Chapter

Lignocellulosic Ethanol: Technology and Economics

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Abstract

The accelerated global warming calls for fast development of solutions to curb excessive Greenhouse gas emission. Like most of other forms of renewable energy, lignocellulosic ethanol can help the human beings mitigate the climate deterioration and gain independence from fossil fuels. This chapter gives a survey of bioethanol production in the U.S. and world, describes classifications of three generations of bioethanol, provides an overview of all the stages of currently adopted process for the second-generation bioethanol production, briefs on new development on enzymes for hydrolysis and fermentation and new processes for ethanol generation, summarizes on recent life-cycle assessments of greenhouse gas emission and techno-economic evaluation of ethanol production. To sustain the infant cellulosic ethanol industry, substantial improvement in the following areas need to happen in a timely manner: (1) Effective and low-cost biomass pretreatment method, (2) efficient fermentation of all sugars released during the pretreatment and hydrolysis steps, (3) development of enzymes that tolerate various inhibitors including monosaccharides (mainly glucose) and ethanol, and (4) heat-tolerant fermentation microbes and enzymes for efficient simultaneous saccharification and fermentation. Genetic engineering is expected to play a key role in addressing most of the issues in these areas.

Keywords: global warming, lignocellulosic biomass, second-generation bioethanol, saccharification, fermentation, life cycle analysis, techno-economic evaluation

1. Introduction

The need to slow down and eventually stop global warming has driven commercial production of the bioethanol in the past two decades because the use of renewable fuel is one of the few ways to mitigate climate change as it helps reduce GHG emissions. Multiple independently produced datasets confirm that between 1880 and 2012, the global average land and ocean surface temperature increased by 0.85 [0.65–1.06]°C [1]. Since 1979 the rate of warming has approximately doubled (0.13°C/decade, against 0.07°C/decade) [2, 3]. The scientific consensus as of 2013 stated in the intergovernmental panel on climate change (IPCC) Fifth Assessment Report is that it "is extremely likely that human influence has been the dominant cause of the observed warming since the mid-20th century." In 2018 the IPCC published a Special Report on Global Warming of 1.5°C which warned that, if the current rate of greenhouse gas (GHG) emissions is not mitigated, global warming is likely to reach 1.5°C between 2030 and 2052 causing major crises. The report said that preventing such crises will require a swift transformation of the global economy that has "no documented historic precedent" [4].

A mandate required developed countries to take the lead in reducing their emissions and was sustained in the Kyoto Protocol to the United Nations Framework Convention on Climate Change (UNFCCC), which entered into legal effect in 2005. In ratifying the Kyoto Protocol, most developed countries accepted legally binding commitments to limit their emissions. Biofuel mandates are set in more than 60 nations and incentives are provided by the governments to boost bioethanol production [5].

In the U.S., production, transportation and fermentation of the corn was adapted quickly by industry for fuel ethanol production, primarily because corn was the only crop that had the existing infrastructure to easily modify for this purpose, especially when initially incentivized with tax credits, subsidies and import tariffs. **Figure 1** shows total U.S. corn use from 1986 to 2018. The amount of corn used for ethanol production increased substantially between 2001 and 2010, as nearly all gasoline was transitioned to 10% ethanol. From 2013, the trend remains consistent with production and usage remaining relatively constant.

There is still some debate on whether biofuel production from food feedstock can truly reduce GHG emissions. The United Nations Intergovernmental Panel on Climate Change released two of its Working Group reports state that "Biofuels have direct, fuel-cycle GHG emissions that are typically 30–90% lower than those for gasoline or diesel fuels. However, since for some biofuels indirect emissions—including from land use change—can lead to greater total emissions than when using petroleum products, policy support needs to be considered on a case by case basis" (IPCC 2014 Chapter 8). The report lists many potential negative risks of ethanol production from food feedstock, such as direct conflicts between land for fuels and land for food, other land-use changes, water scarcity, loss of biodiversity and nitrogen pollution through the excessive use of fertilizers.

Also, the potential of using bioethanol from food feedstock to replace petroleum fuels is limited. The United States will use over 130 billion gallons of gasoline in 2014, and over 50 billion gallons of diesel. On average, one bushel of corn can be used to produce just 2.8 gallons of ethanol. If all of the production of corn in the U.S. were converted into ethanol, it would only displace 25% of that 130 billion.

On the other hand, there is less controversy over GHG reduction from production of lignocellulosic ethanol production as cellulosic materials are mostly the wastes of the agriculture and forest industry. The shift from food crop feedstocks to waste residues and native grasses offers significant opportunities for a range of players, from farmers to biotechnology firms, and from project developers to

