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Wyman, Charles E
Kumar, Rajeev
Cai, Charles M

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Charles E. Wyman[1,2,3], Charles M. Cai[2,3] and Rajeev Kumar[2,3]
[1]Department of Chemical and Environmental Engineering and Center for Environmental Research and Technology (CE-CERT), Bourns College of Engineering, University of California Riverside, Riverside, CA, USA
[2]BioEnergy Science Center (BESC), Oak Ridge National Laboratory (ORNL), Oak Ridge, TN, USA
[3]Center for Environmental Research and Technology, Bourns College of Engineering, University of California Riverside, Riverside, CA, USA

Article Outline

Glossary

Biomass	Plant matter of recent (nongeologic) origin.
Cellulase	One or more enzymes that catalyze the reaction of water with cellulose to release shorter glucose oligomers and ultimately monomeric glucose sugar.
Cellulose	A glucose polymer composed of up to about 15,000 glucose molecules covalently joined by β 1–4 linkages in long, straight chains that can hydrogen bond with parallel cellulose chains to form crystalline regions. About 35–50% of the structural portion of plants is cellulose.
Cellulosic biomass	Also known as lignocellulosic biomass, the structural part of plants that is not edible by humans and contains cellulose, hemicellulose, pectin, and lignin. Examples include grass, wood, and agricultural and forestry residues.
Cellulosic ethanol	Ethanol made from lignocellulosic biomass by biological, chemical, or chemo-biological processes.

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Enzymes	Proteins produced by living cells or organisms that are able to catalyze chemical reactions in organic substances.
Ethanol	An alcohol with the formula C_2H_5OH that is a high octane (greater than 100 Motor Octane Number) transportation fuel and also used commercially in alcoholic beverages, solvents, cosmetics, and other products. Ethanol can be made by microbial fermentation of sugars derived from sugarcane, starch, or cellulosic biomass or made catalytically from synthesis gas. Ethanol is hygroscopic (attracts water molecules) and biodegradable.
Hemicellulose	An amorphous, noncrystalline polymer typically made up of some combination of arabinose, galactose, glucose, mannose, and/or xylose sugars but also containing less amounts of other compounds such as methoxyl, acetyl, and free carboxyl groups. Hemicellulose joins with lignin to glue long cellulose fibers into a very strong composite material. Although the proportions can vary widely, hemicellulose often makes up about 15–30% of the overall dry weight of cellulosic biomass.
Hydrolysis	The reaction of water with a sugar polymer or other compound to form other compounds.
Lignin	A complex phenylpropanoic acid polymer chemically linked with hemicellulose to bind cellulose chains together. Lignin often represents about 7–35% of the dry weight of cellulosic biomass. Lignin is more difficult to break down into its component molecules and is not fermentable at an appreciable rate.
Sugars	Ring-shaped compounds consisting of five to six carbon atoms along with hydrogen and oxygen at a ratio of two hydrogen atoms and one oxygen atom to one carbon atom. Sugar is obtained from the juice of such plants as sugarcane and sugar beets and can be obtained by hydrolysis of starch in corn and other starch plants and by hydrolysis of cellulose and hemicellulose in cellulosic biomass. Sugars typically contained in cellulosic biomass include arabinose, galactose, glucose, mannose, fructose, rhamnose, and xylose. Sugars are typically sweet.

Definition of the Subject

Cellulosic ethanol can be made from the structural portion of plants that are generally known as lignocellulosic or simply cellulosic biomass. Large quantities of cellulosic biomass can be obtained at costs competitive with oil at about 20 US$/bbl [1, 2]. Such plant matter is composed mostly of cellulose, hemicellulose, and lignin along with other typically lesser ingredients that include oils, pectin, proteins, free sugars, starches, and minerals. Cellulose that makes up about 35–50% of the total dry weight of structural plant matter on a dry basis usually represents the largest portion of most plants. Hemicellulose contributes about another 15–30% to the dry weight of many forms of cellulosic biomass and is thereby typically the second greatest fraction of structural plant matter [3, 4]. The rest of the plant is mostly lignin.

Cellulose and hemicellulose are both polysaccharides, that is to say, they are made up of chains of sugars. Glucose sugar forms the long linear cellulose chains, most of which align in parallel and hydrogen bond to adjacent chains to form crystalline fibers [5–7]. Hemicellulose, on the other hand, is comprised of up to five sugars (arabinose, galactose, glucose, mannose, and xylose) as well as other lesser ingredients such as acetyl groups that form highly branched amorphous polysaccharides [4, 8, 9]. Lignin is a complex structure built around random joining of phenylpropene units that works with hemicellulose to form a glue that holds the cellulose fibers together [10, 11].

The cellulose and hemicellulose from such plant resources can be broken down or hydrolyzed to the sugars that make up these two major fractions, glucose from cellulose and some combination of arabinose, galactose, glucose, mannose, and xylose from hemicellulose. Once these sugars are released through combining with water during the hydrolysis reaction, they can be fermented into ethanol or other metabolic products in a way similar to that used to make large amounts of ethanol from corn starch and cane sugar [12–14]. However, although hemicellulose can be broken down relatively easily with reasonably high yields by applying enzymes and/or dilute acid to form its component sugars [15–17], cellulose hydrolysis is much more difficult due to the its inherent structure and complex lignin-hemicellulose shield that protects the cellulose in addition to gluing the cellulose chains together in a natural composite material [13]. As a result, although cellulosic biomass itself is low in cost, the recalcitrance to sugar release resulting from this complex structure presents a major cost barrier to cellulosic ethanol production [18], and many approaches have evolved over the years in an attempt to find a cost-effective route [19–22]. However, to date, many challenges remain to overcoming biomass recalcitrance to sugar release and in deriving value from lignin and other noncarbohydrate portions of cellulosic biomass that are not amenable to fermentation to overcome the barrier to low-cost cellulosic ethanol production [2, 23, 24]. Alternatively, thermal routes such as gasification and pyrolysis can be applied to form intermediates that can in turn be catalytically and/or biologically converted to ethanol. Unfortunately, these routes also face challenges for realizing high yields at low costs. Thus, the primary challenge to producing cost-competitive ethanol from cellulosic biomass requires developing new routes that can capitalize on the low-cost, large amount of cellulosic biomass for production of cost-competitive ethanol.

Introduction

Ethanol is a member of the alcohol family of chemicals, has a molecular formula C_2H_5OH, and is often simply referred to as “alcohol.” Humans have fermented sugars and starch to alcoholic beverages such as wine and beer for millenia and concentrated the ethanol contained in fermentation streams to produce various spirits, liquor, and liquors [25, 26]. In addition, ethanol has many domestic and industrial uses for products ranging from a major ingredient in mouthwash to solvents. However, ethanol also has excellent fuel properties including an octane of about 103 and high heat of vaporization that are of advantage for powering race cars such as those competing in the Indianapolis 500 run in the USA over the Labor Day Holiday. In fact, Henry Ford used ethanol to fuel early vehicles manufactured by the Ford Motor Company, but the emergence of petroleum soon displaced ethanol as a motor fuel. Ethanol gained importance in the USA during World War II for such uses as powering vehicles, propelling torpedoes, and providing a platform for making rubber, but it was once again displaced by petroleum after the War ended [27]. In addition, ethanol can be used as a feedstock for its catalytic conversion to “drop-in” fuels, jet fuel, hydrogen, and chemicals [20, 28, 29].

During the fuel crises of the 1970s and 1980s, ethanol made from mostly corn and a few other starch crops and from cane sugar reemerged to address fuel shortages, and the need for oxygenates in high-altitude cities such as Denver, Colorado, and Phoenix, Arizona (both in the USA), drove consumption to reduce emissions of unburned hydrocarbons and carbon monoxide from carbureted engines. Ethanol use has grown to a point that it now contributes about 10% of the total liquid fuel used for internal combustion engines in the USA and about 50% of that fuel class in Brazil, respectively [30]. Despite this remarkable recent growth in use, starch and sugar crops are limited in their availability to displace a large portion of the huge transportation fuel market, and use of these resources raises questions about competition with food production and environmental impact from such aspects as water demand and fertilizer runoff polluting rivers, lakes, and oceans [14, 31, 32]. In addition, carbon dioxide accumulation in the atmosphere due to use of fossil fuels is driving global climate change, and the almost total dependence of transportation on petroleum results in this sector being the largest single contributor to this global concern about the future of planet Earth [33–35]. Although first-generation ethanol from corn and cane sugar contribute less to global climate change than petroleum or other fossil alternatives, limitations in the ultimate availability of these resources restricts their ability to mitigate global climate change [34, 36–38].

