pentoses released from hemicellulose were considered of little value and no attempt was made to recover them or prevent their degradation. Since biomass is a relatively expensive feedstock, recovery of both hexoses and pentoses is now considered essential to the economic viability of any lignocellulose-to-sugars process. Toward this end, acid hydrolysis processes have incorporated pretreatments not for the purpose of increasing cellulose accessibility but to separately recover pentose from hemicellulose to prevent its degradation under the harsh conditions of acid hydrolysis.

An example of a lignocellulose-to-ethanol plant based on concentrated acid hydrolysis with pretreatment to recover hemicellulose is illustrated in Figure 7.4. Milled biomass is conveyed to a prehydrolysis (hemicellulose) reactor for the purpose of converting hemicellulose into pentose. The biomass is treated with a stream of 9% sulfuric acid and dissolved glucose is recycled from the concentrated acid (cellulose) reactor. The slurry is heated to 100°C for 2–3 hours, which is sufficient to hydrolyze the hemicellulose. The liquid, containing both pentose and hexose, is pressed from the biomass, neutralized with limestone, filtered to remove the resulting gypsum, and sent to a fermentor. The prehydrolyzed biomass enters one or more stages of a concentrated acid (cellulose) reactor where it is treated with 70% sulfuric acid at 100°C for 2–4 hours. Hydrolysis yields hexose and lignin, which is removed by a filter press. The acidic sugar solution is recycled to the prehydrolysis reactor. When cornstover is used as feedstock, alcohol recovery is 315 L per ton of biomass.

A dilute acid hydrolysis plant with pretreatment to recover hemicellulose is illustrated in Figure 7.5. The process resembles the concentrated acid plant except

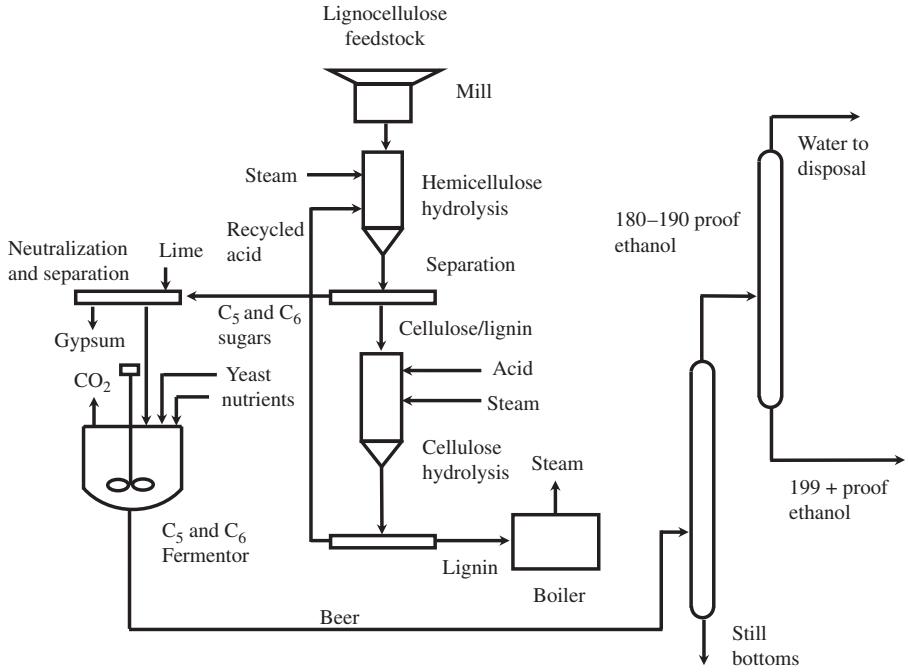

FIG. 7.4 Concentrated acid hydrolysis of lignocellulosic biomass.

that pentoses are recovered and fermented separately from the hexose and cellulose is hydrolyzed with dilute sulfuric acid. Biomass is impregnated with 1% sulfuric acid and heated with saturated steam at 1.24 MPa for 4 minutes to prehydrolyze hemicellulose. The pentoses are separated from the residual lignocellulose by filtering, neutralized with limestone, and sent to a pentose fermentor. The residual lignocellulose is impregnated with 3% sulfuric acid and heated with steam at 2 MPa for 4 minutes to hydrolyze the cellulose. The hexose solution is filtered from the lignin residue, neutralized with limestone, and fermented in a hexose fermentor. The lignin is used as boiler fuel. The yield of alcohol from poplar wood is 250 L per ton.