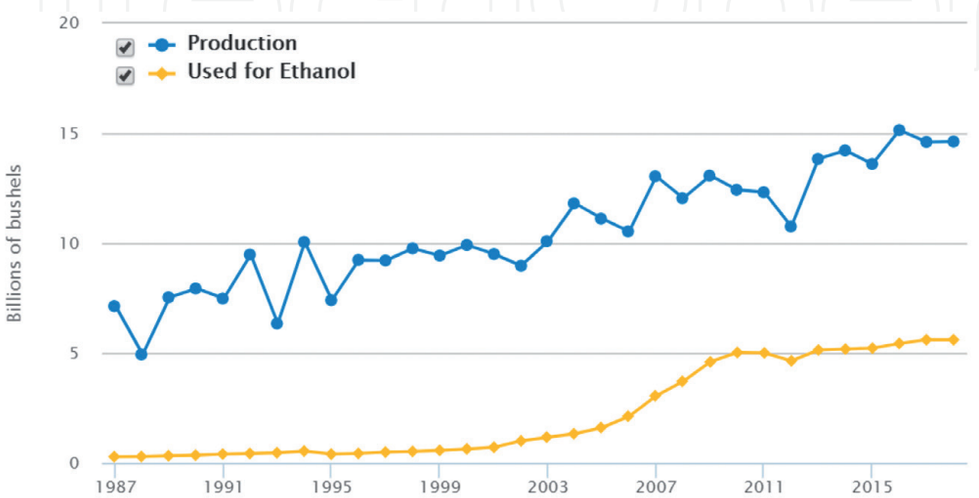

Figure 1.
The U.S. corn for fuel ethanol, feed, and other use. Source: the United States Department of Agriculture Economic Research Service Feed Grain Yearbook.

Company	Location	Feedstock	Capacity (mg year^{-1})	Status
Abengoa Bioenergy	Hugoton, KS	Wheat straw	25–30	2013–2016 Bankrupt [8]
BlueFire Ethanol	Fulton, MS	Multiple sources 19	20	Construction halted 2011 [9]
DuPont	Nevada, Iowa		30	Sold to Verbio in Nov. 2018 [10]
Mascoma	Kinross, MI	Wood waste	20	Construction halted in 2013 [11]
POET LLC	Emmetsburg, IA	Corn stover	20–25	Operational in Sep. 2014 [12]

Table 1.
The status of the U.S. commercial lignocellulosic ethanol facilities.

investors [6]. However, the process to convert lignocellulosic materials to ethanol is much more complex than that used to covert starch and sugars into ethanol.

Cellulosic ethanol industry is still in its infancy. In the U.S., as of 2013, the first commercial-scale plants to produce cellulosic biofuels have begun operating. In the following 5 years, cellulosic ethanol production grown from 0 to 10 million gallons [7], and most likely topping 15 million in 2018. However, that is far from the Renewable Fuel Standard's original target of 7 billion gallons of cellulosic biofuel by 2018 and 16 billion by 2022. Of all five commercial cellulosic ethanol plants that were built/to be built in the U.S. from 2010 to 2016, only POET's Emmetsburg, Iowa facility is still in operation in 2019 (**Table 1**). In 2017, the total cellulosic ethanol produced was less than half the nameplate capacity (25 million gallons year^{-1}) of this single plant [13].

The future of bioethanol generation from lignocellulosic materials is not clear at this point of time. The sustainability of this renewable fuel business will depend on the success of development of cost-cutting technologies for every stage of lignocellulosic ethanol production.

2. Ethanol generation from biomass

2.1 First-generation bioethanol

First-generation biofuel includes biodiesel produced from vegetable oils through transesterification and bioethanol generated from food feedstock, mainly starchy materials (e.g., corn, wheat, barley, cassava, potato) and sucrose-containing feedstock (e.g., sugarcane, sugar beet, sweet sorghum) [14]. First-generation bioethanol is produced from fermentation of these starchy and sucrose-containing materials in four basic steps: enzymatic saccharification or hydrolysis of starch into sugars, microbial (yeast) fermentation of sugars, distillation, and dehydration.

Figure 2 shows global ethanol production by country or region, from 2007 to 2017. Together, the U.S. and Brazil produce 85% of the world's ethanol. The vast majority of Brazil ethanol is produced from sugarcane.

The United States is the world's leading producer of ethanol, with nearly 16 billion gallons in 2017 alone, mainly produced from corn. The annual U.S. production of ethanol from 1981 to 2018 is shown in **Figure 3**.

2.2 Second generation bioethanol

Second and subsequent generations of biofuels including bioethanol are produced from non-food raw materials [16]. Second-generation bioethanol is typically produced

