Chapter 11
Biorefinery of Plant-Based Products

Youn Young Shim, Shahram Emami, Kornsulee Ratanapariyanuch, and Martin J.T. Reaney

Chapter Highlights

- Biorefining is defined as the sustainable processing of biomass into a spectrum of marketable products and energy.
- Similar to the value of refinery to fossil fuel resources, biorefinery is important in the utilization of plant biomass/seeds for the production of bioproducts.
- The process of biorefining crop seeds varies according to seed type but displays some common factors.
- Biorefinery is a key step in ethanol fermentation and can be further developed for the utilization of fermentation by-products.
- The development of biorefinery systems is a key factor in the production of plant bioproducts with high efficiency and low cost.

11.1 Introduction

Throughout most of humankind's existence, humans have used plant materials to meet the technological needs of society. Wood has been used to generate heat and to make weapons, farming tools, and dwellings for thousands of years. Similarly, vegetable oil and animal fats have been used to generate light for over 40,000 years. Early civilizations harvested grasses and grew grains to feed animals that provided

Y. Y. Shim · M. J. Reaney (✉)
Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Prairie Tide Chemicals Inc., Saskatoon, SK, Canada

Guangdong Saskatchewan Oilseed Joint Laboratory, Department of Food Science and Engineering, Jinan University, Guangzhou, Guangdong, China
e-mail: martin.reaney@usask.ca

S. Emami · K. Ratanapariyanuch
Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

G. Chen et al. (eds.), *Plant Bioproducts*,
https://doi.org/10.1007/978-1-4939-8616-3_11

power and transportation. However, with the introduction of coal as a source of energy, a shift began away from the use of bioproducts as a major supply of materials and moved instead toward the use of fossil resources. Initially, technological advancements with regard to bioproducts progressed in parallel with those attained relating to the utilization of fossil fuels. For example, the production of alcohol and key chemicals from wood was a significant advancement, as was the development of technology allowing the separation of cellulose fiber from wood for the production of paper and cardboard. Similarly, advances in the fermentation of natural products enabled the conversion of biomass to alcohol, ketones, acids, and other products (Brown 2003; Zhou et al. 2014). Despite this technological progress, the low cost of fossil fuel-derived carbon has led to the prevalence of its use for societal needs, but with increasing demand for renewable products from a growing world population, an interest in the utilization of renewable materials is flourishing once more. The rising price of fossil fuel and its destructive impact on the environment are further driving this newfound interest.

Part of the success of fossil fuels and natural gas derives from our ability to convert them into a small number of intermediate products that are easily transformed or incorporated into a wide range of final products. Indeed, the reactions or unit processes in a petroleum refinery enable the refiner to shift the ratio between several output products so that waste may be minimized or even eliminated. It has been proposed that similar concepts, if employed in the processing of biomaterials, would lead to a more efficient use of these materials and greater competition with fossil-based resources. A refinery that utilizes biological material in a similar manner to a refinery that utilizes fossil-based material would be termed a biorefinery and would produce products that compete directly with petroleum products, such as platform chemicals, fuel, and fiber, and also potentially materials such as food, fertilizer, and feed for animals. When processing crops, waste is typically discarded. For instance, the processing of pulse grains results in a hull fraction that is not readily used. Increasingly, however, such materials are being processed to make fuel, electricity, chemicals, and other products. The transition of a traditional process to biorefinery occurs with the conversion of waste to coproducts of manufacturing.

According to the International Energy Agency (IEA) Bioenergy, Task 42, **biorefining** has been defined as "the sustainable processing of biomass into a spectrum of marketable products and energy" (IEA Bioenergy 2009). Much of the literature on biorefinery focuses on the processing of dedicated energy crops, which are typically fast-growing plants that produce the maximum possible potential biomass. These biomass crops tend to be burned to produce energy with little diversity of manufactured products. However, the refining of materials used for food and animal feed can also provide the raw materials for producing chemicals and energy while simultaneously improving food quality. In this chapter, the similarities and differences between plant biomass- and fossil fuel-derived resources will be briefly discussed. With this basic information, the concept of biorefining will be discussed, and specific biorefinery processes, including the processing and treatment of seeds (oilseeds and starch seeds) for ethanol fuel fermentation, will be considered. The concept of biorefining can also be applied to the extraction of proteins from plants, such as zein and gluten from corn and wheat, respectively. This topic is discussed in Chap. 9.

11.2 Biorefinery, Petroleum Refinery, and Plant Biomass- and Fossil Fuel-Derived Materials: Similarities and Differences

A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, heat, and value-added chemicals from biomass. Food and feed could also be considered as products of biorefinery processing (Fig. 11.1). The petroleum refinery is a flexible system that allows the operators to change input products to lower the costs of production and to change operating conditions to control the ratio of output products. The latter capability allows the refiner to respond to variance in supply as well as market demand for products. Similarly, the biorefinery should, where possible, have the flexibility and control to meet the demand for manufactured materials.

