Chapter 6
Plant Carbohydrates and Production of Renewable Biofuel from Starch, Sugar, and Cellulose

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Chapter Highlights

- Carbohydrates are a group of molecules with various structures that are composed of carbon, oxygen, and hydrogen.
- Sugars and polysaccharides (e.g., starch, cellulose, hemicellulose, and pectin) are major plant carbohydrates that are important feedstocks for bioproducts.
- Sugarcane and corn are the most widely cultivated crops and are important sources of sugar and starch, respectively, whereas fiber-rich switchgrass is an emerging energy crop.
- Corn starch and sugarcane sucrose are the most important feedstocks for ethanol production.
- Lignocellulosic biomass has a high potential to be used as feedstock for ethanol production, but technologies need further development for commercial-scale production.

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6.1 Introduction

Biologists define carbohydrates as molecules with three or more carbon atoms that are composed of only carbon (C), oxygen (O), and hydrogen (H), typically with a general formula of $(CH_2O)_n$. Carbohydrates are abundant in most plant organs and tissues since they are the primary products of photosynthesis and form polymers that are important in carbon and energy storage, as well as in mechanical support. The starch that accumulates in potato tubers, for example, is used as an energy supply in the initial development of the next generation of potato plants (Turesson 2014). Carbohydrates also have other roles in plants, for example, making up components of nucleic acids and glycoproteins (Nelson and Cox 2017).

Due to their properties, carbohydrates are also important feedstocks for the production of food, food additives, pharmaceuticals, biofuels, paper, construction materials, textiles, and various chemicals. Plant-derived carbohydrates have been gaining attention in recent years as a source of industrial products due, at least in part, to the depletion of fossil fuel-based resources and threat of global warming. Plant carbohydrates have the potential to provide renewable substitutes for fossil-based raw materials, and as an added bonus, their biosynthesis consumes large amounts of carbon dioxide. This means that they form part of a closed carbon cycle, whereby carbon dioxide released by burning the fuels can be removed from the atmosphere through the regrowth of the plant source of feedstock. While carbohydrates (usually in the form of wood) have been burned as fuel for tens of thousands of years and combustion remains a simple method to extract energy from carbohydrates, recent surges in fuel and energy costs, as well as environmental concerns, have spurred interest in the conversion of carbohydrate feedstocks to liquid fuels (especially ethanol). Indeed, recent legislation (e.g., Brazil) requires the addition of fuel ethanol to gasoline, which highlights the importance that carbohydrates will have in the near future supply of fuels and in the reduction of greenhouse gas (GHG) emissions (Table 6.5). This chapter builds upon the information on carbohydrates presented in Chap. 2 and discusses the relevance of carbohydrates to bioproducts and production of fuel ethanol. The discussion on carbohydrates that follows is based on information from Nelson and Cox (2017), Voet et al. (2016), and Heldt and Piechulla (2010).

6.2 Structure and Classification of Carbohydrates

Carbohydrates are classified according to their chemical structures (Table 6.1). Many carbohydrates are polymers, or polysaccharides, made up of monomers called monosaccharides (Nelson and Cox 2017). A disaccharide is a dimer of two monosaccharides, and an **oligosaccharide** is a polymer of several (e.g., roughly 3–20) monosaccharides. Monosaccharides and disaccharides are also commonly known as sugars. With reference to the general formula $(CH_2O)_n$, a monosaccharide may be classified based on the number of carbons it contains; **pentoses** ($n = 5$) and **hexoses** ($n = 6$) are most relevant to our discussion.

Table 6.1 Classification of carbohydrates according to their number of monomers, carbons, and identity of functional groups

According to number of monomers		
Classification	*Number of monomers*	*Example*
Monosaccharide	1	Glucose, fructose
Disaccharide	2	Maltose, lactose
Oligosaccharide	3–20	Raffinose, fructo-oligosaccharides (FOS)
Polysaccharides	>20	Starch, cellulose
According to functional group		
Classification	*Functional group*	*Example*
Aldose	Aldehyde	Glucose, mannose
Ketose	Ketone	Fructose, ribulose
According to number of carbon		
Classification	*Number of carbons*	*Example*
Pentose	5	Arabinose, xylose
Hexose	6	Glucose, fructose

6.2.1 Monosaccharides

All monosaccharides contain a chain of C atoms, to which hydroxyl (-OH) groups, or simply H or O atoms, are attached. The C chain can take either a linear or cyclic form. In the linear form, one of the C atoms is attached by a double bond to an O atom, forming a carbonyl group (C=O). If the carbonyl is at the end of the carbon chain, the monosaccharide is considered an **aldose**; otherwise it is a **ketose** (Fig. 6.1; Nelson and Cox 2017). In both aldoses and ketoses, the carbonyl group and a hydroxyl group may form an intramolecular covalent bond, which changes at least part of the open chain into a ring. It is the cyclic forms of monosaccharides that polymerize to form di-, oligo-, and polysaccharides. If the ring contains five members (4 C, 1 O), it is called a **furanose**. If the ring contains six members (5 C, 1 O), it is called a **pyranose** (Fig. 6.1). In both furanoses and pyranoses, the term anomeric carbon is used to refer to the C atom that formed the carbonyl group when the molecule was in the linear form. The C atoms of a cyclic molecule are often numbered for easy reference, and the anomeric carbon is always designated as C1 (see Fig. 2.7 in Chap. 2). The anomeric carbon is also central to defining α- and β-forms of sugars as described below.

Glucose (Glc, $C_6H_{12}O_6$) is an example of a hexose and is itself also an aldose. Glc is produced during photosynthesis by the reduction and fixation of CO_2 (Nelson and Cox 2017). Glc, however, is not the only hexose present in plants. For example, galactose, mannose, and fructose also each have the formula $C_6H_{12}O_6$ but differ from each other structurally. Like Glc, galactose and mannose are both aldoses, whereas fructose is a ketose. Pentoses that are most relevant to bioproducts are arabinose, xylose, and apiose, which are all aldoses.