An alternative resource from which ethanol can be produced in much larger quantities is lignocellulosic biomass in the form of grass such as switchgrass, wood such as poplar, agricultural residues such as corn stover, and a large portion of municipal waste that includes wastepaper [4, 39]. With a cost of 60 US$/$t_{DM}$ (DM dry matter) for such forms of cellulosic biomass equivalent in energy content cost to petroleum at about 20 US$/bbl, this resource can be very inexpensive. Furthermore, well over a 10^9 t/a of cellulosic biomass could be collected at this price or less, from which enough ethanol or other biofuels could be produced to displace about half of the 470 billion liters of gasoline now used annually in the USA [40, 41]. A common feature of these diverse types of biomass is that about two-thirds to three quarters of their dry mass is composed of polysaccharides that can be broken down to form sugars that can in turn be fermented to ethanol. However, despite the low cost and large potential availability of cellulosic biomass, breaking it down to form fermentable sugars is difficult and requires an extensive and expensive combination of operations to realize the high yields vital to economic competitiveness. Although many routes have been applied over the years to overcome the recalcitrance of biomass as an economic obstacle [21, 24, 42, 43], only a few have been able to realize the high ethanol yields that are paramount to distributing the costs over as much product as possible. It has also been challenging to derive value from the noncarbohydrate portion of biomass that would enhance revenues and reduce costs for processing residues left after carbohydrate utilization [11, 44].

In the following sections of this paper, the fundamental aspects of converting cellulosic biomass to ethanol will be summarized. First, the importance of developing sustainable fuels for transportation such as cellulosic ethanol will be described. Then, a brief overview will be provided of ethanol properties to underline its value as a fuel and challenges in integrating into the current transportation infrastructure. The composition and structure of cellulosic biomass will be described to provide the foundation from which to understand how biomass lends itself to conversion into ethanol, portions of biomass that cannot be converted to ethanol, and factors that contribute to the recalcitrance of biomass to ethanol production. That will follow with an overview of the chemistry involved in converting cellulosic biomass into ethanol, the biochemistry of enzymes that are effective in breaking covalent bonds that join glucose molecules to form cellulose, and features of fermentations that can convert all of the sugars released into ethanol. Next, acid, enzymatic, and thermal processes for converting cellulosic biomass into ethanol will be reviewed to help understand the strengths and challenges in current approaches to low-cost cellulosic ethanol production. Then more details will be provided on the steps involved in enzyme-based cellulosic ethanol production in light of this path being currently favored for commercial implementation. Current estimated costs for cellulosic production will be outlined and its benefits for reducing greenhouse gas (GHG) emissions summarized. Against this background, key barriers to low-cost cellulosic ethanol production will be highlighted, and advancements needed to realize lower costs and more completely utilize biomass to enhance revenues and profitability will be outlined. References will be given throughout the chapter to more detailed treatments of each subject to facilitate the reader accessing additional information if desired.

Importance of Sustainable Transportation Fuels

Over the years, many drivers have come and gone to foster interest in developing and commercializing sustainable transportation fuels. In the mid-1970s, the Organization of the Petroleum Exporting Countries (OPEC) realized that US petroleum production had peaked as had been predicted by Hubbert in 1956 [45] and cut production to demonstrate OPEC's dissatisfaction with various political events of that time. The result was long lines of motorists waiting their turn to fill their cars with fuel, high prices, and shortages of gasoline and other petroleum products. At that time, the USA and other countries turned to development of alternative fuels via such routes as conversion of biomass and coal to liquid fuels [46, 47]. However, when prices dropped in the mid-1980s, interest in such fuel alternatives waned for replacing petroleum. In that same time period, high altitude cities such as Denver, Colorado, and Phoenix, Arizona (both in the USA), were experiencing severe air pollution that resulted from carbureted engines that were employed at that time running too rich in fuel due to the lower oxygen pressure in such cities compared to the low-altitude locations where vehicles were built and tuned. These problems triggered the need for oxygenates whose molecular oxygenate content compensated for lower air pressure to dramatically reduce the release of unburned hydrocarbons and carbon monoxide (CO). Ethanol was a leading option to meet this need, and its importance became even greater with the demise of methyl tertiary butyl ether (MTBE) due to the migration of the latter into groundwater and migration of this foul smelling ingredient into drinking water [35, 48]. Although air quality improved substantially through use of oxygenates, the emergence of better catalytic converters that could more completely eliminate pollutants, improved fuel formulation to reduce emissions, and transition to computer controlled fuel injection systems that could self-tune to compensate for lower air pressure reduced the need for ethanol or other oxygenates to control air pollution.

Although such needs have been met by such measures as greater petroleum production by fracking, catalytic converters, and fuel injection, a more critical need has emerged that has severe long-term consequences: global climate change. In particular, the carbon dioxide released when fossil fuels are burned is a powerful greenhouse gas (GHG) that captures infrared radiation and reemits it toward the Earth. The use of petroleum and other fossil fuels has increased carbon dioxide concentrations from less than 320 ppm (parts per million) in 1960 to over 400 ppm now [49]. The result is increasing temperatures that are projected to become far higher in the years to come and result in such dire consequences as sea level rise and attendant coastal flooding, extinction of various species, and increased spread of diseases [50, 51]. Because burning petroleum-derived products is the largest source of carbon dioxide emissions in the USA, it is vital to replace petroleum with sustainable resources to reduce carbon emissions. Furthermore, because over 70% of petroleum is used for transportation and transportation derives about 97% of its power from petroleum, it is vital to reduce its use for this sector. In that regard, three options are feasible: (1) drive less, (2) drive more fuel-efficient vehicles, and (3) replace fossil-derived fuels with fuels with a low carbon footprint [19, 52, 53]. All three are important strategies that should be addressed. However, ultimately with growing populations and continual improvements in lifestyle that demand more energy, it is vital to develop and employ fuels from sustainable sources that can dramatically reduce net carbon dioxide accumulation in the atmosphere. As will be shown in more detail later, fuels such as ethanol that can be made from cellulosic biomass can dramatically reduce carbon emissions by these plants combining the carbon dioxide released during biofuel production with water to form new plants to replace those harvested for fuel production. The key attribute of cellulosic fuels is their ability to minimize the use of fossil fuels for planting, harvesting, transporting, and converting biomass to fuels with a resulting low carbon footprint [34]. Furthermore, such fuels provide the only known option with the potential for low-cost production on a scale that can meaningfully reduce the need for petroleum for transportation.

Basics

Ethanol as a Transportation Fuel

Ethanol is a clear colorless liquid that looks like water and is completely miscible with water. Ethanol diluted in water as in alcoholic beverages has a somewhat sweet flavor, but concentrated ethanol solutions have a much more burning, pungent taste. In addition to its being the essential ingredient for alcoholic beverages, ethanol is widely used in medicines, lotions, mouthwash, tonics, colognes, rubbing compounds, and solvents. However, a key property for its use as a transportation fuel is its ability to be readily burned with the oxygen in air according to the following stoichiometry (Eq. 1):

$$C_2H_5OH + 3\ O_2 ----> 2\ CO_2 + 3\ H_2O \tag{1}$$

Ethanol burns with a light blue flame. In addition to its flammability, ethanol has a number of important properties that are particularly favorable for use in spark ignition engines. First, it has a high octane number of 103 based on the standard average of research and motor octane values. Furthermore, ethanol has a heat of vaporization of 907 J/g compared to a value of only about 395 for gasoline [54]. The combination of high octane number and high heat of vaporization translates into the ability to employ considerably higher compression ratios in dedicated ethanol engines that in turn result in more efficient use of ethanol compared to gasoline in lower compression ratio engines [55, 56]. These fuel properties explain why ethanol is the fuel used in the Indianapolis 500 motor race: racecars fueled by ethanol can realize equivalent performance in smaller and lighter engines than possible with gasoline. These properties also mean that even though the energy density of ethanol at about two third that of gasoline implies that it would require 50% more ethanol to travel the same distance as a given volume of gasoline, a dedicated ethanol engine can be tuned to realize about 80% of the range of a gasoline fueled engine. In addition, ethanol is much lower in toxicity than any other fuel as evidenced by the fact that ethanol is the key ingredient in alcoholic beverages, while drinking other fuels would be fatal. Ethanol is also less environmentally problematic in that ethanol is completely soluble in water and natural microorganisms readily metabolize it to carbon dioxide and water, thereby facilitating remediation of spills and leaks far more easily than possible with petroleum-based products [1].