Fresh plant biomass and fossil-based resources have a common origin in living materials. Their elemental composition, however, is quite different. The elemental composition of most biomass is rich in the elements hydrogen, oxygen, carbon, nitrogen, sulfur, phosphorous, and essential minerals. Fossil oils and bitumen products, on the other hand, are composed mostly of carbon and hydrogen. The amount of oxygen, nitrogen, phosphorous, and other minerals is typically quite low. Depending on the fossil source, the amount of sulfur present can be several percentages of the product.

The carbon in both biomass and fossil carbon was largely converted from atmospheric carbon dioxide to plant and algal carbon. Photosynthetic organisms use the energy in light for photosynthesis. Carbon and some of the oxygen from the carbon dioxide are combined with other plant nutrients to make plant biomass. The remaining oxygen in plant biomass mostly arises from water. Other elements in biomass such as nitrogen, phosphorous, and sulfur are present in soil and water. Nitrogen can come from atmospheric sources where symbiotic nitrogen-fixing organisms convert the nitrogen gas (N_2) to useful nutrients.

Energy production is a major application of both fossil carbon and biomass. Biomass varies in its energy content per gram. Generally, biomass that is rich in oxygen has less energy than biomass that has little oxygen. Carbohydrates (CH_2O)

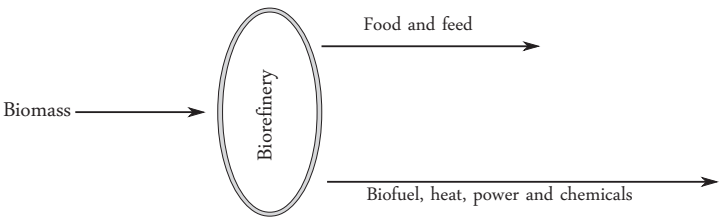

Fig. 11.1 Schematic depicting the sustainable processing of biomass into different value-added products in a biorefinery

have much less energy per gram than hydrocarbons (CH_2). The energy-embodied biomass is largely due to the substantial energy contained in bonds between carbon atoms and the energy in bonds that join carbon and hydrogen. This energy may be released by combustion with oxygen. The bonds between oxygen and carbon or oxygen and hydrogen have little energy and release comparatively less energy when reacted with oxygen. The most common biomass materials are the cell walls of plants and algae, which are rich in carbohydrate polymers including cellulose and hemicellulose. Lignin-rich materials are also present in many plant biomass sources. This material has a higher ratio of carbon to oxygen than cellulose and, therefore, a greater energy density. Hydrocarbons are among the highest-energy biomass materials. They are not common in most biomass crops. While most biomass also contain protein and a wide range of small molecules, these components are usually present at comparatively lower concentrations.

11.3 Processing of Seed Materials

11.3.1 Oilseeds

Vegetable oils are broadly used as a source of dietary lipids, renewable biomaterials, and biofuels. The three most widely grown oilseed crops are soybean, *Brassica* oilseed species (*Brassica* spp., including canola-type, *B. napus*), and sunflower. In this section, canola will be used as an example to discuss the processing of seed oil. *Brassica* spp. oilseeds are grown throughout the world as a source of vegetable oil and protein-rich animal feed (Kimber and McGregor 1995). According to statistical data from the Canola Council of Canada (2017), the average annual production of Canadian canola over the period 2013–2016 was 18.2 million tonnes. The Canadian oilseed-crushing industry crushed an average of 8.0 million tonnes of canola and produced an average of 3.5 and 4.6 million tonnes of canola oil and canola meal annually, respectively, in the same period. Commercial oilseed extraction may include solvent extraction, mechanical expeller-press extraction, or combinations of mechanical and solvent extraction to produce oil and meal (Kimber and McGregor 1995).

Seed crushing is required to separate oil and meal components from seed oil. Nevertheless, pretreatments including seed cleaning, flaking, and conditioning need to be accomplished prior to mechanical extraction. Seed cleaning is an important process to obtain high-quality finished products. A rotary screener is one example of a machine used to clean *Brassica* seeds. The waste associated with seed cleaning can amount to several percent of the total seed mass, which is a substantial amount of biomass and is therefore typically used as an ingredient in animal feeds due to its fat and protein content. Dehulling, which is necessary for some limited markets, makes use of aspiration and/or a fluidized bed sorter. After cleaning, the seeds are conditioned via heat treatment and mechanically flaked by passing through two

cast-iron rollers to increase the efficiency of oil extraction. Conditioning and flaking allow oil bodies to be broken open, reduce oil viscosity, and increase the diffusion rate of prepared oil cake. In addition, hydrolytic enzymes, especially myrosinase, are destroyed by heat. Consequently, glucosinolates do not break down and thus do not release sulfur-containing products into the oil but remain in the meal (Carr 1995). Typically, crude vegetable oil can then be extracted by several means, including mechanical pressing and/or solvent extraction, which are known as conventional methods (Strop and Perry 1994). A series of low-pressure continuous screw presses or expellers are generally utilized to remove as much oil as possible from flaked seeds, and for industrial purposes, this is usually followed by solvent extraction (Carr 1995).