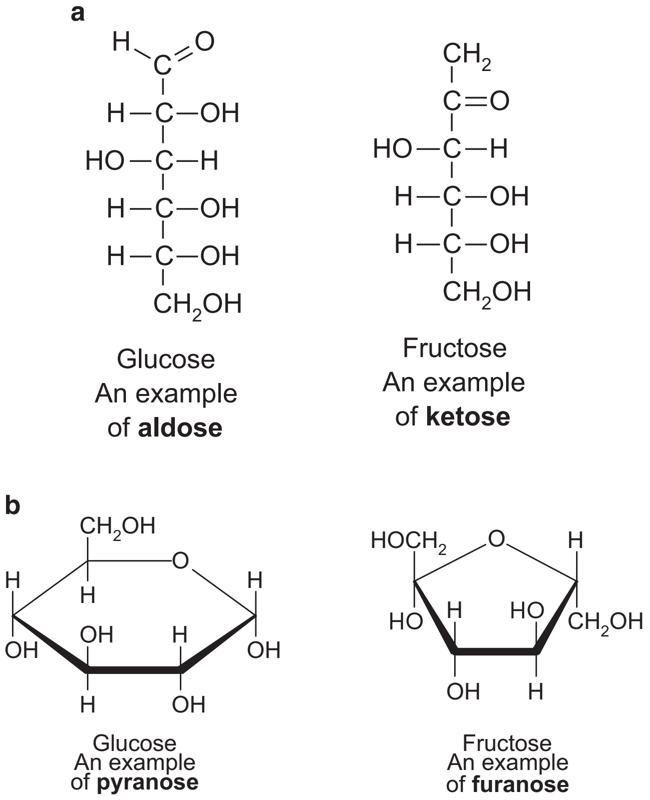


Fig. 6.1 Classification of monosaccharides. (**a**) Example of an aldose and a ketose. (**b**) Example of a pyranose and a furanose

Deoxysugars and acidic sugars are two derivatives of monosaccharides (Nelson and Cox 2017; Voet et al. 2016). The major **deoxysugars** relevant to bioproducts are rhamnose and fucose, which are both deoxyhexoses with the formula $C_6H_{12}O_5$, and have an H atom in place of the terminal hydroxyl group found in other hexoses. The major **acidic sugars** are galacturonic acid and glucuronic acid, which are also both derived from hexoses and have a carboxyl (-COOH) group in place of the -CH_2OH in the sixth carbon position. The Haworth projection of the monosaccharides is shown in Fig. 6.2.

Although the common monosaccharides are all very similar in their elemental compositions, differences in their structures have profound effects on their biological functions. For example, Glc, galactose, and mannose are stereoisomers of each other, because they differ only in the arrangement of some hydroxyl groups relative to other hydroxyl groups in the same molecule (Nelson and Cox 2017). It is also important to note that for each monosaccharide, there are two possible configurations that are mirror images of each other (enantiomers), known as the **D- and L-forms** (Fig. 6.3). Usually, only one of the enantiomers is found within

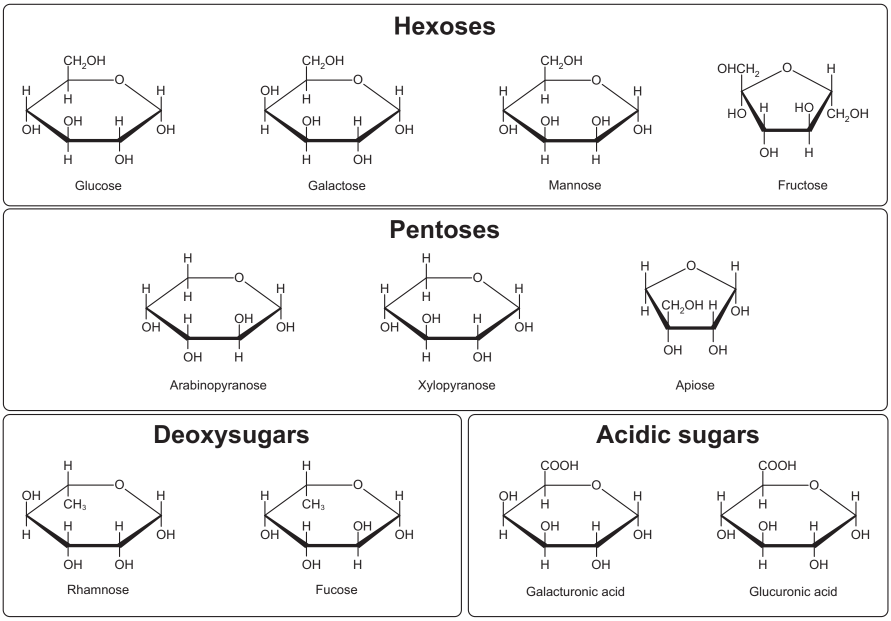
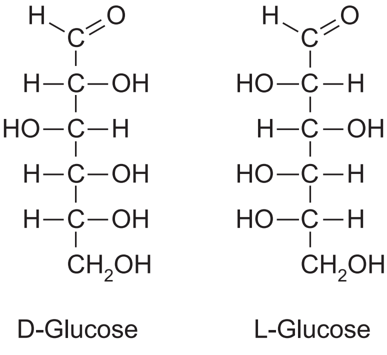


Fig. 6.2 Haworth projection of relevant monosaccharides

Fig. 6.3 Example of linear D- and L-glucose



a cell. For the main hexoses (Glc, galactose, mannose), pentoses (xylose, apiose), and sugar acids (galacturonic acid, glucuronic acid), it is the D-*form* that is biologically relevant. For arabinose, rhamnose, and fucose, it is the L-*form* that is most relevant. Finally, in cyclic molecules, the anomeric carbon is the C atom that was part of the carbonyl group when the molecule was in the linear form. The anomeric carbon is always bound to a hydroxyl group, and the position of the hydroxyl relative to other groups on the ring defines a cyclic monosaccharide as being in either α- or β-anomeric configuration. Most monosaccharides convert freely between the open-chain, α- and β-configurations when in solution, although one of these forms may be more favored than another.