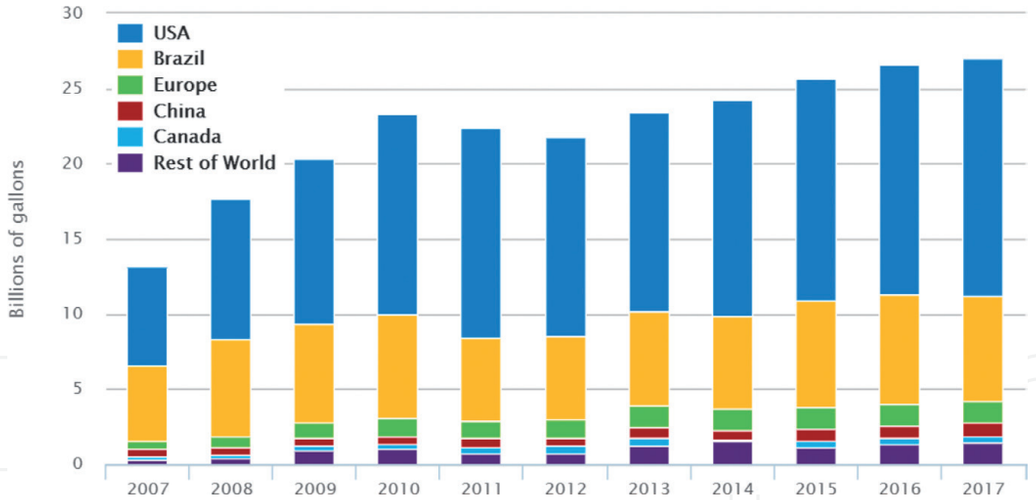

Figure 2.
Global ethanol production by country or region, from 2007 to 2017. Source: Renewable Fuels Association. Last updated October 2018.

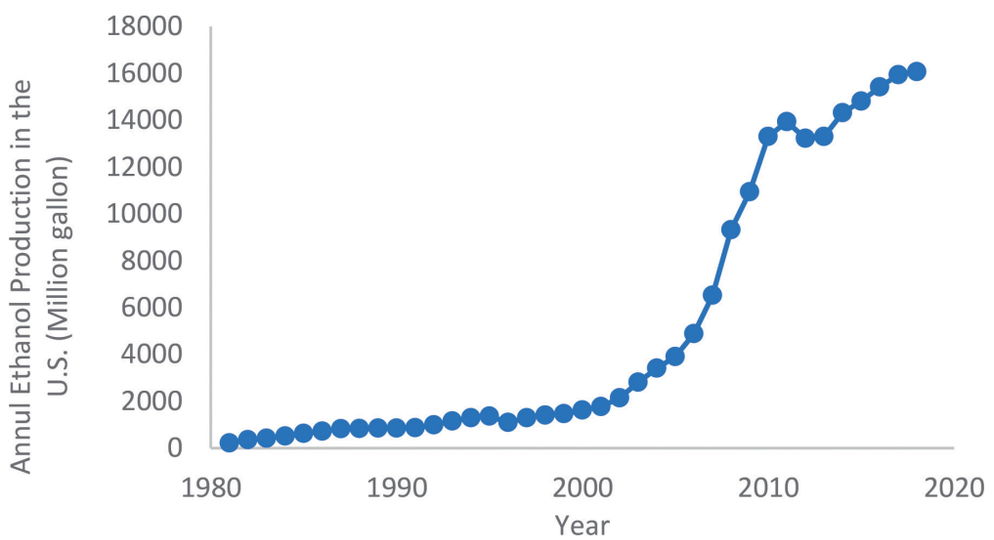

Figure 3.
The U.S. annual production of ethanol from 1981 to 2018 [15].

from sugars derived from lignocellulosic biomass. Various types of biomass have been studied for production of biofuels including agricultural wastes (e.g., corn stover, wheat straw, corn cob, rice husk, and sugar cane bagasse), energy crops which grow on low-quality soil (perennial grasses such as *Miscanthus sinensis* and *M. giganteus* and switchgrass), forest-based woody wastes (bark, sawdust, softwood trimmings and hardwood chips), waste from parks and gardens (leaves, grasses, and branches), municipal solid wastes such as food waste, kraft paper and paper sludge, the whey-a byproduct of the cheese industry, and crude glycerol from the biodiesel industry.

The amount of available lignocellulosic biomass far exceeds the amount of food feedstock that can be used for biofuel production. However, the production of lignocellulosic bioethanol requires feedstock preparation prior to fermentation and finding/developing microbes that are able to hydrolyze polysaccharides and ferment sugars from cellulose and hemicellulose breakdown.

2.3 Third generation bioethanol

The term third generation biofuel refers to biofuel derived from algae and has only recently enter the mainstream. Previously, algae were grouped with other

non-food biomass types as feedstock for second generation biofuels. However, the uniqueness in algae's production methods and potential of much higher yields of biofuel production warrants its separation from other types of non-food biomass to form their own category.

When it comes to the potential to produce fuel, algae is unique in several ways. First, algae produce an oil that can easily be refined into diesel or even certain components of gasoline [17]. Second, it can be genetically manipulated to produce a wide list of fuels including biodiesel, butanol, gasoline, methane, ethanol, vegetable oil, and jet fuel [18]. Third, it is also capable of producing outstanding yields. In fact, algae have been used to produce up to 9000 gallons of biofuel per acre, which is 10-fold what the best traditional feedstock have been able to generate. Yields as high as 20,000 gallons per acre are believed to be attainable. According to the US Department of Energy, yields of 10-fold high mean that only 0.42% of the U.S. land area would be needed to generate enough biofuel to meet all the U.S. needs.

Algae do have a down side: they require large amounts of water, nitrogen and phosphorus to grow. So much that the production of fertilizer to meet the needs of algae used to produce biofuel would produce more greenhouse gas emissions than were saved by using algae-based biofuel. It also means the cost of algae-base biofuel is much higher than fuel from other sources. This single disadvantage means that the large-scale implementation of algae to produce biofuel will not occur for a long time, if at all. In fact, after investing more than $600 million USD into research and development of algae, Exxon Mobil came to the conclusion in 2013 that algae-based biofuels will not be viable for at least 25 years which was calculated on strictly economical term without considering the environmental impacts that have yet to be solved [19].