Crude vegetable oil is generally unacceptable for most usages due to high levels of phosphorus compounds (i.e., phospholipids, phosphatides, phosphoglycerides), gum, and minerals (calcium, magnesium, and iron) (Strop and Perry 1994). In addition, it tends to be composed of approximately 3% solid matter termed "foot," which requires roughly 3 h of gravity-induced settling in a screening tank for its removal prior to further refining processes. Oil remaining in the foot may be re-extracted and recycled back to the screening tank using a separate foot screw press or screening and centrifugal separation. The foot remaining in suspension in the screening tank is called "fines," and it is removed using filtration or centrifugation. Oil remaining in the meal/cake from the expeller is accessed using extraction with a solvent (most often hexane). Solvent remaining in the meal/cake is then desolventized (Carr 1995).

After removing foot and fines, the oil goes through several refining processes, including degumming, neutralization (alkali refining), bleaching, and deodorization (Nawar 1996). When soluble phospholipids are present in the oil, alkaline-refining method is sometimes used to remove them to give a good final oil quality, but the treatment results in oil losses. In this case, water degumming is performed to extract and harvest phosphatidylcholine (lecithin) because this polar lipid fraction is a valuable compound used for multiple applications. If nonpolar phospholipids are present in the oil, acid degumming is preferred to precipitate them out. Free fatty acids, odor, flavor, and some color from phospholipids remaining after the degumming process are removed by alkali refining, which involves mixing the oil with refining agents. Carotenoids and chlorophyll pigments are eliminated from the oil by mixing with bleaching clay, which is called a bleaching process. Steam distillation is utilized to remove natural flavor and odor components (Carr 1995).

Refined vegetable oils can be utilized as edible oils, but they can also be used to produce biodiesel (see Chap. 4) and other bioproducts (see Chap. 5). Oilseed meal is the main by-product of the oil extraction process (Bell 1995), and in the case of canola, it is widely used as a protein source in poultry, swine, beef, fish, and dairy cattle feeds because of its excellent amino acid profile (Canola Council of Canada 2015). However, even though defatted canola meal has a high protein content, it also contains various anti-nutritional factors such as glucosinolates, tannins, phytates, other phenolic compounds, and crude fiber, which require removal prior to its use in some nonruminant animal and fish rations (Bell 1993; Khajali and Slominski 2012; Wickramasuriya et al. 2015; Mejicanos et al. 2016; Higgs et al. 1995).

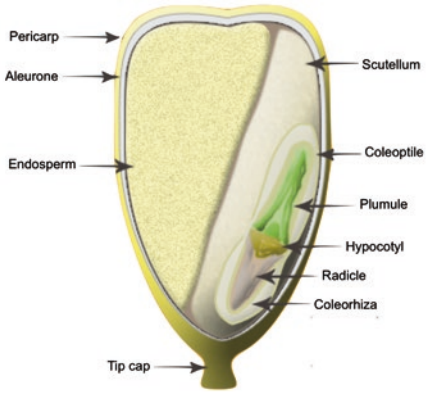

Fig. 11.2 Diagrammatic illustration of corn anatomy (This figure was reproduced with permission from geochembio.com)

11.3.2 Starch-Enriched Seeds

Cereals are rich in starch, which is useful in food, animal feed, hygiene, and pharmaceutical, paper, cosmetic, and textile industries. The five most extensively grown cereal crops worldwide are maize, rice, wheat, barley, and sorghum. Due to their overwhelming prevalence and consumption, the production of these five cereals has a major impact on governments' economies, human nutrition, and related processing industries. In this section, we will discuss the biorefining process utilized for cereal seeds, with maize as an example.

Maize or corn (*Z. mays*) is the most important cereal in North America. Its seed, or kernel, is made up of an embryo, endosperm, aleurone, and pericarp (Fig. 11.2). The endosperm, which is the major constituent of the kernel, constitutes 82–84% of the kernel's dry weight and 86–89% starch by weight (Riahi and Ramaswamy 2003). Indeed, it is made up of 98–99% starch and is low in fat, while the germ, which comprises the embryo and scutellum (an organ that provides nutrition to the embryo during the initial stages of germination), contains 81–85% fat on a dry weight (Lakner et al. 1993). Corn is used as feed, as well as in the production of ethanol, starch, food products, and various other bioproducts.

Corn processing is carried out either by **dry milling** or **wet milling**. Dry milling processing yields germ and endosperm fractions and consists of dehulling, conditioning, removal of the germ, grinding, sifting, purifying and aspirating of grits, and packaging. The endosperm fraction is classified according to size as flaking grits (particle size of 5.8–3.4 mm), coarse grits (2.0–1.4 mm), medium grits (1.4–1.0 mm), fine grits (1.0–0.65 mm), coarse meal (0.65–0.3 mm), fine meal (0.3–0.17 mm), and flour (<0.17 mm). To carry out this process, the kernels are first cleaned and external objects removed, and then the moisture content is adjusted to approximately 16%, after which time the kernels are stored in a bin for 6–8 h to equilibrate moisture levels. The germ is then removed using a Bella-type de-germination device (also called turbo-crushers in Europe), and the endosperm fraction is subsequently ground using a roller mill and separated into products with different size particles using sieves (Gyori 2010). Medium- and large-sized fractions are obtained from primary