6.2.2 Oligo- and Polysaccharides

Monosaccharides can form covalent bonds with other molecules (including other monosaccharides). The most important type of bond is a **glycosidic bond** or glycosidic linkage, which is formed between the anomeric carbon of a monosaccharide and the oxygen of a hydroxyl group of another molecule (Fig. 6.4; Nelson and Cox 2017). When a glycosidic bond forms between two monosaccharides, a disaccharide is produced (see Fig. 2.8 in Chap. 2), and additional glycosidic linkages produce an oligosaccharide and ultimately a polysaccharide. Any glycosidic bond between sugars can be described by identifying the carbons that are linked and by the relative orientation of the monosaccharides. If the monomers are in the α-form, the linkage is classified as α. If the monomers are in the β-form, the linkage is β. For example, starch and cellulose are both polymers of Glc but differ in their linkages. Starch is a mixture of **amylose** and **amylopectin**. Amylose are chains of glucosyl moieties linked α-1,4, whereas amylopectin contains both α-1,4 glycosidic bonds and α-1,6 glycosidic bonds (see Fig. 2.9 in Chap. 2). In contrast, cellulose contains only β-1,4 glycosidic bonds (see Fig. 2.10 in Chap. 2). Starch is readily digestible

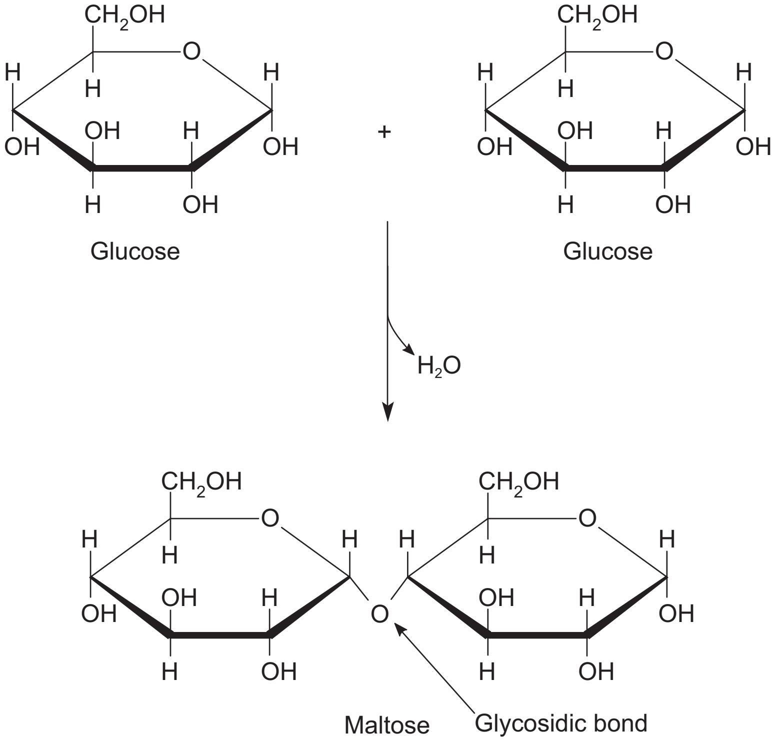


Fig. 6.4 Condensation of two molecules of glucose to form a maltose. This process releases a molecule of water

by most animals, whereas cellulose is insoluble and indigestible (Voet et al. 2016). Thus, the types of glycosidic bonds present in a polysaccharide are of great significance to its function. As shown in amylopectin, a single monosaccharide molecule can form glycosidic bonds with more than one other molecule, which gives polysaccharides the potential for forming highly branched structures.

Many polysaccharides are named based on their constituent sugars, using the suffix -an. For example, mannan is a polymer of mannose, and xylogalacturonan is a polymer of xylose and galacturonic acid. However, to fully describe a polysaccharide, it is necessary to know the identity of all of the constituent monosaccharides; their individual configurations (D- or L-, α- or β-, furanose or pyranose); their sequence in the polymer, including any branches; and the configuration (α- or β-) of the glycosidic bonds. Fortunately, many of the major plant polysaccharides are polymers of fairly simple repeating units, so their primary structures can be inferred without describing every one of their constituents.

6.3 Plant Origins of Polysaccharides

Plant polysaccharides can be categorized as structural or nonstructural polymers. These categories are not mutually exclusive, as some polysaccharides (such as those found in the cell walls of some legumes) provide both energy reserves and mechanical support.

The major structural polysaccharides are located in the cell wall and in secretions such as the mucilage released by the seed coat. All plant cells are surrounded by a primary cell wall, and some cells undergo additional thickening to produce a secondary cell wall. Secondary walls are found most prominently in xylem, where strength is required to support the plant body and to resist the tension created during water transport (transpiration; Plomion et al. 2001). Wood is made of xylem. In softwoods (i.e., conifers), xylem consists mostly of cells called tracheids, whereas hardwood (i.e., woody angiosperms) xylem is composed primarily of cells called vessel elements and non-transporting cells called fibers. Thick-walled cells in other parts of the plant are also commonly called fibers, including the phloem (bast) fibers of crops such as flax and hemp [*Cannabis sativa*] and the hairs (trichomes) on the surface of cotton seeds.

6.3.1 Cell Walls

Cell walls are made mostly of carbohydrates and their derivatives, plus small amounts of other molecules, especially proteins (Heldt and Piechulla 2010). Secondary walls are often also abundant in lignin. The carbohydrate component of the primary cell wall consists of cellulose embedded in a matrix of other polysaccharides, which are classified as either hemicelluloses or pectins (Fig. 6.5).