3. Overview of bioethanol generation from lignocellulosic biomass

3.1 Composition of lignocellulosic feedstock for bioethanol

Dry plant materials are mainly comprised of three types of biopolymers: cellulose, hemicellulose, and lignin. Cellulose and hemicellulose account for more than half of the entire dry biomass (see **Table 2**) [28]. Ethanol yield and conversion efficiency depend on the type of biomass, and benefit from a high content of cellulose and hemicellulose and low lignin content [29]. The domains of the three polymers in plant cell walls are connected strongly through covalent and hydrogen bonds. These bonds make lignocellulosic material resistant to degradation [30] and different methods of pretreatment [31].

Cellulose is a β-glucan linear polymer of 500–14,000 D-glucose units D-glucose linked by β-1,4-glycosidic bonds. Around 36 hydrogen-bonded glucan chains form insoluble microfibrils in secondary cell wall [32]. The cellulose structure is highly crystalline and thus is difficult to break in enzymatic hydrolysis [33]. High temperature (320°C) and pressure (25 MPa) are needed to melt and dissolve this rigid crystalline structure in water, in sharp contrast with the liquefaction temperature 95–105°C of starch at pH = 6.0–6.5, and the saccharification temperatures of 60–65°C at pH = 4.0–4.5 [34, 35].

Hemicellulose is a branched heteropolymer of different monosaccharides including pentoses (D-xylose and L-arabinose) and hexoses (D-mannose, D-galactose, D-glucose) and a small amount of sugar acids called uronic acids [36]. The D-pentose sugars are dominant with occasionally small amounts of L-sugars as well. Among pentoses, xylose is present in the largest amount, although in softwoods mannose can be the most abundant sugar. Typical sugar acids in the hemicellulose structure include D-glucuronic, 4-O-ethylglucuronic and D-galacturonic

Biomass	Cellulose %	Hemicellulose %	Lignin %
Corn stover	37.5	30	10.3 [20]
Corn cobs	33.6	37.2	19.3 [21]
Sugarcane bagasse	45	20	30 [22]
Grasses	25–40	35–50	10–30 [23]
Switchgrass	31.98	25.19	18.13 [24]
Wheat straw	35.9	23.9	19.3 [25]
Oat straw	39.4	27.1	20.7 [23]
Rice straw	44.3	35.5	20.4 [26]
Rice husk	34.4	29.3	19.2 [27]
Hardwood			
Black locust	41.61	17.66	26.70 [24]
Hybrid poplar	44.70	18.55	26.44 [24]
Eucalyptus	49.50	13.07	27.71 [24]
Hardwood stems	40–55	24–40	18–25 [23]
Softwood-pine	44.55	21.90	27.67 [24]
Nut shells	25–30	25–30	30–40 [23]
Newspaper	40–55	24–40	18–25 [23]

Table 2.
Biomass composition.

acids. Meaningful quantities of L-arabinose are contained in corn fiber and specific herbaceous crops [37].

C5 sugars such as xylose and arabinose are mostly found in xyloglucan, xylan, arabinan and arabinogalactan (substructures of pectin), which are components of polysaccharides in the plant cell wall [38]. Xylan is the largest hemicellulose component, consisted of β-1,4-linked xylose residues with side branches of α-arabinofuranose and α-glucuronic acids and contribute to cross-linking of cellulose microfibrils and lignin through ferulic acid residues [39].

Lignin is a natural three-dimensional polymer (600–15,000 kda) bio-synthesized from phenylpropanoid units via radical reactions [40]. Lignin accounts for 20–35 wt% in woody biomass (40–50 wt% in bark) and 10–20 wt% in agricultural stems [41]. In lignin, phenolic units are connected by more than eight different linkages, among them arylglycerol β-aryl ether (β-O-4) is the dominant linkage in both softwood and hardwood in most plants, consisting of ~50% of spruce linkages and 60% of birch and eucalyptus linkage [42]. It has long been recognized as the major renewable source of aromatic chemicals such as phenols and aromatic hydrocarbons.

Due to the complex polymer structure and heterogeneity in the ways monomeric units are linked, lignin is particularly difficult to biodegrade, making it an undesirable component in plant cell walls for bioethanol production. In plant cell wall, lignin functions like a glue to hold all components together [43]. As such, its recalcitrant character makes this three-dimensional polymer molecule a physical barrier to the enzymes that act on cellulose and hemicellulose.

In biorefinery, around 62 million tonnes of lignin is obtained in the commercial production of lignocellulosic ethanol. A large amount of lignin is also being generated in the pulp industry as lignin has also to be separated from cellulose for a different reason: the aromatic components in lignin can turn yellow as it is oxidized

slowly in air. Despite that lignin has mainly been burned to supply heat and to generate electricity, it has long been recognized as the major renewable source of aromatic polymer and chemicals [44].