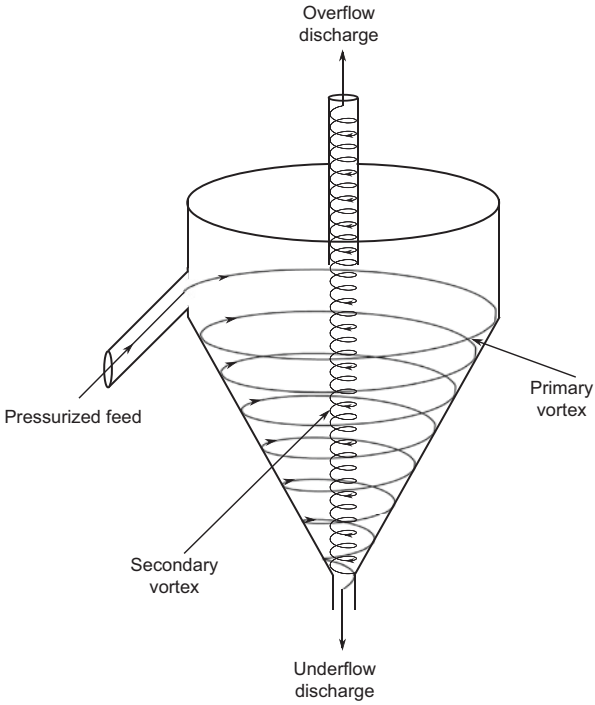

Fig. 11.3 Schematic representation of the spiral flow in a hydrocyclone (This figure was modified from Rijkswaterstaat Leefomgeving http://rwsenvironment.eu/)

grinding, while the small fraction results from secondary grinding. It is very important to separate germ prior to grinding because oil extraction from clean germ is more efficient and the endosperm would suffer from rancidity due to the presence of oil in the germ.

Wet milling begins with dehulling followed by steeping of the seeds in water containing 0.2% sulfur dioxide at a temperature of 48–52 °C until the corn moisture content reaches 45%. The purpose of this process is to soften the seed and facilitate separation of hull, germ, and endosperm. On an industrial scale, steeping is typically carried out in ten tanks with the corn moving from tank one to ten and water moving in the opposite direction. The bisulfite ion generated by the sulfur dioxide reacts with S-S bonds in proteins and breaks the protein matrix into two hydrophilic molecules. As a result, starch-protein separation is facilitated and the starch yield is increased. At the end of this process, the steeping water contains 5–7% dry matter, which is concentrated by reverse osmosis filtration to about 55% and then mixed with bran to be used as feedstuff (Hoseney 1998; Gyori 2010). The resulting soft grain is ground coarsely using an attrition mill in order to release the rubbery germ. This ground slurry is passed through a hydrocyclone to collect germ, which makes up the lower-density fraction (overflow), and the starch, which makes up the higher-density fraction (underflow) (Fig. 11.3). The underflow fraction is then sieved to separate the coarse fraction, which is reground to release starch, protein, and fiber. The fiber fraction is separated by sieving followed by several washes to remove

adhering starch, while starch-protein separation is carried out using a continuous centrifuge or hydrocyclone. The resulting starch and protein fractions are dewatered by centrifugation and then dried (Hoseney 1998).

11.4 Industrial Production of Ethanol from Plant Carbohydrates

The United States (USA) is the world leader in ethanol fuel production from cereal grains, and it is predicted that world ethanol production will reach approximately 155 billion liters by the end of 2020 (OECD/FAO 2011). In the sections below, we will discuss the process of obtaining ethanol from agricultural feedstocks. Both corn (starch) and sugarcane (sugar) will be examined as they make up the most widely used crops for ethanol production. Although carbohydrate-enriched biomass as source starting material for ethanol production was discussed in Chap. 6, the information below emphasizes the actual processes involved in the context of the biorefinery.

11.4.1 Production of Ethanol from Corn

Corn is the preferred feedstock for the production of ethanol in the USA and as discussed earlier, there are two main industrial processes through which it is obtained, termed dry milling and wet milling. The main difference between the two processes concerns whether the starch is separated from the other components of the kernel before hydrolysis; this is the case with wet milling, but not dry milling. The aim of dry milling plants is mainly the production of ethanol, which requires less investment in equipment units and increases the return per unit of ethanol compared to wet milling (Bothast and Schlicher 2005; Vohra et al. 2014). Conversely, while wet milling plants require higher investments because of the additional separation steps, the intent of this process is to not only produce ethanol but also many other corn derivatives, such as corn oil, gluten meal, gluten feed, high-fructose corn syrup, and dextrose (Bothast and Schlicher 2005; Vohra et al. 2014). The main steps and products of dry milling and wet milling are depicted in Fig. 11.4.