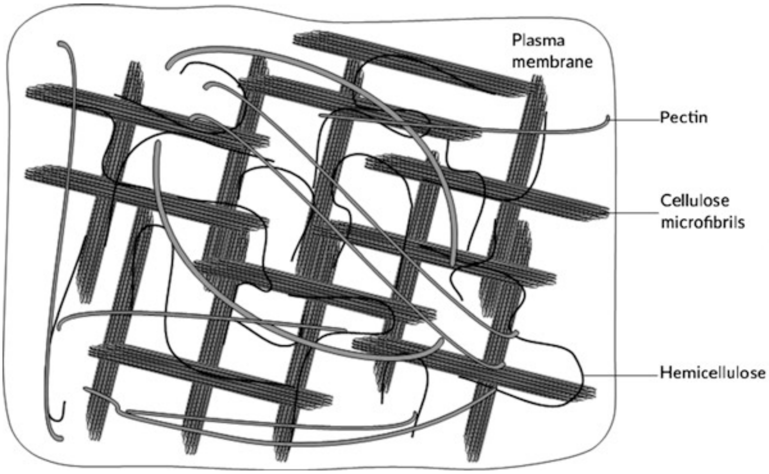


Fig. 6.5 Structure of a plant cell wall

As previously shown, cellulose is an unbranched β-1,4 glucan (see Fig. 2.10). It is the most abundant terrestrial organic polysaccharide (Wüstenberg 2015). Within the cell wall, cellulose is found in microfibrils, which are bundles of approximately 36 cellulose molecules (Voet et al. 2016; Rayon et al. 2014). The parallel alignment of the cellulose chains allows for the formation of many intra- and intermolecular hydrogen bonds within the microfibril. Regions of the microfibrils with extensive hydrogen bonding are considered to be crystalline and are particularly strong and resistant to chemical or enzymatic degradation. Microfibrils may also contain amorphous regions in which the cellulose molecules are not crystalized. The degree of crystallinity, therefore, affects the use of cellulose in bioenergy and bioproduct applications. Microfibrils are themselves bundled together to make macrofibrils, which form a network throughout the cell wall.

Hemicelluloses usually make up less than half of the dry mass of cell walls. Unlike cellulose, they do not form microfibrils or extensive crystalline regions. In the primary walls of cereals and other grasses, the major hemicellulose is arabinoxylan, which comprises a β-1,4 xylan backbone to which side chains consisting mostly of arabinose are attached (Grandis et al. 2014). In most other seed plants (i.e., eudicots, gymnosperms), the major hemicellulose is xyloglucan. Xyloglucan has a β-1,4 glucan backbone, to which are attached short side chains that begin with xylose and may additionally contain arabinose or terminal galactose or fucose. Other hemicelluloses include mannans, glucomannans, and galactoglucomannans.

Pectins are defined by a polymer backbone that is rich in galacturonic acid (Heldt and Piechulla 2010). Pectins comprise part of the cell wall and are also abundant in the middle lamella, which connects adjacent plant cells. Under specific circumstances, carboxyl residues of galacturonic acid form intramolecular ionic linkages, mediated by Ca^{2+}. Within the plant, these linkages stiffen cell walls. Methylation of the carboxyl group prevents the formation of ionic linkages, and therefore the degree of methylation of a pectin molecule greatly affects its function. The simplest pectin is homogalacturonan, which is a α-1,4 polymer of galacturonic acid. Pectins of the rhamnogalacturonan II group have a backbone similar to homogalacturonan, to which are attached complex side chains consisting of combinations of up to ten sugars. Rhamnogalacturonan I is also a type of pectin, but its backbone is made of repeats of a rhamnose, galacturonic acid disaccharide. Many different polysaccharide chains may be attached to the rhamnogalacturonan backbone.

6.3.2 Starches

Starches are the most important nonstructural polysaccharides and are a major source of stored carbon and energy. Starch is found in many cell types throughout the plant but is most abundant in vegetative storage tissues (e.g., tubers of potatoes) and in the seeds of some species (e.g., endosperm of cereals; Heldt and Piechulla 2010). Starch is synthesized mainly within the chloroplast, or in specialized organelles called **amyloplasts**, although some synthesis may also occur in the cytosol. Starch is deposited in granules, the diameter of which affects their digestibility and physical properties.

As indicated previously, starch is made up of two types of polymers (see Fig. 2.9 in Chap. 2), namely, amylose (α-1,4 glucan) and amylopectin (α-1,4 glucan with α-1,6 branch points; Heldt and Piechulla 2010). Despite its name, amylopectin is chemically unrelated to pectin. The highly branched structure of amylopectin gives it very different physical properties than amylose. The linear nature of amylose allows for adjacent molecules to tightly associate and form hydrogen bonds; thus amylose tends to have higher crystallinity and lower solubility and digestibility than amylopectin. Natural starches contain more amylopectin than amylose, and some crop varieties have been selected for specific starch characteristics. For example, starch in sticky rice (also called glutinous rice) is made almost entirely of amylopectin and contains no amylose, whereas short grain rice is approximately 85% amylopectin/15% amylose and long grain rice is 75% amylopectin/25% amylose (Lian et al. 2014; Cameron and Wang 2005; Ayres et al. 1997). Waxy-type starches (~ 100% amylopectin) are produced by varieties of several species and are preferred in some bioproduct applications.

6.4 Plant Carbohydrates as Industrial Feedstocks

Any component of the plant that is rich in polysaccharides is a potential industrial feedstock. Many factors affect the utility of a feedstock (US Department of Energy 2012), including **uniformity** (feedstocks that do not vary in their composition are preferred), **availability** (ideally a reliable supply should be available year-round), **proximity** (distance between the harvest location and the end user), and

extractability (the ability to separate desirable and undesirable components of the feedstock). Effort may also be expended for **densification** (i.e., compaction) of the feedstock prior to transportation. The price of a feedstock therefore depends not only on the cost of growing and harvesting the crop but also any necessary densification and transportation and many other market factors. Not all feedstocks are suitable for all applications, and most established industries are reluctant to adopt new feedstocks (e.g., a different crop or plant component) for existing processes.