Enzymatic hydrolysis was developed to better utilize both cellulose and hemicellulose from lignocellulosic materials. Pretreatment solubilizes hemicellulose under milder conditions than those required for acid hydrolysis of cellulose. Subsequent enzymatic hydrolysis of the cellulose does not degrade pentoses released during prehydrolysis.

Cellulose is a homopolysaccharide of glucose linked by β-1,4'-glycosidic bonds. Thus, enzymatic hydrolysis of cellulose proceeds in several steps to break glycosidic bonds by the action of a system of enzymes known as cellulase. Native cellulose is hydrolyzed by the isoenzymes cellobiohydrolase I and cellobiohydrolase II to yield

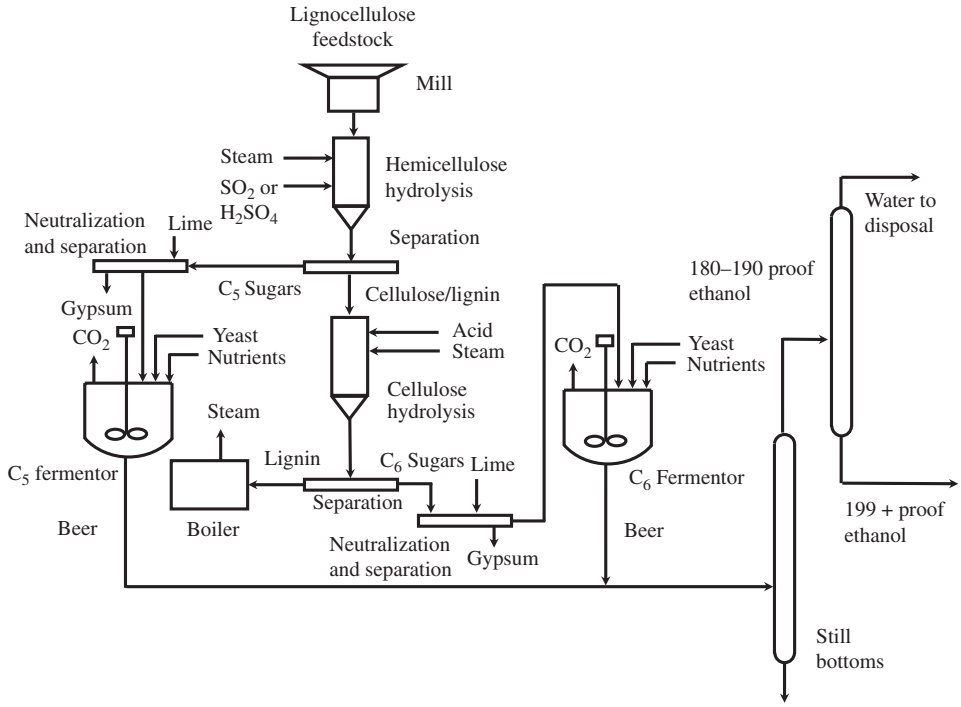

FIG. 7.5 Dilute acid hydrolysis of lignocellulosic biomass.

cellodextrins and cellobiose. The cellodextrins are further hydrolyzed to cellobiose, a disaccharide of glucose, by the isoenzymes endoglucanase I and endoglucanase II. The cellobiose is hydrolyzed to monosaccharide by β-glucosidase. The system of enzymes also usually contains hemicellulase to hydrolyze any hemicellulose not solubilized by prehydrolysis.

A variety of fungi and bacteria produce cellulases both aerobically and anaerobically. The aerobic mesophilic fungus *Trichoderma reesei* and mutants of it have been the most intensely studied sources of cellulases. Other fungal cellulase producers include *Trichoderma viride*, *Trichoderma lignorum*, *Trichoderma koningii*, *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp., *Chrysosporium pannorum*, and *Sclerotium rolfsii*. The problems with enzymatic hydrolysis are relatively low specific activity, low rates of conversion, and sensitivity to end product inhibition. The low specific activity leads to high enzyme loading requirements: approximately 1 kg of enzyme is needed for hydrolysis of 50 kg of cellulose fibers. Conversion rates are as low as 20% in 24 hours; thus, up to 7 days are required to digest lignocellulose. Simultaneously hydrolyzing cellulose and fermenting hexose as it is released, a technique described in the next section, can substantially overcome end product inhibition.