Due to the lower oxygen content in lignin as compared to that in cellulose, the energy value of lignin could be as high as cellulose despite of its lower weight percentage in lignocellulosic biomass. This has generated a lot of interest in converting lignin into liquid fuels using thermochemical and biological methods including pyrolysis, hydrothermal liquefaction, and enzymatic decomposition [45]. Among these methods, hydrothermal liquefaction has been more investigated recently and appears to be a promising way to decompose lignin into bio oil which could be further processed into liquid transportation fuels.

3.2 Biochemical conversion of biomass into ethanol

Second-generation bioethanol is produced using a process involving the four primary steps of (i) pre-treatment, (ii) hydrolysis to sugars, (iii) fermentation, and (iv) product/coproduct recovery [46]. During pre-treatment, the feedstock is subjected to physical (heat, steam) or chemical (acid or base) conditions that disrupt the fibrous matrix of the material, resulting in the separation of the hemicelluloses from the cellulose chains and the lignin that binds them together. Hydrolysis follows pre-treatment, releasing individual glucose from cellulose and hexose and pentose from hemicellulose. These monomers can then be fermented to ethanol by yeasts that have been modified to ferment both hexose and pentose sugars and adapted to deal with the inhibitors that are produced during pre-treatment and unavoidably associated with the hexose and pentose sugars [34]. Distillation and dehydration of the aqueous ethanol solution produces ethanol of 99.9% purity. Coproduct recovery will depend upon the feedstock and pre-treatment process used and can include a range of products such as extractives, lignin, and unhydrolyzed cellulose [47].

In the following three sections (Sections 4–7), each of the four primary steps will be reviewed. Current topics of research, which are concentrated on recombinant fermentative microbes development and a consolidated process of hydrolysis and co-fermentation of hexoses and pentoses, will be covered in Section 8. A review on cost analysis is given in Section 9 to present opportunities for cost reduction for second-generation bioethanol production.

4. Pretreatment of lignocellulosic biomass

4.1 Objectives of pretreatment and basic methods

Without pretreatment before the enzymatic saccharification stage, the non-biodegradable lignin in lignocellulosic material presents as a major obstacle to the enzymatic hydrolysis of crystalline cellulose and hemicellulose which themselves already have low digestibility [48]. Pretreatment removes or decomposes the lignin (delignification) [49] and thus makes cellulose and hemicellulose more readily available to cellulases and hemicellulose's.

In principle, there are three methods for pretreatment: biological, chemical and physical processes. Some processes, where chemical and physical actions are inherently inseparable, are termed physiochemical. Two or all of these basic methods can be used in combination to gain benefits from each method. Various pretreatment methods have been described and compared critically in a recent review [50].

Biological treatment uses microorganisms such as white, brown or soft rot fungi which break up the structure of lignin via the action of extracellular lignolytic enzymes released by the fungi [51]. Further research is needed to overcome the issues of selectivity, cost, retention time and effectiveness to make it a practical choice [50].

Chemical treatments include treatment with bases, diluted acids, and oxygen as an oxidizer. These reagents react with lignin and cause the polymer to breakdown into smaller and more soluble fragments. Physical pretreatment is usually performed before chemical or biological treatment to reduces cell wall crystallinity and particle size by physical milling or grinding [50]. In some treatment methods, both physical action and chemical reaction play important roles in lignin removal. Such physico-chemical pretreatment can involve steam explosion, liquid hot water, ammonia fiber explosion, ammonia recycle percolation or a supercritical carbon dioxide.

Pretreatment contributes a vital role in the cost evaluation process of whole technology, because they contribute about 30–35% of overall production cost [52]. There are many issues that arise from this process [50] including loss of sugars (mainly pentose sugars derived from hemicellulose degradation), and generation of toxic substances that inhibits the downstream fermentation process. Both need to be minimized to make ethanol production more efficient.

4.2 Steam explosion

Steam explosion has become one of the most adopted pretreatment processes, where hydrolysis of hemicellulose also happens which improves cellulose digestibility. It is a physiochemical method that uses both physical changes caused by sudden pressure reduction and heat- and catalyst-induced chemical changes. An impregnation agent is sometimes used before the pretreatment step. Upon steam explosion after 1–5 min soaking in 160–270°C and 20–50 bar steam, fibers loose up and sugar polymers (mainly hemicellulose) partially degrade into sugars via hydrolysis of glycoside bonds in polysaccharides and lignin into soluble fragments including some inhibitors and phenolic products [50]. The process allows for subsequent solubilization of hemicellulose in water and lignin in organic or alkaline solvent. Cellulose undergoes some degree of polymerization but is still insoluble in water or organic solvents and remains in the solid phase. Acid (sulfuric acid and sulfur dioxide) impregnation before steam explosion reduce the time and temperature necessary for proper depolymerization of the feedstock, increases the efficiency of enzymatic hydrolysis of polysaccharides to glucose and xylose and reduce enzyme consumption [53]. Compared to other methods of biomass fractionation, steam explosion uses less dangerous chemicals, less demanding on investment and energy consumption [54]. Steam explosion is not recommended for agricultural and hardwood wastes with high contents of pentoses and low levels of lignin, due to the susceptibility of pentoses to thermal degradation. Steam explosion is recommended for processing straw and bagasse.