Although ethanol has important fuel properties, it also has some limitations. For one, the high octane number means that it has a low cetane number, thereby making it less suitable for use in compression ignition engines. Its low energy density compared to jet fuel also hurts its prospects for use in air travel. The preference of ethanol for water compared to hydrocarbons makes it favorable for dealing with water inadvertently occurring in vehicle gasoline tanks but is not desirable for transport in pipelines that frequently accumulate pools of water. In addition, even though ethanol has a substantially lower vapor pressure than gasoline (16 kPa Reid vapor pressure vs. ~71 kPa for gasoline), nonideal interactions between the two result in higher vapor pressures for mixtures containing low ethanol concentrations [57]. Because ethanol requires less oxygen to burn than gasoline, only dedicated vehicles tuned for ethanol or flexible fuel vehicles equipped with instrumentation that adjusts the air/fuel ratio can accommodate ethanol, with the result that current US regulations limit ethanol concentrations in gasoline to 10% for older vehicles and 15% for newer cars.

Ethanol has some other notable properties that favor its production and purification compared to other fuels that could be produced biologically. For one, the long history of ethanol production in beverages dating back to the Babylonians making beer in about 6000 BC has resulted in evolution of organisms that can realize high rates of ethanol production to concentrations of over 12% in water. In addition, the low boiling point of ethanol 78 °C compared to water facilitates its recovery and purification, with distillation of ethanol from wine practiced in Italy in about the eleventh century.

Composition and Structure of Cellulosic Biomass

The primary components making up cellulosic/lignocellulosic biomass are cellulose, hemicellulose, and lignin, typically in that order of prevalence, plus generally lesser amounts of various other ingredients such as proteins, pectin, minerals, and sugars [3, 18, 58].

- Cellulose is a conceptually quite simple linear polymer of β-(1→4)-linked D-glucose (D-glucopyranose) monomer units whose degree of polymerization (DP or number of glucose molecules in a cellulose chain) ranges from 5,000 to 15,000 glucose molecules, depending on the source [59]. Although made up of glucose units, the basic repeating cellulose unit is cellobiose, a molecule made up of two covalently linked glucose molecules; cellobiose is also known as a β-(1→4)-linked disaccharide of D-glucopyranose [60, 61]. Although its chemical makeup appears quite simple, cellulose chains are organized in complex assemblies with increasing size scales. These long cellulose chains align and hydrogen bond to one another to form large crystalline regions interdispersed with much smaller amorphous segments. Large segments of these long cellulose chains arrange in a regular, repeating crystalline structure in which these long chains pack together as typically from around 30 to 200 independent chains to form bundles or microfibrils [62, 63]. Long sections of these individual chains are in an extended conformation in these microfibrils, and segments of adjacent chains pack together as highly regular microcrystalline regions. However, the periodic disruption of these long segments of ordered chain conformations by shorter irregular amorphous regions gives plants both great strength and flexibility. Another result is that cellulose tends to be extremely insoluble in water, an important attribute for its structural function in plant cell walls [64, 65].
- Hemicelluloses are amorphous and highly branched chains that generally contain more than one of the following sugars in their chains: arabinose, galactose, glucose, mannose, and xylose [4, 66, 67]. Arabinose and xylose contain five carbon atoms each and are typically referred to as five carbon sugars, while the other three sugars are referred to as six carbon sugars in recognition of each containing six carbon atoms. Hemicelluloses also contain other components such as acetyl groups. Typically, xylose and mannose are the most prominent sugars in hemicelluloses; however, the relative amounts of the various components vary widely among different plants. Although hemicelluloses are grouped according to various subclasses, they can be categorized according to their ability to be hydrated with water. The branched structure of hemicelluloses generally enhances water solubility and serves primarily to stabilize cell walls through hydrogen bonding to cellulose and covalent bonding to lignin [4, 10, 67]. This interplay among these three components strengthens the cellulose microfibrils. Pectins, non-cellulosic acidic cell wall polysaccharides, complex and reinforce this matrix further and help retain water and ions as well as facilitate cell wall-modifying enzymes, cell wall porosity, cell-to-cell adhesion, cell expansion, cell signaling, developmental regulation, and defense [68].
- Lignin is the third major polymer making up the structural components in biomass. Lignin is a complex three-dimensional phenol-propene polymer that partially encases the plant cell wall polysaccharides and cellulose microfibrils [11]. As such, it is not composed of sugars that can be released for fermentation to ethanol or other products. Lignin provides mechanical and elastic strength and facilitates water and nutrient transport in the plant. It also resists

attack by microbes and thereby protects the plant from natural predators. The contribution of lignin to fighting off biological breakdown is believed to also provide the major barrier to the release of sugars for production of ethanol from cellulosic biomass; that is to say, lignin is a primary source of biomass recalcitrance [64, 69, 70].

Table 1 illustrates the considerable variability in the distribution among cellulose, hemicellulose, and lignin within plants. The composition, structure, and interactions among these three biopolymers in the complex cellulosic matrix provide many key plant functions including providing mechanical support and transporting water, nutrients, and photosynthates in the plant. Cellulosic biomass can be pictured as nature's composite material in which cellulose fibrils can be viewed as analogous to the fibers in fiberglass, while lignin and hemicellulose can be viewed as similar to the epoxy that holds the fibers together.

Chemistry of Converting Cellulosic Biomass into Ethanol

For ethanol production from cellulosic biomass by biological routes, enzymes or acids can break down the xylan and arabinan in hemicellulose into its component sugars via the hydrolysis reaction presented in Eq. 2 in which n refers to the number of xylan or arabinan entities in the chain. Equation 2 can be described in words: xylan or arabinan + water ——> xylose or arabinose.

$$(C_5H_8O_4)_n + n\,H_2O - -- > n\,C_5H_{10}O_5 \quad (2)$$

A large fraction of the xylan and arabinan in the hemicellulose can be recovered as xylose or arabinose, respectively, when this reaction is catalyzed by acids [15, 17, 72, 73]. However, the combination of the heterogeneity in bonds within hemicellulose and the specificity of enzymes makes it more challenging to realize high yields of sugars from hemicellulose [74, 75]. The stoichiometry represented by this equation means that 132 mass units of xylan or arabinan will form 150 mass units of xylose or arabinose sugars, a mass gain of 13.6%.

The hydrolysis of cellulose or the six carbon anhydrous sugars of galactose, mannose, or glucose in hemicellulose proceeds according to the stoichiometry shown in Eq. 3; it can be described in words: glucan (galactan or mannan)+water ——> glucose (galactose or mannose).

$$(C_6H_{10}O_5)_n + n\,H_2O - - - - > n\,C_6H_{12}O_6 \quad (3)$$

The six carbon sugars in hemicellulose can be readily broken down by acids due to the amorphous structure of hemicellulose, while enzymes are again challenged by the variety of bonds among the variety of sugars and other components that make up hemicellulose. However, in the case of cellulose, acids are unable to realize high yields of glucose via this reaction due to the high crystallinity of cellulose. But, the high specificity of cellulase enzymes coupled with the homogeneity of glucan bonds in cellulose makes it possible to realize virtually full recovery of glucose from cellulose [59]. Unfortunately, very high doses of expensive cellulase enzymes are typically needed to achieve these high yields.

Once the sugars that comprise cellulose and hemicellulose have been released into solution by the hydrolysis reactions above, they can be fermented to ethanol. In the case of the five carbon sugars arabinose and xylose, the fermentation reaction results in the following stoichiometry (Eq. 4; arabinose or xylose ——> ethanol + carbon dioxide):

$$3\,C_5H_{10}O_5 - - - - > 5\,C_2H_5OH + 5\,CO_2 \quad (4)$$

Thus, 450 mass units of five carbon sugars can form 230 mass units of ethanol, resulting in a maximum theoretical mass yield of $230/450 \times 100 = 51.1\%$.