Tuber or seed-derived starch is usually used as a food source because starch is easily digested by humans and animals. Although starch and cellulose are both polymers of Glc, cellulose is not digestible by animals (except ruminants and others with specialized microbial symbionts). Cellulose and other cell wall polysaccharides are abundant in wood and straw and other crop or forest residues. These are normally less expensive than starch and are also commonly referred to as biomass.

Some major crops are cultivated to provide components that are rich in carbohydrates. Sugarcane [*Saccharum* spp.] is rich in sucrose, corn accumulates starch, and switchgrass [*Panicum virgatum*] is a source of cellulose. Corn and sugarcane are the most widely cultivated crops, while switchgrass is being developed as a potential energy crop. Due to their relevance as sources of carbohydrates, these plants will be discussed as representatives in the sections below. Although all three are agronomically important crops, both sugarcane and switchgrass in particular are very much limited by the fact that their growth is restricted to warm climates.

6.4.1 Corn/Maize

Corn or maize [*Zea mays*] is a crop of great relevance because it is an important source of carbohydrates in the western diet and it serves a feedstock for many industrial applications. Corn was the second most widely cultivated crop in 2014, and its production was estimated to reach more than 1 billion tonnes (FAO 2017). The production of corn is highly concentrated in a few countries, such as the United States (USA) and China, which make up approximately 55% of total production. Indeed, ten countries (Table 6.2) make up more than 80% of the global production of this crop. Corn produced in the United States is very important because it can be used as a feedstock in the production of biofuels and animal feed. In the 2015/2016 production cycle, 38% of the corn harvested in the United States was consumed in the production of fuel ethanol, and 37% was used in livestock feed (USDA 2017). Exports (14%), food, and other industrial uses (10%) also represent important markets for the corn produced in the United States (Table 6.3).

Corn kernels contain around 70% of their weight (on a dry weight basis) as starch, which is composed mainly of amylopectin (73%; Corn Refiners Association 2006). The composition of this starch can be altered using breeding and genetic techniques, allowing the amount of amylose to be reduced or increased according to the application. The remainder of the corn kernel is composed of fibers, proteins, and oils, which also have commercial value.

Table 6.2 Main producers of corn in the world – 2014 (FAO 2017)

Country	Corn production (million tonnes)
United States of America	361.1
China	215.8
Brazil	79.9
Argentina	33.1
Ukraine	28.5
India	23.7
Mexico	23.3
Indonesia	19.0
France	18.3
South Africa	14.3

Table 6.3 Consumption of corn in the United States by segment in the 2015/2106 production cycle (September to August; USDA 2017)

Segment	Consumption (%)
Fuel ethanol	38.2
Feed and residual use	37.4
Exports	13.9
High-fructose corn syrup (HFCS)	3.5
Glucose and dextrose	2.5
Starch	1.8
Cereals and other products	1.5
Alcohol for beverages and manufacturing	1.0
Seed use	0.2

Technological advances over the last century, such as the adoption of fertilizers and herbicides, mechanization, and the use of hybrids, have resulted in increased production yield per area and reduced cultivation costs (Crow 1998; Pruitt 2016). Genetically engineered plants have also contributed to increased yields and profit more recently. For example, varieties that are simultaneously resistant to herbicides and insects (via the introduction of Bt protein, which is toxic to insects but not humans) are currently on the market, which has diminished the use of insecticides and has improved management practices (Vercesi et al. 2006; Pruitt 2016).

Despite these advances, there are certain areas that could still use improvement, such as resistance to drought, which often affects production and therefore impacts corn prices and the cost of associated bioproducts (Adonizio et al. 2012). In addition, although high levels of starch are stored in kernels, for certain industrial processes, the polysaccharide must first be broken down into its monomers, which means additional steps and costs. The enzymatic hydrolysis of starch, for example, releases monomers of Glc that can be used as a substrate for microbial fermentation (Lin and Tanaka 2006; Vohra et al. 2014). However, genetics and breeding techniques for corn plants are well-established, and their application may provide new varieties that overcome this limitation in the future.

6.4.2 Sugarcane

Sugarcane is mainly cultivated for the production of sucrose, which is an important feedstock in the food and manufacturing sectors. The production of sugarcane in 2014 was 1.9 billion tonnes, making it the most abundantly produced crop in the world (FAO 2017). Similar to corn, most of its production is concentrated in a handful of countries such as Brazil and India, which make up almost 60% of its production, with 83% of its global production occurring in only ten countries (Table 6.4).

Ethanol and sugar are the most relevant products of sugarcane, with 1 hectare of sugarcane yielding 140 kg of sugar (Vohra et al. 2014). The dry matter within the sugarcane juice, which is the syrup extracted from pressed sugarcane, comprises 77–90% of total sugars (Carneiro et al. 2015). **Bagasse** is the biomass residue, which is usually burned to generate power in processing plants. Bagasse, however, contains cellulosic fibers (8–14%) that also have the potential to be used in the production of cellulosic ethanol (Bizzo et al. 2014).

The advantages of using sugarcane as a feedstock for industrial processes are its high content of sucrose, the high yields of the crop, and low processing costs (Vohra et al. 2014). Sugarcane, however, rapidly deteriorates after harvest. Consequently, it must be processed no longer than 72 h postharvest to prevent a loss of sugars in the stalks (Solomon 2009; Vohra et al. 2014). Thus, the proximity of sugarcane fields to processing plants is an important consideration.

While traditional breeding methods and the use of genetic markers have allowed the development of diverse varieties of sugarcane (Jackson 2005; Mohan 2016), the complexity of its genome in terms of the number of repeats, polyploidy, and degree of heterozygosity has limited the use of advanced molecular techniques (Mohan 2016).