4.3 Inhibitors generated in pretreatment

One of the lasting issues in the second-generation bioethanol production is the formation of inhibitors during the pretreatment. The inhibitors create unfriendly environments for fermentative microbes, increases the length of lag phase, causes loss of cell density and lower growth rates of fermenting microbes, and consequently decreases ethanol yields [55]. The commonly observed inhibitors are aldehydes such as 5-hydroxymethyl-2-furaldehyde and 2-furaldehyde (furfural), weak organic acids (formic, acetic and levulinic acids) and phenolic compounds [56]. Acetic acid is the major organic acid found in hydrolysates coming from the

hydrolysis of acetyl side-chain groups in hemicellulose [57]. Cell growth of fermentative microbes is inhibited by the intracellular process of anions of weak acids. Furan aldehydes are poisonous for microbes and phenolic compounds interfere with the function and integrity of cell membranes [58].

There are several methods used for the removal of inhibitors [59]. The detoxification of lignocellulosic hydrolysates can be performed using inhibitor sorbents such as excess of lime, active carbon or lignite (brown coal).

5. Enzymatic hydrolysis of polysaccharides

After pretreatment to partially remove lignin and loose up polysaccharide structures, polysaccharides need to be hydrolyzed into sugar molecules which will be converted into ethanol by fermentation [38]. The hydrolysis can be accomplished chemically via acid-catalyzed cleavage of glycosidic bonds or by enzymes produced by microbes. Enzymatic method is more popular due to less impact on the environment and higher selectivity in the hydrolysis. Glucose and xylose are the main products in hydrolysates from the enzymatic breakdown of polysaccharides.

Enzymes produced by the filamentous fungi such as *Aspergillus nidulans*, *Aspergillus niger*, *Penicillium* spp. and *Trichoderma reesei* are dominant in commercial biorefinery [38]. Among different types of cellulases, endoglucanases attack the internal glycosidic bonds in the amorphous cellulose regions, causing fragmentation of the cellulose structure, and exoglucanase works of the termini of β-glucan molecules to release glucose molecules one at a time, while β-glucosidase attacks catalyzes the hydrolysis of the glycosidic bonds to terminal non-reducing residues in beta-D-glucosides and oligosaccharides to release one or two glucose units at a time [60]. The costs of cellulases are high, spurring the development of methods to recycle hydrolysis enzymes [61]. Inclusion of hemicellulose's, such as endoxylanases, xylosidases, exoxylanases and other accessory enzymes, such as esterase's and arabinosidase's, in the hydrolysis step improves the efficiency of enzymatic hydrolysis of lignocellulosic biomass and helps reduce enzyme loading and costs [62].

Various strains of yeasts and bacteria are being investigated with the goal of developing a consolidated process of hydrolysis and co-fermentation of glucose and xylose, without the need for adding exogenous cellulases [63].

6. Fermentation of lignocellulosic hydrolysates

Sugars in the hydrolysate are converted into ethanol by fermentation using microorganisms such as yeasts. Ethanol-producing ability of yeasts depends on lignocellulosic hydrolysate, their strain and fermentation conditions (temperature, pH, aeration and nutrient supplementation). For use in industrial bioethanol production, microorganisms (mainly yeasts) must show thermotolerance and high fermentative activity for simple carbohydrates such as glucose and xylose. They should also be resistant to environmental stressors, including inhibitors mentioned in Section 4.3, acidic pH, high sugar level at the beginning of fermentation (causing hyperosmotic stress), and higher temperatures which prevents microbiological contamination, and are able to grow on various lignocellulosic substrates at a fast growth rate [58, 64].

Saccharomyces cerevisiae JRC6 and *Candida tropicalis* JRC1 are recommended for hydrolysates after alkali pretreatment and acid pretreatment, respectively [41]. *Saccharomyces* sp. yeasts are used in biorefineries to ferment glucose released during starch hydrolysis. Apart from glucose, they are capable of fermenting galactose and mannose.

Zymomonas mobilis is a Gram negative, facultative anaerobic, non-sporulating, polarly-flagellated, rod-shaped bacterium. It has notable bioethanol-producing capabilities, which surpass yeast in some respects. However, it only ferments glucose, fructose and sucrose [65]. This prevents them from being used in industrial production of bioethanol. The *Z. mobilis* strains are tolerant to ethanol concentration up to 120 g/L, and have low nutritional requirements for growth [58]. However, its tolerance to acetic acid is low: as little as 2.5 g/L of HOAc. Its recombinant strain AX101 also has low tolerance to acetic acid.

7. Distillation and dehydration (drying) of bioethanol

After fermentation, the mash is heated so that the ethanol evaporates. This process, known as distillation, separates the ethanol, but its purity is limited to 95–96% due to the formation of a water-ethanol azeotrope with maximum 96.5% v/v) ethanol. This hydrous ethanol can be used as a fuel alone, but is not miscible in all ratios with gasoline, so the water fraction is typically removed before ethanol is added to gasoline.