In the case of the six carbon sugars galactose, glucose, and mannose, the fermentation stoichiometry is according to Eq. 5; i.e., galactose, glucose, or mannose ——> ethanol + carbon dioxide:

$$C_6H_{12}O_6 - - - - > 2\,C_2H_5OH + 2\,CO_2 \quad (5)$$

Bioethanol from Lignocellulosic Biomass, Table 1 Typical biomass composition for a variety of feedstocks, highlighting the diversity in chemical makeup (Adapted from Carroll and Somerville [71])

Source	Cellulose	Xylan	Mannan	Galactan	Arabinan	Lignin	Extractives	Ash	Uronic acids
Sugarcane bagasse	39.01	22.05	0.35	0.46	2.06	23.09	3.78	3.66	2.16
Corn stover	37.69	21.61	0.38	0.87	2.42	18.59	5.61	10.06	2.99
Wheat straw	32.64	19.22	0.31	0.75	2.35	16.85	12.95	10.22	2.24
Monterey pine	41.7	5.9	10.7	2.4	1.6	25.9	2.7	0.3	2.5
Hybrid poplar	39.23	13.07	1.81	0.88	0.89	25.18	6.89	2.03	4.31
Eucalyptus	48.07	10.42	1.23	0.74	0.3	26.91	4.15	1.22	4.07
Switchgrass (alamo)	30.97	20.42	0.29	0.92	2.75	17.56	16.99	5.76	1.17
Switchgrass (cave in rock)	33.08	20.93	0.27	1.04	3.01	17.54	13.8	6.42	No data
Sweet sorghum	34.01	14.14	0.2	0.52	1.65	16.09	22.03	5.04	1.07

Thus, 180 mass units of six carbon sugars can form 92 mass units of ethanol, resulting in a maximum theoretical mass yield of $92/180 \times 100 = 51.1\%$, the same as possible from five carbon sugars.

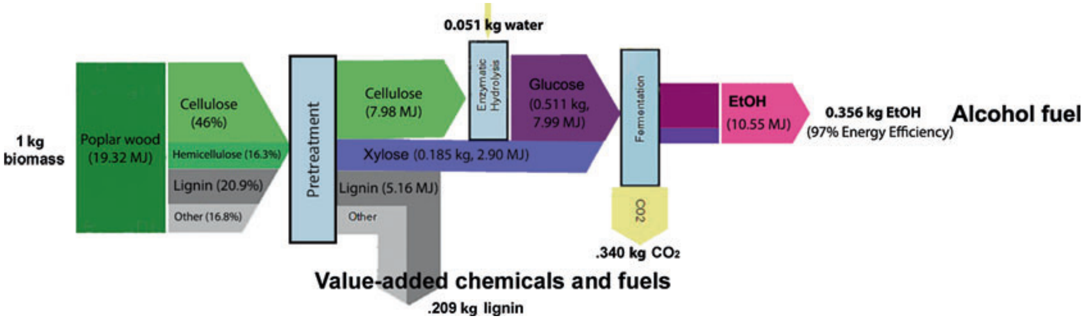
Historically, yeast has been the dominant microorganism for glucose fermentations to ethanol according to reaction (5) by an anaerobic reaction in the absence of oxygen. In this case, a relatively small fraction of the glucose or other six carbon sugars is used to support yeast growth and maintenance, with the rest released as ethanol and carbon dioxide [76, 77]. The result is that yields are typically about 85–92% of the stoichiometric limit, with about 43–47 mass units of ethanol that can be produced for every 100 mass units of six carbon sugars.

Natural organisms lack the ability to ferment five carbon sugars efficiently, limiting the economic viability of utilizing these sugars for ethanol production. However, since the late 1980s, a number of bacterium and yeast have been genetically modified so they can ferment various five carbon sugars to ethanol according to reaction (4) [78–82]. Because these microorganisms are also anaerobic and use only a limited amount of these sugars for growth and maintenance, they achieve a similar practical ethanol mass yield range of 85–92% of the theoretical maximum.

A concern often expressed about fermentation of sugars to ethanol is the loss of mass as carbon dioxide. In particular, combining Eqs. 2 and 3 with Eqs. 4 and 5 and the use of some of the sugars noted for organism growth and maintenance results in less than half the mass of polysaccharides in cellulosic biomass being captured as ethanol. For chemicals that are sold based on mass of product, these losses are important as they will approximately double the cost of the sugars used to produce that product. However, it is critical to realize that the value of fuels is based on their energy content and not their mass. In addition, for fuels production, an important objective is to densify the energy content as much as possible, that is to say, to increase the energy density of the fuel. In this regard, the energetics of Eqs. 2–5 are very favorable in that about 100% of the heat of combustion of cellulose and hemicellulose is retained in the sugars formed by hydrolysis reactions (2) and (3). Furthermore, because carbon dioxide has no heating value and reactions (4) and (5) are only slightly exothermic, about 97% of the heat of combustion in the sugars formed by these reactions is preserved in the ethanol produced by fermentation. The overall result is that about 97% of the energy content in cellulose and hemicellulose sugars is contained in a final ethanol product that has a mass of about half that of cellulose and hemicellulose, thereby approximately doubling the mass energy density, a highly desirable outcome for making fuels [54].

Figure 1 illustrates the difference between mass and energy flows by outlining the fate of hemicellulose, cellulose, and lignin in hybrid poplar wood (*Populus* sp.) energy crop (composed of 46% cellulose, 16.3% hemicellulose, 21% lignin, and 16.8% other) as it is fractionated and its sugars converted to fuel ethanol at theoretical yields. The boxes represent individual unit operations of pretreatment, enzymatic hydrolysis, and fermentation, and the stream thickness is proportional to relative mass contributions for each component. The total higher heating value (HHV, MJ) is also shown in parenthesis for each of the sugars and lignin components starting from 19.32 MJ total in 1 kg of poplar wood. In this pathway, the pretreatment reaction (Stage 1) first applied to the biomass hydrolyzes hemicellulose sugars to sugar monomers (represented simply by xylose, a primary monosaccharide from hemicellulose) followed by an enzymatic hydrolysis step (Stage 2) to produce fermentable glucose from the solid cellulose fraction remaining from Stage 1. The glucose and xylose sugars (10.88 MJ together) are then fermented, either individually or combined, by microbial yeast to produce 0.356 kg of ethanol with a net higher heating value (HHV) of 10.55 MJ. Thus, although the bar widths show loss of mass during fermentation, almost all of the energy content (about 97%) of the sugars is captured in fuel ethanol, thereby increasing the fuel energy density.

Depending on the feedstock, lignin makes up from about 7–35% of its mass but has a higher energy density (24.68 MJ/kg) than sugars. For instance, a lignin energy content of about



Bioethanol from Lignocellulosic Biomass, Fig. 1 A mass and energy flow Sankey diagram illustrating biological conversion of poplar wood to fuel ethanol at the theoretical yields (thickness of the individual component streams is proportional to their relative mass with respect to 1 kg of initial biomass (poplar wood)); energy of combustion (in MJ, HHV), mass flow, and net energy efficiency of ethanol produced from cellulose and hemicellulose are shown (Adapted from Cai [84])

5.16 MJ would correspond to the 20.9% lignin content for the poplar example in Fig. 1 but would be considerably lower for grasses and agricultural residues. In any event, due to challenges in deriving value from lignin, it is conventionally pictured as being burned to provide heat and power for a biorefinery, thus capturing limited value from lignin. It would be advantageous to employ lignin in higher-value products such as liquid transportation fuels, bioplastics, and chemical solvents [11, 44, 83].

Enzyme Biochemistry

Biomass polysaccharide efficient deconstruction requires synergistic action of several catalytic and non-catalytic proteins, such as cellulases, hemicellulases, accessory enzymes, and oxidative enzymes, and non-catalytic proteins such as swollenin and expansins [75, 85–87]. The most frequently studied and industrial relevant cellulase from the filamentous fungi *Trichoderma reesei* is comprised of enzymes including two cellobiohydrolases (CBH I and CBH II), at least five endoglucanases (EG I–EG V), and at least two β-glucosidases [88–91]. Most of these components contain two domains – a catalytic domain and a cellulose-binding domain (CBD) – that are connected by o-glycosylated peptide linkers [86, 92]. In a heterogeneous reaction, cellulase components cellobiohydrolase I and cellobiohydrolase II processively deconstruct an insoluble cellulose chain from reducing and nonreducing ends, respectively, to produce mostly cellobiose and small amounts of glucose and cellotriose [93, 94]. The reaction end products cellobiose and glucose in turn inhibit cellulase catalytic activity [95–98] as well as adsorption [99]. However, β-glucosidases hydrolyze cellobiose and higher soluble cellooligomers via a homogenous reaction into glucose, thereby reducing inhibition of cellobiohydrolases and endoglucanases [97, 98, 100].