Table 6.4 Main producers of sugarcane in the world – 2014 (FAO 2017)

Country	Sugarcane production (million tonnes)
Brazil	736.1
India	352.1
China	126.2
Thailand	103.7
Pakistan	62.8
Mexico	56.7
Colombia	36.5
Australia	30.5
Indonesia	28.6
United States of America	27.6

6.4.3 *Switchgrass*

Switchgrass is a perennial plant that is currently used in the production of forage hay or the control of erosion (Parrish et al. 2012). In addition, there is a growing interest in the development of switchgrass for the production of bioenergy because the cultivation of switchgrass has particular advantages compared to other crops. For example, certain upland ecotypes are adapted to drought conditions and grow well in marginal areas (Perlack et al. 2011; Parrish et al. 2012; Sands et al. 2017), which means that competition with other crops that are used for food production can be avoided. Furthermore, its production requires low inputs (such as fertilizers or pesticides) and does not require specialized machinery (Parrish et al. 2012).

Unlike corn and sugarcane, switchgrass is composed mainly of fibers (77–84%) and does not accumulate sugars or starch (Brown et al. 2016). Instead, cellulose and hemicellulose composed mainly of Glc and xylose are the main carbohydrates in this plant, representing an average 35% and 29% of the dry weight, respectively (David and Ragauskas 2010). Due to this property, switchgrass is mainly cultivated for the production of biomass, and most interest revolves around its potential to be developed as a bioenergy crop. Indeed, switchgrass has the potential to either be burned to generate electricity or used in the production of cellulosic ethanol. These technologies, however, are not viable on a commercial scale as of yet, and further research will be necessary to render production economically viable. In addition to its potential use for bioenergy production, the use of switchgrass as a raw material for pulp and the manufacturing of other bioproducts has also been proposed (Parrish et al. 2012).

6.5 Plant Carbohydrates as Feedstocks for Ethanol Production

6.5.1 *Applications of Ethanol*

One of the oldest uses of ethanol is in the production of fermented alcoholic beverages such as wine and beer. It is also used in many industrial sectors, such as the food and beverage industries, whereby it is used in the extraction of flavors and aromas that will be used in food products (ePURE 2017a). In the pharmaceutical industry, ethanol is used in cosmetics and medicines, while in the chemical industry, it is used as a solvent in the production of different chemicals and paints. Ethanol is also an important intermediate in the synthesis of valuable chemicals, such as ethylene, which is the precursor of a myriad of compounds and can be obtained from the dehydration of ethanol (Harmsen et al. 2014). Despite having many applications, the majority of ethanol produced is used as a fuel. While many vehicles use a blend of gasoline and ethanol, there are also those that are able to run using only ethanol, while others accept ethanol and gasoline in any proportions (flex-fuel vehicles), which are especially popular in Brazil.

Table 6.5 Blend of gasoline and ethanol in selected regions

Region	Amount of ethanol in gasoline (% v/v)
United States of America[a]	10
Canada[b]	5
European Union[c]	5
Brazil – regular gasoline[d]	27
Brazil – fuel ethanol only[e]	100[f]

[a] US Department of Energy (2017b), [b] ECCC (2017), [c] ePURE (2017b), [d] Petrobras (2017), [e] ANP (2015)
[f] This fuel is not pure ethanol, but a mixture with up to 7% of water (ANP 2015)

The recent increase in the production of ethanol is in part due to regulations to diminish pollution and emissions of GHGs. For example, methyl tertiary butyl ether has been used in gasoline as an antiknocking agent (to avoid engine knocking), octane enhancer (to substitute lead), and to oxygenate the fuel to improve its combustion (Keller et al. 2000; Shapouri and Salassi 2006). However, due to groundwater contamination, it has been banned in the United States. When added to gasoline, ethanol provides the same functions as methyl tertiary butyl ether and is even considered superior due to the fact that it contains higher amounts of oxygen, which may lead to more complete combustion (Bothast and Schlicher 2005).

Fuel ethanol is typically sold as a mixture with gasoline. These blends are termed E5, E10, E15, and E85, with the number indicating the percentage of ethanol in the mix (US Department of Energy 2017a). Some countries, such as Brazil, have regulations concerning the amount of ethanol in gasoline blends (Table 6.5), where all gasoline sold must contain a certain percentage of ethanol (Petrobras 2017). Brazil also provides hydrated ethanol (a mixture of 95% ethanol and 5% water) as a fuel (ANP 2016); since most cars in Brazil are flex-fuel vehicles and ethanol prices are competitive with that of gasoline, there is a high demand for ethanol-based fuels.

6.5.2 Industrial Production of Ethanol

Ethanol (or alcohol) is a flammable liquid that has the chemical formula C_2H_5OH. It is naturally produced by some yeasts under anaerobic conditions, a process that has been exploited by humans since ancient times for the production of beer and wine. Currently, ethanol has many uses and is produced on a very large scale, with global production reaching approximately 100 million m^3 in 2016 and 85% of this production (Fig. 6.6) being contributed by the United States (57%) and Brazil (28%; RFA 2017).