Water can be removed by passing hydrous ethanol vapor through a bed of molecular sieve beads. The bead's pores are sized to allow adsorption of water while excluding ethanol. Two beds are often used so that one is available to adsorb water while the other is being regenerated. This dehydration technology can save 3000 BTUs/gallon over the azeotropic distillation and has been adopted by most modern ethanol plants.

Recent research has demonstrated that complete dehydration prior to blending with gasoline is unnecessary. When the azeotropic mixture is blended directly with gasoline, water separates from the gasoline/ethanol phase and can be removed in a two-stage counter-current setup of mixer-settler tanks with minimal energy consumption [66].

8. LCA on GHG emissions and techno-economic evaluation of lignocellulosic ethanol production

Numerous life cycle analyses (LCAs) of lignocellulosic ethanol have been published over the last 15 years and several reviews of these LCA studies have been completed and are cited in a more recent review [67]. These studies show a clear reduction in GHG emissions for lignocellulosic ethanol compared to gasoline. However, accurate quantification of GHG emission reduction is hard to obtain as gaps remain in understanding life cycle performance due to insufficient data, and model and methodological issues. Critical unresolved issues that are expected to impact its energy/GHG emissions performance include feedstock-related emissions, consequential versus attributional life cycle aspects, choice of system boundaries, and allocation methods.

Decisions regarding feedstock, process technology and co-products can significantly impact GHG emissions calculations. Predicted life cycle GHG emissions vary widely depending on how the following key parameters are considered: nitrogen-related emissions due to supplemental fertilizer requirements and the N content of feedstock, cellulase requirements, farming energy, ethanol yield, and how the value of co-products such as lignin are realized, among others.

Government support (i.e., Ethanol mandate, tax credit, etc.) is not expected to last forever. To be sustainable, lignocellulosic biofuels production must meet or exceed the economic performance of their first-generation counterparts.

The growth in the capacity of commercial lignocellulosic ethanol production has been slow in the past decade, despite significantly better predicted performance on various environmental and energy security criteria than corn-based ethanol in the various techno-economic evaluations published before 2010 [68]. The slow growth has been due to both large technological risk, large capital cost, and the poor predicted economic performance of biorefineries in the short term.

An LCA of US softwood cellulosic ethanol was reported in 2012 by Stephen et al. [68]. In the paper, the base case (capacity: 50 mL ethanol $year^{-1}$) softwood ethanol production cost was compared with costs of ethanol produced from corn and sugarcane found in the literature. Softwood lignocellulosic ethanol was predicted to have a production cost of \$0.90 L^{-1}, 250–300% higher than US corn and Brazilian sugarcane ethanol production costs, which were in the range of \$0.30–\$0.40 L^{-1}. The lignocellulosic base case scale of 50 mL $year^{-1}$, compared to 150 mL $year^{-1}$ of US corn and 365 mL $year^{-1}$ of and Brazilian sugarcane, is much smaller as it was chosen based both on the projects funded under the US Department of Energy's commercial biorefinery program and those operating in other places such as Denmark. Production costs of sugar- or starch-based ethanol are expected to continue to decline to \$0.22–\$0.25 L^{-1} by 2020. Thus, second-generation ethanol is not going to catch up with first-generation ethanol on production cost soon.

Another very recent techno-economic evaluation was performed on production cost of ethanol produced from corn stover using either biochemical or thermochemical methods. For heat integrated biochemical route, the predicted bioethanol product costs at \$2.00 for a production capacity of 43,300,000 gallon $year^{-1}$ [69]. This result was clearly an underestimation of lignocellulosic ethanol as a major cost item, capital investment cost, was not included. Furthermore, the corn stover price of 46.8 \$/ton was an underestimation, and feedstock transportation cost was not included in LCA. Feedstock cost can impact total cost by 40 percent according to a Lux Research report of 2016 [70]. The Brazilian birefinery company Raizen has the lowest projected minimum ethanol selling price of \$2.17 per gallon while Abengoa's capital-intensive \$500 million Hugoton facility has the highest price of \$4.55 with feedstock cost emerging as the most critical variable. The low cost of Raizen's cellulosic ethanol is largely attributed to its access to low cost sugarcane straw and sugarcane bagasse (\$40 and \$38 per dry metric ton), respectively, compared with corn stover (\$90) used by Abengoa and POET-DSM and wheat straw (\$75) used by Beta Renewables [71].

9. Opportunities for cost reduction

It is apparent that second-generation ethanol is currently much more costly to produce than first-generation ethanol. It is hard to predict when the cost of lignocellulosic ethanol will be reduced to the level of corn/sugar cane ethanol. Dramatic reductions in the capital and operational costs must occur before the potential superior environmental benefits from cellulosic ethanol relative to corn ethanol can be realized. Pretreatment, enzymatic hydrolysis and distillation are responsible for much of the cost of producing bioethanol. Currently, intensive research is being conducted to improve each of the processes to make them more economical.

9.1 Pretreatment

An effective pretreatment increases specific surface area of biomass, making cellulose better available for the action of hydrolytic enzymes obtained from fungi and bacteria, minimizing reductions in enzyme activity, and thus improving the rate of biomass hydrolysis and providing the highest possible concentration of fermentable

sugars. Effective pretreatment also reduces the degradation of monosugars [72]. In selecting pretreatment methods, factors such as their environmental impact and recycling of chemical compounds (for example ammonia in the ammonia fiber explosion process [73, 74]) must be considered. Different pretreatment methods and their combinations are being explored for different types of biomass [50].