Although the distinctive and definitive role of these individual cellulase components is still unclear, as per the classical model, endoglucanases are believed to attack cellulose disordered or amorphous parts creating chains ends for cellobiohydrolases to feed on [93, 94]. However, as per the new model, in addition to endoglucanases attacking disordered region of cellulose, the oxidative enzymes called lytic polysaccharides monooxygenes (LMPOs) are believed to disrupt the crystalline region of cellulose creating the new attacking sites for cellobiohydrolases [101]. The synergism between cellobiohydrolases and endoglucanases and cellulases and lytic polysaccharides monooxygenes (LMPOs), however, has been shown to be dependent on cellulose and biomass type and structure [102–104].

Hemicellulose in most agricultural residues, hardwoods, and energy crops such as switchgrass is mainly composed of xylan with small amounts of substituents and other sugars, whereas softwood hemicellulose is comprised of both (galacto) glucomannan and xylan with mannan-based hemicellulose being a major constituent [4, 8, 9, 105]. Therefore, hemicellulose hydrolysis requires concerted action of various main and accessory enzymes. For example, hydrolysis of hemicellulose xylan requires endoxylanse, β-xylosidase, and accessory enzymes such as acetyl xylan esterase to remove acetyl substituent from the xylan backbone, α-arabinofuransoidases to remove arabinose [106]. Hydrolysis of hemicellulose (galacto) glucomannan requires synergistic action of β-mannanase, β-mannosidase and β-glucosidase, and α-galactosidase to hydrolyze mannan backbone to oligomers and oligomers to monomers and to remove galactose side chain, respectively [8, 105, 107].

Process Options

Due to the millennial-long history of brewing ethanol from sugars for production of alcoholic beverages, ethanol fermentations are so well developed as to not present a cost barrier. In addition, dilute acids can easily deconstruct hemicellulose to form its component sugars via reaction (2). However, because breaking down cellulose to release imbedded glucose is controlled by the natural resistance of cellulosic materials to deconstruction, cellulose hydrolysis by reaction (3) presents the primary barrier to taking advantage of the low cost of cellulosic biomass. Thus, biological conversion of cellulosic biomass to ethanol revolves around the three routes to catalyze cellulose hydrolysis to form fermentable glucose by (1) concentrated acids, (2) dilute acids, and (3) enzymes, followed by fermentation of the sugars released to ethanol in the next step. In addition, thermochemical conversion approaches can be employed to produce ethanol. The basic features of these four pathways will be summarized in the following, while the next section will provide more details on the enzymatic route in light of its leading position for large-scale implementation.

Enzymatic Routes for Biomass Deconstruction

Enzymes can break the covalent bonds linking sugars in cellulose and hemicellulose and have the advantage of being highly selective, thereby avoiding degradation of the sugars released from either polysaccharide by hydrolysis. However, coupling the heterogeneity of hemicellulose bonds with the selectivity of enzymes challenges realizing high yields of sugars by enzymatic hydrolysis of hemicellulose. In addition, the recalcitrance of biomass to deconstruction to sugars with high yields by enzymes alone makes it vital to employ pretreatment before or during enzymatic hydrolysis [20, 108, 109]. As a result, almost all processes that employ enzymatic hydrolysis pretreat biomass first to reduce its resistance to break down and make the structure of the solids from pretreatment amenable to deconstruction to sugars with high yields by enzymes.

Three basic process configurations have been applied to enzymatically hydrolyze the solids produced by pretreatment. The first applies three operations in a rather obvious sequence of (1) producing cellulase enzymes, (2) adding the enzymes to a second vessel for release of sugars from the polysaccharides in the pretreated solids, and (3) fermentation of the sugars released to ethanol. This combination is often referred to as separate hydrolysis and fermentation or SHF [110]. Conventional cellulase enzymes have to be produced separately as organisms such as *Trichoderma reesei* require aerobic conditions (i.e., aeration to grow the organism), while sugar fermentations are anaerobic (i.e., grown in the absence of oxygen). These enzymes can then be added to pretreated solids to release glucose and other residual sugars at temperatures of about 50–55 °C and pH values of about 4–5 that are most favorable for rapid release of sugars with high yields. Once enzymatic hydrolysis is complete, yeast such as *Saccharomyces cerevisiae* or other fermentative organisms such as the bacterium *Zymomonas mobilis* are added to the sugar solution after the temperature has been lowered to a value that is optimal for fermentation performance, typically about 32 °C. The primary challenge to this separate hydrolysis and fermentation (SHF) configuration is that the sugars released during enzymatic hydrolysis slow and eventually stop enzyme action and thereby require larger vessels for enzymatic hydrolysis and fermentation and produce dilute ethanol streams that require more energy for product recovery. Furthermore, because cellobiose and other long chained oligomers of sugars such as xylose are much more inhibitory than glucose or ethanol, it is important that high levels of β-glucosidase activity be in the enzyme mixture to keep cellobiose concentrations low [111–113]. It can also be highly desirable to include hemicellulase activities in the enzyme mixture if the pretreatment applied prior to separate hydrolysis and fermentation (SHF) leaves large amounts of hemicellulose in the solids fed to SHF [87, 114–116]. Such activities can also be valuable to minimize accumulation of soluble hemicellulose oligomers that have been shown to be even more inhibitory than cellobiose [116, 117].

To reduce inhibition of enzymes by the sugar they release, Takagi et al. combined enzymatic hydrolysis with sugar fermentation in a single vessel in an approach they labeled simultaneous saccharification and fermentation or SSF [118–120]. In SSF, the yeast so quickly consumes the glucose released by cellulase enzymes that sugar concentrations remain low enough to not substantially affect enzyme action [121]. In addition, ethanol produced by the yeast has a much lower impact on enzymatic hydrolysis than glucose and cellobiose [96, 120, 122]. Other advantages for simultaneous saccharification and fermentation (SSF) include the low concentrations of sugars and presence of ethanol reduce the potential for invasion by *Lactobacillus* or other unwanted organisms. As for separate hydrolysis and fermentation (SHF), it is important that high levels of β-glucosidase activity be in the enzymes used for SSF to keep cellobiose concentrations low. In this line, yeast that directly ferment cellobiose to ethanol can be very effective in enhancing hydrolysis rates and reduce or eliminate the need for β-glucosidase supplementation [123]. However, the simultaneous saccharification and fermentation (SSF) temperature must be kept below 50–55 °C that is generally optimal for enzymes to accommodate the lower temperature tolerance of yeast of about 32 °C. The overall result is to operate simultaneous saccharification and fermentation (SSF) at about 37 °C to achieve reasonable hydrolysis rates while maintaining yeast viability [110]. The combination of the low sugar concentrations, ethanol buildup, and non-optimal temperature operation has made it challenging to achieve ethanol concentrations above about 5% by weight, even when organisms are employed that would be thermotolerant for fermenting pure sugar [76, 124].

An approach termed consolidated bioprocessing or CBP is a third option for biological conversion [110, 125]. In this case, an organism such as the thermophile *Clostridium thermocellum* that can produce cellulolytic enzymes and ferment the sugars they release is employed. Consolidated bioprocessing (CBP) thereby eliminates the need for the separate very costly enzyme production step required for both separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). In addition, CBP enjoys the SSF advantages from combining biomass saccharification and fermentation in a single vessel. Evidence is mounting that the unique cellulosome enzyme complex employed by *C. thermocellum* for CBP is more effective at deconstructing biomass to sugars than levels of enzymes required for SHF or SSF yields to be economically viable [126]. The major challenge for consolidated bioprocessing (CBP) is to develop an organism that produces effective cellulolytic enzymes and converts the sugars they release to ethanol with high yields [20, 127]. In addition, *C. thermocellum* is very sensitive to oxygen and presents challenges that could impede commercial use.