In both cases, the starch is broken down using enzymes since yeasts are unable to use starch as a substrate for ethanol production. A thermostable α-amylase is added initially, and the temperature is increased to above 100 °C using a jet cooker (Bothast and Schlicher 2005). This reduces the size of starch chains by breaking α-1,4 glycosidic bonds via the enzyme's catalytic action as well as the mechanical shear provided by the jet cooker. This consequently lowers the viscosity of the solution. The dextrins, which are low molecular mass products of starch digestion, are then broken into Glc monomers by the action of glucoamylases, which catalyze the hydrolysis of both α-1,4 and α-1,6 glycosidic bonds. Dextrin formation from

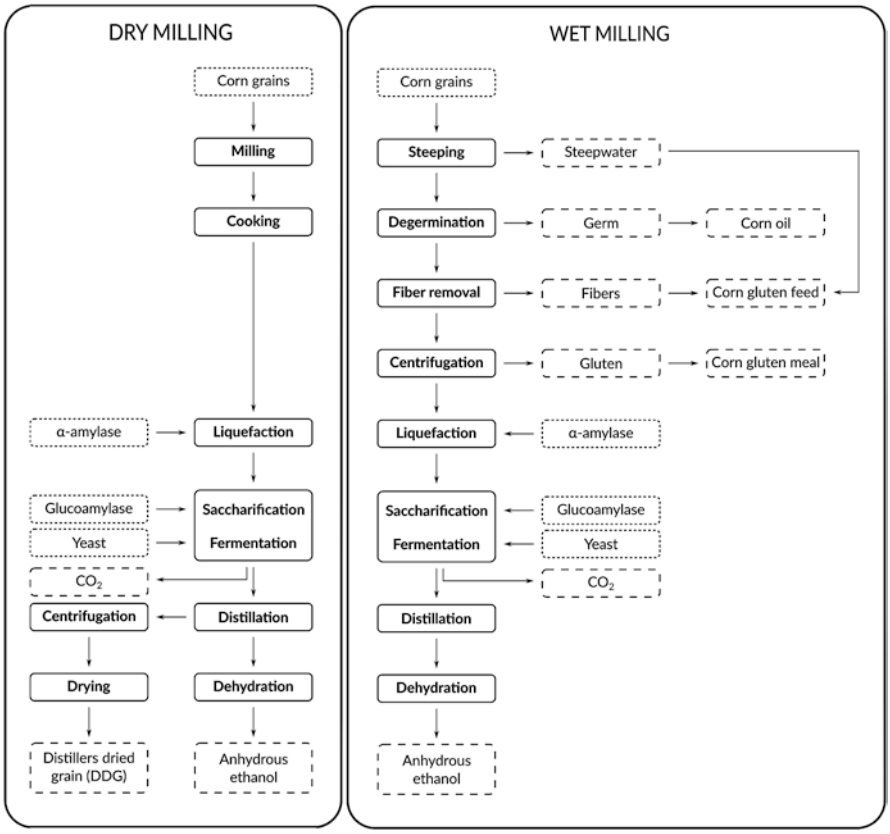

Fig. 11.4 Schematic representation of dry milling and wet milling processes for the production of corn-derived ethanol

starch is sometimes referred to as **liquefaction**, whereas production of Glc monomers from dextrins is referred to as **saccharification**. Both of these enzymes are produced on an industrial scale by microorganisms such as bacteria or fungi (Sauer et al. 2000; Souza and Magalhães 2010); however, new technologies regarding the production of amylases are being developed to reduce industrial production costs. Among the innovations, a variety of corn expressing a thermostable α-amylase has recently been developed by Syngenta (Sainz 2009). Dry milling using grains from these varieties of corn does not require additional microbial enzymes, which reduces processing costs.

Another new technology is "cold hydrolysis," which is the hydrolysis of starch granules at lower temperatures (Cinelli et al. 2015). In this process, the enzymes (mainly α-amylase) catalyze the degradation of starch initially in the outer surface of the granules and then continue catalyzing the reaction radially. The lower temperatures reduce operational costs, eliminate the need to deal with viscous solutions, and reduce the number of undesirable reactions.

Yeasts are added to the fermenter in the fermentation step, which often occurs simultaneously with the saccharification step and allows the conversion of Glc into ethanol and carbon dioxide. This reaction occurs at room temperature in the presence of a nitrogen source (such as urea or ammonium), which is added to help the yeasts grow (Bothast and Schlicher 2005). Certain processing plants also add proteases to break down corn proteins, resulting in the release of amino acids that the yeasts can then consume. During fermentation, the concentration of ethanol in the broth can reach 12%, which limits further fermentation (Schobert 2013). Since carbon dioxide is a by-product of fermentation, certain corn-processing plants capture this gas and sell it for the manufacturing of dry ice or carbonated beverages (Bothast and Schlicher 2005).

In the distillation step, the fermented broth is transferred to distillation columns, where the ethanol is separated from the mixture. This process, however, does not remove all of the water from ethanol (Bothast and Schlicher 2005). The hydrated ethanol is passed through a molecular sieve for dehydration. The resulting anhydrous ethanol can be blended with gasoline, and a denaturant is often also added to discourage human consumption. The remaining broth contains fibers, oils, and proteins; it is centrifuged to remove excess water and then dried to produce DDG (Bothast and Schlicher 2005; Vohra et al. 2014) following the dry milling process. The wet milling process yields only minor amounts of solids after fermentation because most parts of the kernel were removed in previous steps (Vohra et al. 2014).