Currently, most ethanol is produced via the **fermentation** of agricultural feedstocks, followed by distillation. A smaller fraction, however, is synthetically produced from petroleum derivatives and is used mainly for industrial purposes (IEA – Industrial Ethanol Association 2007; Schobert 2013). This synthetic route

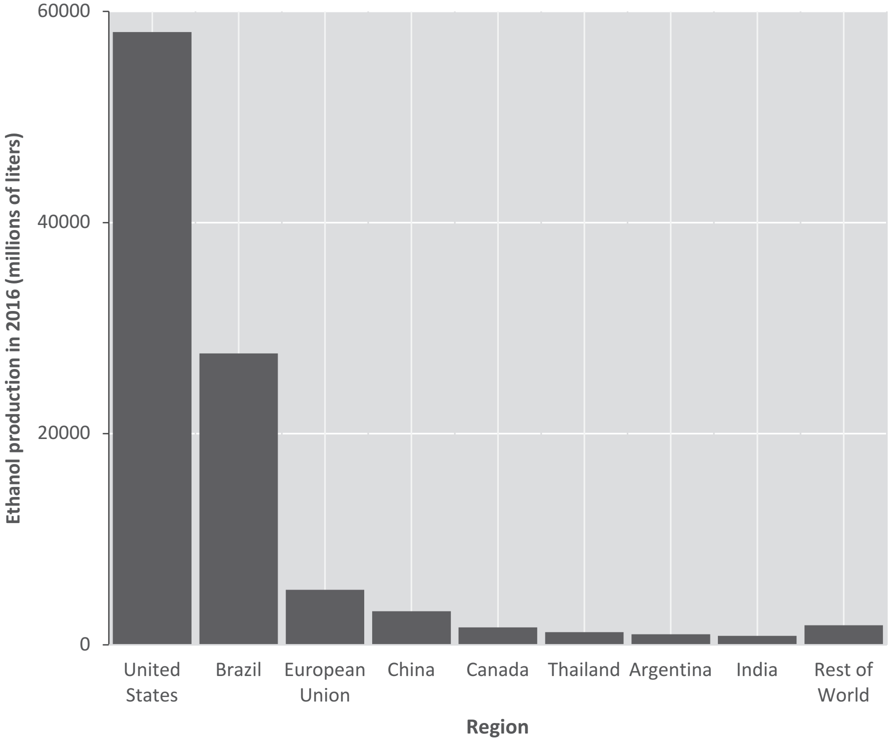
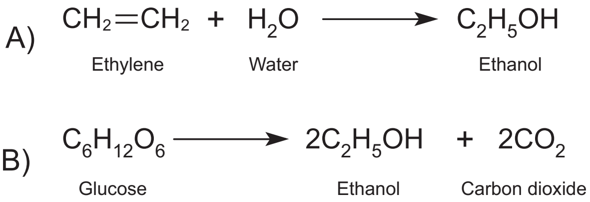


Fig. 6.6 Production of ethanol by country in 2016. Based on RFA (2017)

Fig. 6.7 Ethanol production via chemical synthesis (**a**) and biological fermentation (**b**)



involves the hydration of ethylene in the presence of an acid catalyst (Fig. 6.7a) at high temperatures. This process produces acetaldehyde as a by-product, but the process is not economically viable, and petroleum-derived ethanol is not considered a renewable fuel (Schobert 2013; Mohsenzadeh et al. 2017). When produced from agricultural sources, on the other hand, the resulting ethanol is considered a renewable fuel (also known as bioethanol). In this case, the fermentation is performed by certain microorganisms under far milder conditions.

Fermentation is an intracellular process that occurs in the absence of oxygen, whereby cells use organic compounds (e.g., pyruvic acid and Glc or other carbohydrates) for the production of ethanol or lactic acid (depending on the microorganism or cell type) and carbon dioxide (Fig. 6.7b). It guarantees electron flow in the cell and the consequent production of energy (in the form of ATP) under anaerobic conditions. Humans have long been exploiting microorganisms that are capable of fermentation for the manufacture of a myriad of products, such as alcoholic beverages, yogurt, bread, antibiotics, and fuels.

Saccharomyces cerevisiae is an example of a fast-growing yeast that produces ethanol during fermentation and is widely used in industrial fermentation. Mankind learned how to exploit microorganisms in the manufacturing of a myriad of products, including alcoholic beverages, yogurt, bread, antibiotics, and fuels.

Feedstocks for first-generation bioethanol production are sucrose (mostly from sugarcane, but also from sugar beet [*Beta vulgaris*] and sorghum [*Sorghum bicolor*]) or starch (mostly from maize, but also from barley [*Hordeum vulgare*], wheat [*Triticum aestivum*], and other cereals). Starch that is released from milled grains must first be saccharified to yield Glc, and the resulting Glc or sucrose is fed to yeast growing in large liquid cultures (refer to Chap. 11 for the biorefinery process). The ethanol produced through alcoholic fermentation is then purified through distillation. By-products of this process include bagasse (leftover biomass) or distillers dried grains (the remnants of cereals from which the starch has been removed). An important economic consideration to the use of bioethanol is going to be our ability to find a market for these by-products in order to prevent their accumulation and yield maximal value from the crops. Currently, distillers dried grains have some value as animal feed, while bagasse can be used in energy production, in place of low-quality wood fiber in manufacturing, or in second-generation ethanol production as described below.

6.5.3 Industrial Production of Ethanol from Plant Carbohydrates

Ethanol derived from sugarcane and corn grains may not be able to meet our fuel demand, and there are also ethical concerns regarding the use of food crops for the production of biofuels. Therefore, the use of agricultural residues and other sources of cellulose is a promising alternative in the production of renewable fuels. Agricultural residues such as sugarcane bagasse or **corn stover** are by-products in the processing of these crops that usually have few applications (Tuck et al. 2012). Corn stover refers to the cobs, leaves, and stalks that remain in the field after harvest. Indeed, the production of these by-products often exceeds their demand, which makes these feedstocks very abundant and cheap. Other cellulosic sources, such as fast-growing trees (e.g., poplar [*Populus* spp.]) and switchgrass, also have potential to be used in the production of biofuels. As is the case with agricultural residues, they would not compete with food crops because they could be grown in marginal areas.

Despite these advantages, technology for the production of lignocellulosic-derived ethanol is not currently developed enough to allow commercial-scale production. Saccharification of cellulose is the main challenge, since biomass fibers are difficult to break. In addition, enzymes that catalyze the hydrolysis of cellulose and hemicellulose are very expensive; lowering this cost will be essential for large-scale production (Kim and Kim 2014). Fermentation using lignocellulosic-derived sugars also has some limitations, since *S. cerevisiae*, which is commonly used for ethanol production, is not able to use pentoses as substrate (Gupta and Verma 2015). While other microorganisms do have this ability, their conversion times are slower than *S. cerevisiae.* Therefore, metabolic engineering has the potential to yield an ideal microorganism that can ferment both the hexoses and pentoses with a high productivity.