Pretreatment Prior to Enzymatic Hydrolysis

Most types of cellulosic feedstocks must be subjected to some type of treatment either before or during enzymatic hydrolysis in order to realize commercially relevant ethanol concentrations [108]. Although many pretreatment technologies have been investigated for this service over the years, treatment of biomass at moderate temperatures generally with chemicals added emerged as most suitable to achieve high yields. In 2010, a team of pretreatment experts called the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) completed comparisons of total glucose plus xylose yields from pretreatment coupled with subsequent enzymatic hydrolysis of corn stover, poplar wood, and switchgrass by pretreating these three feedstocks with (1) ammonia fiber expansion (AFEX), (2) dilute sulfuric acid, (3) hot water, (4) lime, (5) soaking in aqueous ammonia (SAA), and (5) sulfur dioxide. Comparative results showed that thermochemical pretreatments with just hot water and at high and low pH over a wide range of temperatures and times were effective in achieving high yields of xylose and glucose from cellulosic biomass in the combined operations of pretreatment and enzymatic hydrolysis [39, 128–130]. However, only sulfur dioxide and lime with much different pH and temperature optima achieved similar high yields from all three feedstocks: corn stover, switchgrass, and poplar. In addition, differences in sugar yields from different sources of poplar and switchgrass have been shown. Nonetheless, further optimization of enzyme formulations could erase some of these differences. Techno-economic evaluations conducted in concert with this project projected similar capital costs for all the pretreatments and relatively small contributions of each pretreatment to overall capital costs for a cellulosic ethanol process due to the high costs of the rest of the plant [131]. Thus, the primary driver to the minimizing of the ethanol selling price required to cover all operating and annualized capital costs for an ethanol production plant was glucose plus xylose yields from the combined operations of pretreatment and enzymatic hydrolysis. For pretreatments that achieve similarly high yields, other contributors including chemical costs, sugar concentration achieved, and pretreatment materials of construction and residence time become important differentiators [129, 130, 132]. Because pretreatment impacts both upstream and downstream operations, other factors such as xylose and glucose release in pretreatment vs. enzymatic hydrolysis, feedstock to be used, enzyme formulation requirements, fermentative organism sugar preferences, and formation of inhibitors by pretreatment must be taken into consideration in the selection of a pretreatment technology. The take-home message is that one pretreatment is unlikely to be the best fit for all situations.

Solvents have also been employed for pretreatment to separate most of the cellulose, hemicellulose, and lignin components in relatively pure streams with the goals of improving the susceptibility of cellulose to enzymes and producing a relatively pure lignin stream that could lend itself to conversion into valuable products [133–140]. Mixing methanol with water and dilute sulfuric acid in a so-called organosolv pretreatment was employed for a number of years followed by substitution of ethanol for methanol. Another option that has been revitalized is pretreatment with ionic liquids to fractionate biomass at mild reaction conditions [23, 141, 142]. The use of gamma-valerolactone (GVL) was introduced more recently to remove lignin from biomass and promote its deconstruction [143]. Most recently, adding tetrahydrofuran (THF) in solution with water significantly enhanced dilute acid deconstruction of biomass at lower reaction temperatures by simultaneously promoting high sugar recovery from hemicellulose into the liquid, producing highly digestible glucan-rich solids, and extracting lignin by precipitation from solution after removing low boiling tetrahydrofuran (THF) [144]. For solvent pretreatments to be commercially successful, most of the solvents must be recovered and reused; however, expensive solvents such as ionic liquids and gamma-valerolactone (GVL) are difficult to remove from water at low cost due to their high boiling points. Ethanol and THF are particularly attractive in this regard in that their high volatilities facilitate recovery, and both can be produced from biomass. Ethanol not recovered and recycled after pretreatment can be recovered during final ethanol product purification. However, removing lignin from an ethanol organosolv stream typically involves dilution with water that hurts downstream processing and economics. Compared to other solvents, tetrahydrofuran (THF) offers several important attributes including high volatility that facilitates recycle, the ability to be made from furfural that can be a pretreatment byproduct, and lignin recovery by dilution, evaporation, or extraction [121, 145, 146].

Concentrated Acids for Biomass Deconstruction

In the presence of concentrated acids, e.g., 72% sulfuric acid, the hydrogen bonds responsible for cellulose crystallinity become overwhelmed by the ion concentration of the acid, with the result that crystals disaggregate to form amorphous cellulose that is readily broken down by dilute acid to form glucose [147–150]. Furthermore, this reaction can be carried out at ambient temperatures and pressures, thereby minimizing energy requirements for biomass hydrolysis. Unfortunately, although the technology appears conceptually attractive, several considerations complicate commercial competitiveness. First, biomass must be dried prior to adding concentrated sulfuric to avoid violent release of heat when the water in the biomass and concentrated acid meet; drying biomass requires considerable energy inputs due to the high heat of vaporization of water. In addition, the process requires addition of such large amounts of acid that a large portion of the acid must be recovered and recycled to achieve acceptable acid costs, but acid recovery requires such very expensive operations as evaporation and ion exchange [151–153]. Despite many attempts to commercialize processes for release of glucose from cellulosic biomass by concentrated acids, these and other challenges have stood in the way of commercial implementation.

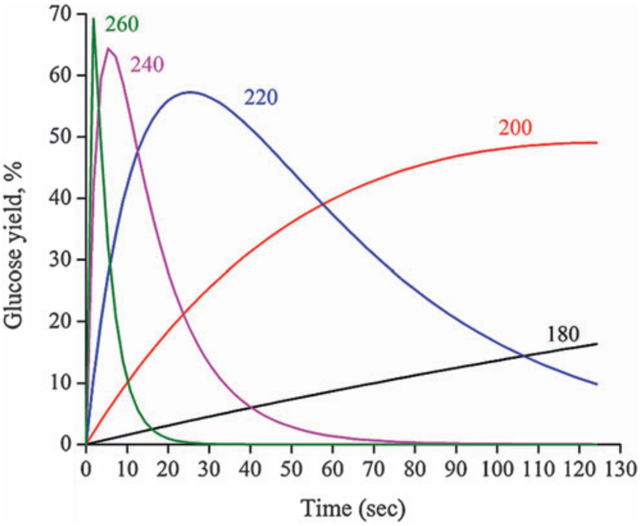
Dilute Acids for Biomass Cellulose Deconstruction

After removing most of the hemicellulose from lignocellulosic biomass via dilute acid pretreatment such as described above, heating the remaining solids that are highly enriched in cellulose to about 220–260 °C in about 2.0–4.0% aqueous sulfuric acid solutions will rapidly break down cellulose to release glucose [152, 154–158]. However, these conditions also degrade glucose to form 5-hydroxymethylfurfural (HMF) that in turn hydrolyzes to form levulinic acid and formic acid [159, 160]. Thus, to achieve high glucose yields, reaction conditions must be applied that promote cellulose hydrolysis to glucose compared to glucose breakdown. Because the activation energy for cellulose hydrolysis is greater than that for glucose breakdown [157], glucose yields increase with increasing temperatures (Fig. 2). Increasing acid concentrations also increase yields. However, high temperatures also shorten reaction times, with the result that to achieve yields of about 69% of the theoretical maximum possible at 260 °C, the reaction time drops to only about 2 s. Controlling reaction times so precisely is very difficult to implement commercially. In addition, high temperatures promote pyrolysis reactions that form insoluble tars that attach to reactor walls and other equipment [161]. The result is the reaction time continually drops as the reactor diameter for fluid passage shrinks, and flow soon stops when the reactor is fully plugged by tars [155, 157]. Temperatures must be kept below about 190 °C to avoid tar formation by pyrolysis; however, as evident in Fig. 2, the yields drop significantly at these lower temperatures. Water pressures also become extremely high at these temperatures, reaching nearly 4,700 kPa at 260 °C, as an example. Pressurizing biomass to these levels is very difficult and energy consuming, particularly at high solid loadings needed to achieve adequate sugar and subsequent ethanol concentrations that are important to keep energy costs for ethanol recovery manageable. Another challenge is that because hemicellulose hydrolyzes to the five carbon sugars arabinose and xylose much more rapidly than cellulose breaks down to glucose, reaction (2) must be carried out first and the hemicellulose sugars removed prior to subjecting the resulting cellulose rich solids to dilute acid hydrolysis to form glucose. Applying this sequence introduces more equipment and operating costs, further hurting process competiveness.