7.3.3 Fermentation

The first step in a successful fermentation is removal of toxic compounds from the hydrolysate that would otherwise inhibit the growth of fermentation organisms. These toxic compounds include furfural and acetic acid, which are breakdown products from hydrolysis of hemicellulose. Traditional detoxification methods, such as the addition of activated carbon, extraction with organic solvents, ion exchange, ion exclusion, molecular sieves, over-liming, and steam stripping, can be costly. Another method under development is adaptation of the fermentation organisms to the inhibitory substances.

Numerous yeast species, including common baker's yeast *S. cerevisiae* and two species of bacteria in the genus *Zymomonas*, efficiently ferment six-carbon sugars to ethanol and carbon dioxide. These microorganisms are suitable for fermenting traditional sugar and starch crops, which yield hexoses upon processing. However, they are not able to ferment the pentoses released from lignocellulosic biomass upon hydrolysis of the hemicellulosic fraction. Efficient conversion of lignocellulosic biomass requires fermentation of pentoses, especially xylose and arabinose.

A variety of microorganisms can directly ferment pentose. Among wild-type yeast genera *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* are able to ferment both five-carbon and six-carbon sugars. Maximum yields are on the order of 50 g/L compared to 150 g/L for hexose-fermenting *S. cerevisiae*. A few filamentous fungi, notably within the genera *Fusarium*, *Rhizopus*, and *Paecilomyces*, ferment both five- and six-carbon sugars to ethanol and CO_2, but at low rates and final concentrations of ethanol. Thermophilic bacteria such as *Clostridium thermohydrosulfuricum*, *Clostridium thermosaccharolyticum*, and *Clostridium thermocellum* are able to produce ethanol from both hexoses and pentoses but they have low tolerances for end products. For example, the maximum yield of ethanol by *C. thermocellum* is less than 30 g/L.

Research into using wild-type bacteria and fungi to convert xylose waned during the 1980s, due in part to the widely recognized disadvantages of low rate and/or poor yield of these types of microorganisms, coupled with the fact that moderately productive xylose-fermenting yeasts had been identified. Recombinant DNA techniques are being employed to produce new strains of microorganisms with the desired trait of fermenting both hexoses and pentoses. For example, the bacteria *Escherichia coli* are able to convert both hexoses and pentoses to pyruvate but the end product is acetic acid rather than ethanol. The bacteria *Zymomonas mobilis* are able to convert pyruvate to ethanol but cannot ferment pentose. Using recombinant techniques, researchers transferred pyruvate decarboxylase and alcohol dehydrogenase from *Z. mobilis* to *E. coli*, which resulted in an organism able to ferment up to 90% of the sugars derived from lignocellulose with final ethanol concentrations ranging from 40 to 58 g/L.

Three approaches have been developed for fermenting sugars released from lignocellulose: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and CBP.

The SHF process employs separate steps for the unit operations of prehydrolysis, enzymatic hydrolysis, and fermentation. The primary advantage of this approach is that by separating each step, undesirable interactions are avoided. Use of separate reactors for hydrolysis and fermentation allows these two processes to proceed at their optimal temperatures: 50°C for enzymatic hydrolysis and 20–32°C for fermentation. The disadvantage is inhibition of the enzyme β-glucosidase by the hydrolysis product glucose, thus requiring lower solids loadings to obtain reasonable yields. Low sugar concentrations result in lower ethanol concentrations, which increase the cost of fermentation and subsequent product recovery.

The SSF process combines hydrolysis (saccharification) and fermentation to overcome end product inhibition that occurs during hydrolysis of cellobiose. By combining hydrolysis and fermentation in the same reactor, glucose is rapidly removed before it can inhibit further hydrolysis. The SSF process is illustrated in Figure 7.6. Biomass feedstock is milled and then prehydrolyzed to yield a mixture of pentoses, primarily xylose and arabinose, and fiber. The mixture is neutralized with limestone and mixed with cellulase and hemicellulase enzymes, which are either