A major challenge when dealing with lignocellulosic materials is the variation in composition and structure of biomass, since different sources of biomass have distinct proportions of cellulose, hemicellulose, and lignin. **Softwoods** (e.g., spruce [*Picea* spp.], pine [*Pinus* spp.]), and **hardwoods** (e.g., poplar) are made up of woody biomass that is usually denser than agricultural residues, which is better for transportation (McKendry 2002; Zhu and Pan 2010). They also produce a lower content of pentoses after hydrolysis, which may contribute to fermentation efficiency. The higher content of lignin, however, increases the cost of pretreatment because it increases the energy requirements of these processes (Zhu and Pan 2010). Agricultural residues, such as corn stover, sugarcane bagasse, and rice hulls, have a lower lignin content and are therefore easier to break down compared to woody materials. Agricultural residues, however, have to undergo a compaction process prior to transportation, which can increase the price of the final product (McKendry 2002). **Energy crops** (or biomass crops), such as switchgrass and miscanthus [*Miscanthus sinensis*], have only biomass as their final product. They exhibit high yields, low lignin content, and fast growth. **Industrial waste**, especially from cellulose-processing plants, and **municipal solid waste**, such as paperboard products and woody materials, can also be used as feedstock for the production of bioethanol.

6.6 Pyrolysis of Lignocellulosic Biomass

Thermochemical and biochemical conversions of biomass are the two main routes for the transformation of this feedstock into fuels and other valuable compounds. While biochemical conversion uses enzymes and microorganisms to break down biomass (e.g., fermentation), thermochemical conversion is able to directly generate energy or other compounds from feedstock using high pressure, temperature, and/or chemicals (Basu 2013). Combustion, gasification, torrefaction, and pyrolysis are all used in the thermochemical conversion of biomass. Burning lignocellulosic biomass in the presence of oxygen releases heat in an exothermic oxidation reduction called combustion. Gasification of biomass produces gases (H_2, CO, CH_4) that can be utilized for the production of energy or for the production of liquid fuels. Torrefaction reduces the oxygen content of biomass and produces solid fuels that have a higher energy density (Basu 2013).

Pyrolysis is similar to combustion; however, biomass conversion occurs in the absence of oxygen and under lower temperatures (400–600 °C; Mettler et al. 2012; Basu 2013). The type of biomass, as well as temperature and length of reaction (residence time), affects the final composition of the products. Conventional pyrolysis usually yields gaseous, liquid, and solid products, but their proportions can be adjusted by altering parameters of the process (Basu 2013; Collard and Blin 2014; Stefanidis et al. 2014). The most common solid phase product is biochar, which is mainly composed of carbon and has a higher energy density than biomass. It is the main product of slow pyrolysis and can be used as a form of carbon sequestering (Basu 2013). The gas phase is composed of noncondensable gases such as carbon dioxide, carbon monoxide, hydrogen, and hydrocarbons (Aho et al. 2013; Basu 2013). The liquid phase is made up of a complex mixture that contains water and a high content of oxygen known as bio-oil. Along with water, which can make up to one quarter of its mass, acids, ketones, phenols, aldehydes, ethers, carboxylic anhydrides, furans, and saccharides are among the compounds typically found in this mixture (Alvarez et al. 2014). While phenolic compounds are derived from lignin degradation, the degradation of cellulose and hemicellulose yields ketones. Acids, on the other hand, are derived from the decomposition of all the three polymers. A long residence time at high temperatures favors the production of gases, while lower temperatures favor the production of biochar (Bridgwater 2010). Fast pyrolysis, on the other hand, has a short residence time at intermediate temperatures and maximizes the production of bio-oil (Bridgwater 2010; Basu 2013).

Bio-oil is not suitable for direct use as a fuel in most engines (there are some furnaces and diesel engines that can use bio-oil) because it has a low pH and high moisture and oxygen contents (Bridgwater 2010). However, it can be upgraded to conventional fuels such as diesel or gasoline by converting oxygen to water or carbon dioxide, which are removed in downstream steps (Bridgwater 2010; Si et al. 2017). Gasification to synthesis gas, hydrodeoxygenation, and catalytic cracking are some processes that can convert bio-oil into fuels. Bio-oil can also be used for the production of chemicals for use in many different applications (Bridgwater 2010), although separation processes still need to be developed to effectively and efficiently separate out the most valuable compounds.

Co-pyrolysis is a technology that allows biomass to be processed with fossil fuel polymers to increase the final quality of bio-oil by reducing water and oxygen contents and increasing yield (Abnisa et al. 2017). In this case, tires and plastics are added to biomass, which increases both the energy and oil content of the bio-oil. The use of fossil fuel-derived materials in this process could also reduce the amount waste in landfills, making co-pyrolysis an attractive potential alternative in the production of biofuels.

6.7 Closing Comments

Carbohydrates are a very important output of many cultivated crops. The abundance of resulting feedstocks and the variety of chemical structures are very promising in terms of the potential use of plant-derived carbohydrates for many industrial applications. Among them, the production of biofuel demands enormous amounts of carbohydrates. Although corn starch and sugarcane sucrose are widely used for the production of fuel ethanol, the use of lignocellulosic biomass as an alternative source is gaining attention. This is happening especially because of ethical implications concerning the production of fuels from potential food sources. Unfortunately, the production of ethanol from lignocellulosic materials is not as economically viable as ethanol production from corn or sugarcane, and therefore, virtually every step in the process currently requires improvement to reduce costs and increase yields. The use of plant carbohydrates as feedstocks for the production of bioproducts other than biofuel will be discussed in the next chapter.

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