Supercritical Deconstruction in Water

High energy water molecules at either subcritical or supercritical conditions can cause rapid deconstruction of cellulose and other biomass components [162–165]. By Le Châtelier's principle, the pH of water drops as hydrogen ion concentrations

Bioethanol from Lignocellulosic Biomass, Fig. 2 Effect of various temperatures and times on hardwood flour cellulose hydrolysis in 2 wt. % sulfuric acid (Adapted from Brennan et al. [157])



increase with increasing temperature. At supercritical conditions, heat transfer properties of the fluid are also largely enhanced. These conditions are favorable for breaking down organic matter, and application to biomass was originally adapted from supercritical water oxidation (SCWO) and supercritical water gasification (SCWG) methods for treating mixed wastes and from gasification to synthesis gas or natural gas [166, 167]. Similarly, cellulosic biomass deconstruction is accelerated by supercritical water as cellulose hydrolysis is more rapid than at subcritical conditions. Temperatures above 700 K also cause pyrolysis-type reactions and may create bio-oil from cellulose suitable for gasification. Although hydrolysis reactions are accelerated relative to decomposition, supercritical water processing of biomass creates solid carbonaceous residues such as tars that limit the conversion efficiency and challenge product recovery.

Subcritical “hot water” methods have been applied in the form of steam or high temperature water to facilitate in the breakdown of biomass. Aided by reduced pH, the hot water environment is mostly effective for hydrolyzing amorphous hemicellulose sugars from biomass and opening up the cellulose structure from the lignin matrix to improve its accessibility to cellulolytic enzymes [82]. These methods are ultra clean as they do not involve addition of flammable solvents, corrosive acids, or other hazardous materials. However, the high energy demand of heating water to such high temperatures must be considered.

Thermal Conversion of Biomass to Ethanol

Thermal routes can also be employed to convert cellulosic biomass into fuels. For ethanol production, biomass can be gasified with high temperature steam to produce hydrogen and carbon monoxide [168–171], the mixture being known as syngas, as illustrated by the simplified stoichiometry according to Eq. 6:

$$n\ C_6H_{10}O_5 + n\ H_2O ----> 6n\ CO + 6n\ H_2 \tag{6}$$

Here n cellulose units $C_6H_{10}O_5$ react with steam to produce 6n carbon monoxide molecules and 6n hydrogen molecules. The syngas can then be reacted to form methanol via the following Eq. 7:

$$CO + 2H_2 ----> CH_3OH \tag{7}$$

The methanol can in turn be reacted over a catalyst to form ethanol as shown in Eq. 8:

$$2\ CH_3OH ----> C_2H_5OH + H_2O \tag{8}$$

However, because reaction (6) does not produce the same ratio of hydrogen to carbon monoxide as desired for reaction (7), the carbon monoxide can be reacted with steam to adjust the relative proportions of each via the following water gas shift reaction (Eq. 9):

$$CO + H_2O - - - - > CO_2 + H_2 \quad (9)$$

Alternatively, biological systems can be employed to ferment syngas to ethanol directly by coupling the two reactions (10) and (11) [172, 173]:

$$6\ \ CO + 3\ H_2O - - - - > C_2H_5OH + 4\ CO_2 \quad (10)$$

$$6\ H_2 + 2\ CO_2 - - - - > C_2H_5OH + 3\ H_2O \quad (11)$$

Biological fermentation of syngas offers such advantages as avoiding the need for a water gas shift reaction and operation at near ambient temperatures and pressures.

Although gasification followed by catalytic or biological conversion to ethanol appears straightforward, it faces several challenges. For one, syngas contains many other products formed by breakdown of biomass including tars and mineral compounds that foul equipment and must be removed at some expense [174]. The high capital cost of gasification coupled with power law scaling of equipment costs with process throughput favors large-scale operations to make unit capital costs more attractive. Unfortunately, biomass accessibility is often not compatible with such large facilities [175]. The subsequent catalytic conversion of syngas to methanol is equilibrium controlled, with higher yields favored by lower temperatures and higher pressures and by the need for recycle of unreacted gases at additional costs [176, 177]. The series of reactions required to produce ethanol also results in fuel energy content gains and compounds yield losses. Biological routes face such challenges as providing good mass transfer from syngas that has a low solubility in water to organisms and slow fermentation rates. Approaches that have been employed to overcome these limitations include use of low-priced feedstocks such as waste carbon monoxide and hydrogen from steel manufacture and gasification of municipal waste biomass to compensate for higher capital costs for smaller-scale operations.

Biological Conversion of Sugars to Ethanol

Enzymatic hydrolysis coupled with fermentation has been the platform favored for conversion of cellulosic biomass to ethanol because high yields are possible and little if any degradation results. Such biological conversion approaches for converting cellulosic biomass to ethanol typically involve the following operations:

1. Feedstock storage and feeding to provide cellulosic biomass to the conversion operations
2. Pretreatment to open up biomass structure so high sugar yields can be realized in subsequent enzymatic hydrolysis
3. Production of enzymes that can deconstruct the polysaccharides left in solids from pretreatment
4. Enzymatic hydrolysis of the solids from pretreatment
5. Fermentation to convert the sugars released by pretreatment and enzymatic hydrolysis to ethanol
6. Product recovery and purification to produce ethanol of suitable purity for use as a fuel
7. Disposal of lignin and other biomass components left in the solids after sugar release
8. Wastewater treatment to remove soluble components left in the water so it can be recycled or released to the environment
9. Product storage for sale [13, 58, 178]

Details can be found from various sources on the many possible choices and configurations for these operations and associated costs. The following briefly summarizes a process design developed to estimate ethanol production costs [179] (for more details see [179]). However, it is important to view this particular design as a useful reference point but to understand that other process configurations and unit operation choices could have economic advantages. Over the years, other process designs have been published that can provide a valuable reference point as well [180].

Pretreatment

Two thousand t_{DM} corn stover with a composition of 35% glucan, 20% xylan, and 16% lignin was used as the feedstock for the technoeconomic evaluation [180]. The stover was subjected to deacetylation with 4.0 wt% sodium hydroxide at 80 °C for 2 h followed by reacting biomass with dilute sulfuric acid for pretreatment. This combination was introduced to improve hemicellulose and enzymatic conversion yields and increase fermentation yields by reducing inhibitor concentrations. This strategy also reduced acid loadings required for pretreatment to be effective and reduced downstream ammonia neutralization and conditioning costs.

Enzyme Production

The cellulase enzymes described above that are needed to break down the cellulose in pretreated biomass are generally produced commercially using a filamentous fungus such as *Trichoderma reesei* that produces cellulase when grown aerobically on cellulose or other enzyme inducers. These enzymes can be purchased or made at the ethanol production site, with the latter likely to be more economic for large-scale operations due to a number of factors. Several economic studies were based on the first option of purchasing enzymes. However, other designs estimated the cost of onsite production by a submerged culture of the *T. reesei* fed by glucose, some of which was converted to sophorose, a powerful inducer that can realize much more vigorous enzyme production than possible with just glucose [179, 181]. Enzymes produced by growing this fungus on the glucose/sophorose mixture in an aerated vessel that is vigorously agitated could then be directly fed to the next process step: enzymatic hydrolysis.

Enzymatic Hydrolysis

The reduction in acetic acid and other inhibitors by deacetylation followed by dilute acid pretreatment allowed direct feeding of the whole slurry from pretreatment into subsequent enzymatic hydrolysis after conditioning with ammonia. An enzyme loading of 19 mg protein/g glucan proved effective in converting a 20% total solids loading in the saccharification tank in 84 h (3.5 day) at 48 °C. About 78% of the glucan was converted to glucose by this approach.

Sugar Fermentation

After enzymatic hydrolysis, the hydrolyzed material was cooled prior to fermentation at 33 °C and a pH of 5.8 using an engineered fermentative bacterium for a reaction time of 36 h (1.5 day) using 0.25% corn steep liquor (CSL) as the only nutrient. The combined saccharification and fermentation time was thus 5 days and produced 7.4% ethanol.

Ethanol Recovery

Ethanol was recovered by distillation coupled with dehydration with molecular sieves. Fuel grade ethanol is the purified overhead product, while lignin and other low-volatility products are recovered in the bottoms along with water containing dissolved low-volatility materials.

Wastewater Treatment

Deacetylation prior to dilute acid pretreatment dramatically reduced acid use and the amount of ammonia needed for subsequent neutralization and conditioning prior to hydrolysis and fermentation. It also lowered downstream costs for water removal from the solids by an evaporator system, waste removal and concentration by a membrane reactor and reverse osmosis system, and removal of dissolved wastes by anaerobic and aerobic digestion, and the other equipment scaled on total chemical oxygen demand (COD) loading to the wastewater treatment (WWT) system.