The unevaporated residue of distillation, called **stillage**, is passed through screens and/or a centrifuge to collect the suspended solids (Wall et al. 1983) as a sludge called wet cake (Wilkins et al. 2006). The remaining turbid liquid is called thin stillage (Wall et al. 1983). Usually, the water in thin stillage is evaporated to form thick syrup, which is then mixed with wet cake to make **wet distillers' grains with solubles** (WDGS). WDGS has a high moisture content (65%) and consequently a shelf life of only 1–2 weeks (Bothast and Schlicher 2005). To increase the shelf life and decrease transportation costs, WDGS may be dried to 10–12% moisture to generate a material of commerce called **dried grains with solubles** (DDGS) (Bothast and Schlicher 2005). DDGS is often used in animal feeds (Wilkins et al. 2006). Over 3.8×10^6 tonnes of DDGS is produced annually from ethanol plants in the USA (Bothast and Schlicher 2005).

Drying thin stillage, however, is not energy efficient. The energy required to evaporate the large amount of water entrapped in thin stillage is a major cost in ethanol production. For example, evaporation of water from stillage consumes about 40–45% of the thermal energy and 30–40% of the electrical energy utilized in a dry-grind facility (Wilkins et al. 2006). According to Meredith, (2003), evaporation of 100,000 lb./h of water requires 1,000 kW of heat. In addition, Wall et al. (1983) stated that the cost of purchase and operation of evaporators is 0.03 $/L (Wall et al. 1983). Therefore, rather than evaporating and using thin stillage for feed, it might prove economically viable to isolate or enrich potentially valuable compounds or fractions from thin stillage. According to Thomas and Ingledew (1992), the mass of stillage is more than four times that of the associated ethanol. If global ethanol production does indeed reach 155 billion liters by the end of 2020 as stated previously,

there would be approximately 620 billion liters of thin stillage produced from the bioethanol industry and thus a very large quantity of liquid waste that could potentially be used for other purposes.

11.4.2 Production of Ethanol from Sugarcane

Sugarcane is a crop that has a high yield of sugar per area, and the sucrose obtained does not need to be broken down like cornstarch. Therefore, the production of sugarcane ethanol requires fewer processing steps than corn-derived ethanol, which reduces investment and operational costs (Vohra et al. 2014). On the other hand, sugarcane is a seasonal crop requiring warm climates, which results in a lack of supply during certain periods of the year and could increase the price of ethanol.

The process for the production of ethanol from sugarcane consists of harvesting stalks, washing to remove impurities, and crushing to extract the juice, which contains the sucrose. The juice undergoes a treatment, whereby high temperatures are applied, and calcium oxide is added to aid in the precipitation of fibers and other impurities (Palacios-Bereche et al. 2014; Vohra et al. 2014). The mixture is then filtered and sent for the production of ethanol and/or sugar depending on the configuration of the plant. Juice sent for ethanol production must be concentrated in evaporators to achieve a high efficiency of fermentation.

Brazil is one of the largest producers of ethanol from sugarcane. In the typical Brazilian fermentation process, also known as the Melle-Boinot process, yeast cells are intensively recycled from one fermentation and then reused in the next one (Zanin et al. 2000). The process, however, leads to very high yeast cell density in the fermenter, which in turn contributes to a very short fermentation time (4–12 h) resulting in a relatively low final ethanol concentration compared to corn-processing facilities. The shorter fermentation duration may maximize the amount of sugarcane processed, which is important because sugarcane is a seasonal feedstock (Vohra et al. 2014; Lopes et al. 2016). This fermentation process is extensively used in Brazil and other countries due to its low operational and production costs, high efficiency, and simple operation (Zanin et al. 2000). The resulting liquor, which contains the ethanol, is then passed through distillation columns to produce hydrated ethanol, followed by a molecular sieve or distillation step with cyclohexane to produce anhydrous ethanol (Dias et al. 2015).

11.4.3 Production of Ethanol from Plant Cell Walls

The general process of producing bioethanol from lignocellulosic materials, which varies in its specifics depending on the type of biomass or technology chosen, begins with the reduction of biomass size to increase the surface area for subsequent chemical and enzymatic reactions (Vohra et al. 2014). The main steps of the process are