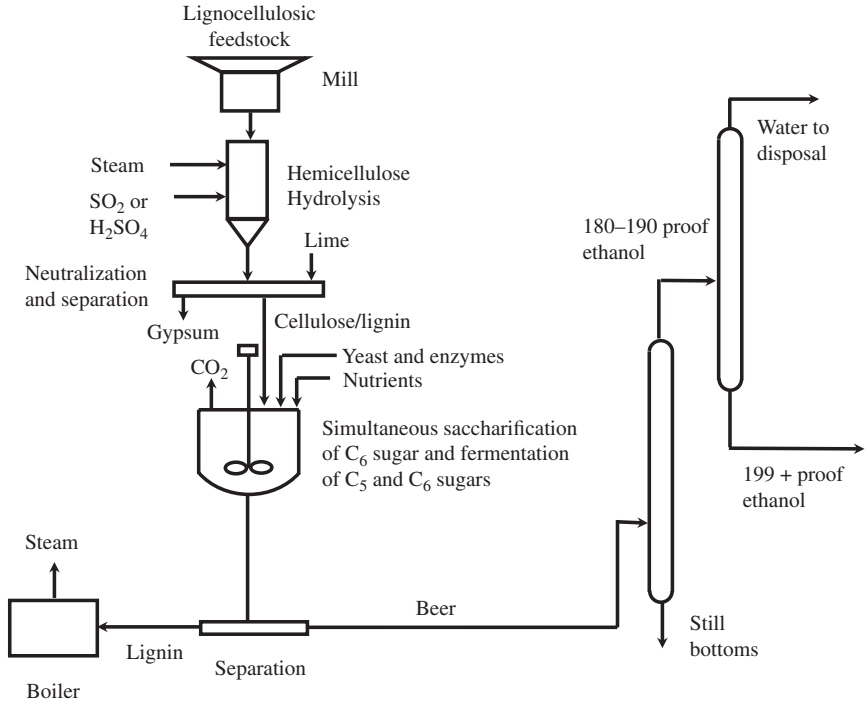

Fig. 7.6 Enzymatic hydrolysis of lignocellulosic biomass.

purchased commercially or produced on site, yeast, and nutrients. The cellulose and any remaining hemicellulose are solubilized to hexose (glucose) and pentoses (xylose and arabinose), which are immediately fermented to ethanol. The rate-limiting step is the hydrolysis of cellulose to glucose. The optimum temperature for the hydrolysis/fermentation reactor is a compromise between the optimum temperature for cellulase activity and the best temperature for the yeast. Lignin is separated from the mixture and used as boiler fuel. The beer is distilled to ethanol in a process identical to that employed after sugar or starch fermentations.

Consolidated bioprocessing, also known as direct microbial conversion (DMC), combines cellulase production, cellulose hydrolysis, and glucose fermentation into a single step. The process is attractive in that it reduces the number of reactors, simplifies operation, and reduces the cost of chemicals. An example of commercially successful application of DMC is anaerobic digestion of sewage sludge and agricultural wastes into methane and carbon dioxide. To date, though, product yields are low, undesired metabolic byproducts are produced, and product inhibition is common. Further development should improve the attractiveness of this approach.

7.4 Distillation

Fermentation can produce gas (such as methane from anaerobic digestion), precipitate (such as calcium acetate during the production of aliphatic acids), or water-soluble compounds (such as ethanol). Gaseous or precipitated products are attractive because of the relative ease of separating them from the spent fermentation broth (beer). Distillation is an energy-intensive process required to recover water-soluble products of fermentation.

The first distillation yields 55% (v/v) ethanol and stillage bottoms, the latter of which contains significant protein in the case of whole-grain fermentation. These stillage bottoms are marketed as animal feed under the name of DDGS. The second distillation produces an ethanol and water azeotrope containing 95–96% ethanol (190–192 proof). If essentially water-free ethanol is desired, purification beyond the azeotrope can be achieved by one of several methods: further distillation in the presence of an entrainer (e.g., benzene, cyclohexane, heptane) that is subsequently recovered; absorption using corn grits or some other solid material; or pervaporation or other membrane-based operation.

Energy consumption in the distillation process is partly responsible for criticism that ethanol production consumes more energy than it produces. Although there is basis for this criticism in older plants, modern plants pay close attention to energy consumption. Some plants are reported to use as little as 5.6 MJ of steam per liter of ethanol produced, with a total energy consumption of 11.1–12.5 MJ/L of product ethanol.

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