Lignin Utilization

Lignin and other low-volatility components left in the solids after water removal are assumed to be burned to provide all the heat and power for the process with energy beyond that needed to run the process sold as power to the grid.

Costs and Benefits of Cellulosic Ethanol Production

Process Economics

Capital cost of about 210 million US$ for a base case design to produce 207 million liters of ethanol per year from 2,000 t_{DM}/d of corn stover costing 58.5 US$/$t_{DM}$ with a yield of 268 l of ethanol/dry metric ton has been calculated. Just over half of this capital cost was for burning lignin and wastewater treatment. Pretreatment was responsible for about 14% of this cost, enzyme production coupled with enzymatic hydrolysis and fermentation accounted for about 20% of the total, and distillation and solid recovery contributed a little under 20% to the total. Coupling the cost for amortizing the equipment with costs for feedstock, chemicals, labor, and other operating expenses resulted in an overall minimum ethanol selling price of 0.568 US$/liter in year 2007 dollars to cover all of these items.

Although the cost estimate is based largely on experimental data, it is important to recognize that this estimate is for a nth plant using mature technologies that benefited from a long learning curve from prior commercial experience. However, costs are much higher for the first plants now beginning operations for which capital costs are about 2.6–4.0 US$/liter of ethanol produced vs. the much lower capital cost estimate of 1.01 US$/annual liter [58]. In addition, the lower ethanol production capacities of between 38 and 114 million liters/year for these first-of-a-kind projects will incur somewhat larger annualized capital costs due to not taking advantage of the economies of scale that the presented design would enjoy with production rates that are 1.8–5.5 times larger. However, correcting for differences in scale of operation can only lower the annualized capital cost to about 1.30 US$/liter in the best case. Thus, conservative designs to assure operability of first plants coupled with differences in technology and other factors make entry into cellulosic ethanol production very expensive.

Greenhouse Gas Emissions

The total net greenhouse gas (GHG) emissions associated with the conversion process outlined above were negative at -0.32 kg CO_2 equivalent/liter of ethanol produced. This offset/negative result is mostly attributable to displacement of electricity in the grid by that exported from lignin burning beyond that needed to run the process, -0.54 kg CO_2 eq/liter. Deacetylation removal of acetic acid also helps lower GHG emissions by reducing pretreatment severity, acid loadings, and subsequent ammonia demand for neutralization and less chemicals needed for wastewater treatment (WWT). The majority of fossil energy demand and associated GHG emissions are associated with enzyme production and the use of chemicals.

Future Directions

Cellulosic biomass provides a unique low-cost resource for sustainable production of liquid fuels on a scale sufficient to have a substantial impact on petroleum use. As a result, such biofuels can address important strategic, economic, and environmental issues that result from virtually total dependence on petroleum for transportation. Ethanol has many favorable fuel properties and is one of the biofuels that can be made from cellulosic biomass. Furthermore, cellulosic ethanol can build from the unique, substantial infrastructure and technology platforms established by current large-scale use of ethanol made from corn starch in the USA and cane sugar in Brazil, an implementation platform not available to most other biofuels. In particular, polysaccharides that make up about two-thirds to three quarters of biomass as cellulose and hemicellulose can be hydrolyzed to sugars for fermentation to ethanol, while the remaining fraction of mostly lignin can be burned to provide all the heat and power needed to run the process plus export extra power to the grid for additional income. Alternatively, thermal routes can be applied to generate synthesis gas that can be catalytically or biologically converted to ethanol.

The challenge to both biological and thermal processes is the difficulty in deconstructing the complex structure of cellulosic biomass to generate sugars or syngas that can then be converted into ethanol, that is, to overcome the recalcitrance of cellulosic biomass to form reactive intermediates [13]. Biological routes that couple biomass pretreatment to reduce biomass recalcitrance with enzymatic hydrolysis to release sugars from the pretreated solids with high yields and subsequent fermentation of the sugars released to ethanol tend to be currently favored [13]. However, substantial costs are incurred for biomass pretreatment, enzyme production, and hydrolysis that still present barriers to low costs. In addition, although burning lignin and other residues left in the solids following ethanol production results in very favorable reductions in greenhouse gas emissions, the cost for producing heat and power from these residues along with wastewater treatment contribute about half the total capital costs of the entire process and present a formidable economic obstacle [180].

Low petroleum prices and price instabilities coupled with the cost of overcoming biomass recalcitrance challenge large-scale commercial implementation. Application of sensitivity studies to technoeconomic models of biological conversion of cellulosic biomass to ethanol has shown that it is particularly vital to develop more effective enzyme preparations that can achieve high ethanol yields with significantly lower enzyme loading requirements [182]. Lower pretreatment capital costs would also improve process economics. It is also expected that learning by doing, i.e., the learning curve effect, will reduce costs as more cellulosic ethanol plants are built and operated. If such technical advances can be coupled with learning curve experience, cellulosic ethanol costs could become competitive with prices for petroleum-based liquid transportation fuels.

Although significant advances in enzyme activity and pretreatment could lower costs for biological conversion of cellulosic biomass to be competitive, many years of enzyme research, development, and production have not yet succeeded in lowering biological deconstruction costs to be competitive when petroleum prices are low [58]. Thus, it is important to consider paradigm shifts that could dramatically alter the costs of biological ethanol production. A promising option now being stressed to circumvent high enzyme production and biomass hydrolysis costs is the consolidated bioprocessing (CBP) strategy outlined above in which enzyme production, polysaccharide hydrolysis to sugars, and sugar fermentation to ethanol are all carried out in a single vessel. The leading organism now being applied for CBP is *Clostridium thermocellum*, a bacterium that grows anaerobically at about 60 °C and produces a cellulosome enzyme complex that has proven to be more effective in deconstructing biomass than conventional fungal enzymes [126]. Recent research has also shown that *C. thermocellum* can realize high breakdown of polysaccharides to sugars in combination with simultaneous or intermittent mechanical milling in an approach called cotreatment and thereby avoid the need for pretreatment before this coupled biological-physical operation [183]. However, several features of this organism must be improved through genetic engineering and adaptation including shifting from production of a diverse array of metabolic products to almost entirely ethanol, improving its ethanol tolerance, and being able to work well at high solids loadings needed to achieve commercially viable ethanol concentrations. It also remains to be demonstrated that cotreatment can be applied effectively with low enough energy requirements to be economically attractive.

Another important paradigm shift is to move from focusing on just using the polysaccharide fraction for fuels production. Lignin, nature's dominant aromatic polymer, is found in most terrestrial plants in the approximate range of 7–35% dry weight and provides structural integrity [11]. Most large-scale industrial processes that use plant polysaccharides have burned lignin to generate power. However, because lignin is wet and has lower energy content than coal, its value in this role is limited to about 20–30 US\$/t_{DM}, and higher-value uses would enhance overall biorefinery competitiveness [11, 44]. Furthermore, the advent of biorefineries that convert cellulosic biomass into liquid transportation fuels will generate substantially more lignin than necessary to power the operation, and efforts are needed to transform it into higher-value products. Solvent-based biomass pretreatment technologies such as that using tetrahydrofuran (cosolvent enhanced lignocellulosic fractionation or CELF) facilitate lignin recovery and enable catalytic modifications for desired chemical and physical properties. The recent novel integration of consolidated bioprocessing (CBP) with cotreatment promises to provide native or near-native lignin that can lend itself to more complete valorization [183]. Novel biological approaches show promise to convert soluble lignin fractions into valuable products as well [184–186]. And a significant fraction of insoluble lignin could be valuable for production of copolymers with enhanced properties [83].

Major detriments to cellulosic ethanol economics are the costs for lignin recovery and combustion for heat and power and for wastewater treatment as the two represent just over half of the total capital costs [179, 180]. Thus, it is vital to search for options to shift the paradigm from the conventional route outlined above to a far more streamlined process. Use of consolidated bioprocessing (CBP) with cotreatment would simplify the primary steps for ethanol production and promises to provide native lignin that could be converted to much more valuable products than can be realized by just burning lignin [183]. Residuals not amenable to conversion into more valuable products could be sold as high energy biomass pellets for use in existing coal power plants or home pellet stoves, thereby avoiding the high capital cost of burning lignin at the ethanol production site. Simplifying the overall system through this strategy would also make the overall process less dependent on economies of scale and lend cellulosic ethanol production to distributed implementation near biomass supplies and avoid the costs and challenges of transporting large amounts of biomass to central processing facilities.

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