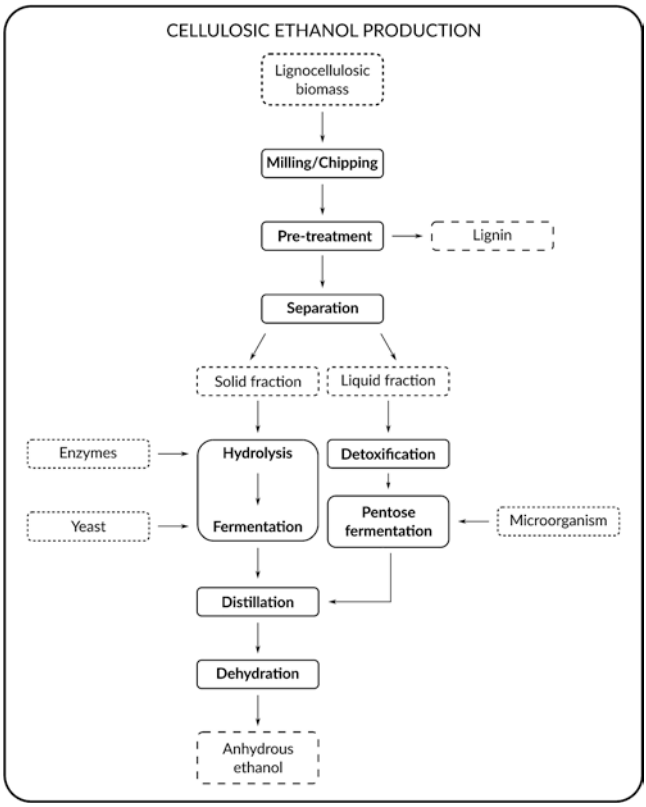

Fig. 11.5 Generic schematic for the production of lignocellulosic ethanol

summarized in Fig. 11.5. The pretreatment of biomass is necessary to expose the cellulose fibers for subsequent enzymatic treatment (Silveira et al. 2015; Kim et al. 2016). Typically, the pretreatment step subjects the biomass to harsh conditions, such as high temperature and pressure, which may cause loss of biomass. In addition, these steps are costly because of the amount of energy required. Due to these reasons, more efficient means of pretreatment still need to be developed to make the production of lignocellulosic ethanol economically feasible. The ideal process would allow efficient hydrolysis without loss of sugars, not require a lot of energy, minimize the number of steps, maximize the recovery of lignin, and not produce inhibitory compounds (Jorgensen et al. 2007; Kim 2013; Silveira et al. 2015; Kim et al. 2016).

Pretreatment methods can be divided into biological, chemical, physical, and chemical/physical methods (Zheng et al. 2009; Bensah and Mensah 2013; Silveira et al. 2015; Kim et al. 2016). Biological methods rely on microorganisms and enzymes to degrade biomass fibers, especially lignin and hemicellulose. They are environmentally friendly processes that do not cause high losses of sugars, but they

require long reaction times for high yields, which increase costs (Silveira et al. 2015). Physical methods are energy-intensive approaches that aim to reduce the size of the biomass and include milling, chipping, and grinding. Chemical methods rely on the use of acids, bases, or organic solvents to break and dissolve fibers. These processes may produce undesirable compounds that affect hydrolysis or fermentation yield and require a further step to neutralize or remove them. In addition, they often require high temperatures, which increase processing costs. A combination of chemical and physical methods produces better results and reduces the cost of pretreatment. For example, milling can be combined with acids, bases, or other catalysts that help break chemical bonds, which saves energy (Silveira et al. 2015). Other chemical/physical methods include the application of steam and ultrasound in combination with use of acids or bases.

Regardless of the pretreatment method, lignin is removed, and the remaining mixture is fractionated to yield a solid fraction (cellulose) and a liquid fraction (a solution of hydrolyzed hemicellulose; Kim 2013; Vohra et al. 2014). Purified lignin can be recovered and sold as a high-value compound or burned to generate power. The liquid fraction contained pentoses and fermentation inhibitors. Once the fermentation inhibitors are removed, the liquid fraction can be used as carbon source for certain microorganisms which can consume pentoses to produce ethanol. The solid fraction can also be used for ethanol production, by first hydrolyzing using an acid or via the catalytic action of enzymes. Both hydroxylation methods, however, have some shortcomings to be overcome. While acid treatment is faster, the process will degrade Glc and thus result in a lower ethanol yield during fermentation. In addition, acid treatment will produce wastewater that has to be further treated (Vohra et al. 2014). The enzymatic method requires the use of cellulases, which are produced by certain microorganisms and are expensive, to break down cellulose. The duration of the enzymatic process is also limiting and requires improvement, since it takes much longer than acid hydrolysis (Vohra et al. 2014). Following fermentation, the broth is distilled to obtain hydrated ethanol, which is then directed to a dehydration unit to obtain anhydrous ethanol.

In order to be as competitive as corn- or sugarcane-derived ethanol, the production of lignocellulosic ethanol must overcome the limitations described above, especially a reduction in the costs of pretreatment and cellulases, as well as the development of processes resulting in high yields of ethanol. In order to achieve this, relevant plant species could potentially be genetically engineered to have altered lignin configuration and/or content, which would reduce the cost of pretreatment (Cheng and Timilsina 2011). Similarly, an alternative to the use of microbial cellulases is the heterologous expression genes encoding these enzymes in plants during their growth (Lambertz et al. 2014). In addition, a proposed configuration called **consolidated bioprocessing** focuses on the simultaneous production of enzymes, saccharification, and fermentation, which would reduce costs by eliminating steps. Finally, improving the productivity of the crops using conventional breeding, biotechnology, and/or superior crop management techniques would increase the amount of cellulose available for ethanol production, which would have a direct impact on the processing prices (Balan 2014).

11.5 Enhanced Biorefinery: Utilization and Refinery of Fermentation By-Products

The treatment and utilization of the large amount of fermentation by-products and liquid waste are challenging; however, with efficient processes and refinery, they have the potential to provide valuable products. Here, thin stillage generated in the ethanol fermentation process is used as an example.

Thin stillage may be used as a medium for dissolving macromolecules present in biomass (Reaney and Ratanapariyanuch 2013) that have mass in excess of a 1,000 molecular weight cut off. These compounds may include proteins, peptides, gums, mucilaginous compounds, polyphenolic compounds, and complex polymers of carbohydrates and gums. Thin stillage also contains numerous ions and organic compounds that are smaller than the molecular weight cut off; these compounds are generally nontoxic and may be recovered following the concentration of the extracted biomass solution.

For example, the manufacture of enriched protein fractions from oilseed meal requires large volumes of treated water, and since thin stillage from ethanol production is available in large volumes, it has been found to be suitable for extracting protein-rich materials (Ratanapariyanuch et al. 2012). The use of thin stillage, in lieu of water, for protein extraction would decrease the energy requirements and waste disposal costs of both the protein isolation and biofuel production processes. Besides the use of thin stillage for protein extraction, it can also be utilized as media for the extraction of other compounds, such as soluble polysaccharide mucilage (Table 11.1).

11.6 Platform Chemicals Arising from the Biorefinery

After using thin stillage to extract macromolecules, organic compounds remain in thin stillage. Ratanapariyanuch et al. (2011) studied the composition and properties of wheat-based thin stillage and found that the osmotic potential of thin stillage was lower than that of water, whereas both the density and viscosity of thin stillage were higher than that of water. The pH of thin stillage was typically 3.7–3.8, and the total

Table 11.1 Primary yield and viscosity of mucilage extracted by 0.5 M $NaHCO_3$ versus thin stillage

Solvent	Time (min)	Grams of mucilage (dry weight)	Viscosity of the mucilage (centipoise)
0.5 M $NaHCO_3$	15	0.61 ± 0.03	3.15 ± 0.07
	30	0.66 ± 0.01	3.20 ± 0.00
	45	0.67 ± 0.01	3.30 ± 0.00
	60	0.68 ± 0.01	3.35 ± 0.07
Thin stillage	30	0.58 ± 0.01	3.25 ± 0.07

Table 11.2 Organic components of thin stillage

Constituent	Concentration (g/L)[a]	Amount of constituent (kilotonnes) from 620 billion liters of thin stillage
Dextrin	10.04	6224.8
Maltotriose	0.62	384.4
Maltose monohydrate	0.59	365.8
Glycerol	5.85	3627
Isopropanol	0.32	198.4
Ethanol	0.30	186
Lactic acid	5.55	3441
1,3-Propanediol	1.22	756.4
Acetic acid	1.27	787.4
Succinic acid	0.79	489.8
Glycerophosphorylcholine	1.00	620
Betaine	0.88	545.6
Phenethyl alcohol	0.29	179.8

[a]Mean values of organic compounds from four batches of thin stillage (Ratanapariyanuch et al. 2011)

Kjeldahl nitrogen was approximately 0.08–0.10% (w/w). The constituents of thin stillage can be categorized into three groups: (1) yeast metabolites including glycerol (Russell 2003), ethanol (Wilkie et al. 2000), succinic acid (Russell 2003), glycerophosphorylcholine (Almaguer et al. 2006), and phenethyl alcohol (Schrader et al. 2004); (2) bacterial metabolites including isopropanol (Lovitt et al. 1988), acetic acid, lactic acid (Chin and Ingledew 1993), and 1,3-propanediol (Cheng et al. 2006); and (3) wheat metabolites such as betaine (Kampen 1993). In addition, yeasts, bacteria, and fungi were also found. Thin stillage was also shown to contain $CaCl_2$, NaCl, K_2SO_4, $NaNO_3$, $Mg(OH)_2$, Na_2SO_4, and KOH. As previously stated, there could be approximately 620 billion liters of thin stillage produced from the bioethanol industry by 2020. Therefore, the amount of organic compounds that could potentially be recovered is very large (Table 11.2), if isolation procedures are developed to recover them.

11.7 Closing Comments

Biorefining is the sustainable processing of biomass into valuable products and energy. Based on the biorefinery concept, it is clear that biomass can be used as material to create new production lines for food, feed, biofuel, power, and chemicals. Value-added compounds could also arise from the biorefinery process. As shown in the description of the processing of seed materials, biorefinery is an efficient process for maximizing the use of plant biomass to produce valuable bioproducts. Depending on the type of starting biomass, it may be possible to produce food and feed in addition to industrial bioproducts. For example, the biorefining of the

seed of a *Brassica* oilseed species can potentially lead to production of oil for biodiesel production and meal to serve as feed for livestock. In addition, along with the development of agricultural biotechnology and the requirement of bioproducts with different properties, biorefinery processes are accordingly under continuous development and optimization.

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