Better results, e.g., improved ethanol yield, have been obtained from combination of two or more pretreatment methods, but have resulted often at the cost of more energy consumption compared to single method of pretreatment. Among single treatment methods, dilute acid pretreatment is more suitable for various types of biomass as it solubilizes most of hemicellulose and partially remove lignin [50].

It is vital to analyze the pros and cons of each pretreatment technology before scaling up for industrial application. However, technoeconomic assessment will only give a rough estimate on capital cost and the final fuel cost in commercial scale production when many research findings are still in pilot scale level and demonstration plant level [52].

9.2 Pentose fermentation

Efficient fermentation of pentoses helps reduce ethanol production cost since pentoses can be 25.8 wt% as in sugarcane bagasse [75, 76] 22.3–74.9 wt% in corn stover (**Table 3**). Wild microorganisms are incapable of producing ethanol in high yields, as they are unable to utilize both pentoses and hexoses. Pentose-specific transporter proteins and enzymatic reactions determining the metabolism of pentoses such as L-arabinose and D-xylose have not been found in naturally occurring baker's yeast.

Owing to large microbial biodiversity, fermentation of pentoses can be achieved either by finding a potent naturally occurring pentose utilizing microorganism or by a genetically engineered C5 utilizing strain [78, 79]. One effective strategy is to create recombinant strain with genes for xylose metabolism [80]. Genetic engineering has been conducted mainly on *Saccharomyces cerevisiae* yeast, [81] the Gram-positive bacteria *Clostridium cellulolyticum* and *Lactobacillus casei* and the Gram-negative bacteria *Zymomonas mobilis*, *Escherichia coli* and *Klebsiella oxytoca* [43]. Recombinant yeasts consume xylose much slower than glucose, thus requiring prolonged fermentation time due to a lack of reaction intermediates and efficient pentose transporters [82].

A common problem of xylose-fermenting strains is the production of xylitol or the reabsorption of ethanol, which lead to low ethanol yield. One grand challenge is glucose repression, which results in di-auxic fermentation of a mixture of glucose and pentoses since glucose prevents the catabolism and/or utilization of other non-glucose sugars, leading reduced volumetric ethanol yield [83]. Approaches and conditions sought to improve glucose and xylose fermentation to ethanol are reviewed in a recent paper with emphasis on microbial systems used to maximize biomass resource efficiency, ethanol yield, and productivity [64].

9.3 Simultaneous saccharification and fermentation (SSF)

Separate processes have been established for enzymatic hydrolysis of cellulose and hemicellulose and fermentation (SHF) of sugars in hydrolysate. In the SHF processes, saccharification and fermentation take place in separate vessels, so the two processes can be optimized separately. One drawback of SHF is that accumulation of simple carbohydrates (such as cellobiose) causes end-product inhibition of hydrolytic enzymes, for example cellulases or cellobioses. To prevent end-product inhibition, extra doses of β-glucosidase are needed together with the commercial cellulase preparations [84].

Biomass	Lignin	Hexoses			Pentoses		Carbohydrate
		Glucan	Mannan	Galactan	Xylan	Arabinan	
Corn stover	18.2	30.6	0.5	0.7	16.0	1.9	49.7 [76]
	20.2	38.1	0.4	0.7	20.3	2.0	61.5 [76]
	17.2	36.1	N/A	2.5	21.4	3.5	65.3 [77]
Corn leaf	N/A	34.2	1.8	2.5	22.1	3.5	64.1 [68]
Corn stalk	N/A	36.5	1.7	2.4	21.6	3.2	65.4 [68]
Corn fiber	6.9	36.5	N/A	2.9	18.4	13.3	71.1 [77]
DDG	3.1	22.0	N/A	0.3	9.5	5.5	37.3 [77]
Wheat straw	14.5	36.6	0.8	2.4	19.2	2.4	61.4 [77]
	16.9	32.6	0.3	0.8	19.2	2.4	55.3 [76]
Switchgrass	23.2	32.2	0.4	0.0	20.3	3.7	56.6 [77]
	23.1	35.9	0.4	0.5	19.6	1.5	57.9 [76]
	27.6	31.9	0.3	0.3	10.6	1.1	44.2 [76]
	24.1	42.6	0.3	0.5	23.1	1.5	68.0 [76]
S. bagasse	18.4	38.1	0.4	0.0	23.3	2.5	65.0 [77]
Softwood							
Spruce	28.3	43.2	11.5	2.7	5.7	1.4	64.5 [76]
Red pine	29.0	42.0	7.4	1.8	9.3	2.4	62.9 [76]
Lodgepole pine	27.9	42.5	11.6	2.1	5.5	1.6	63.3 [76]
Ponderosa pine	26.9	41.7	10.8	3.9	6.3	1.8	64.5 [76]
Loblolly pine	28.0	45.0	11.0	2.3	6.8	1.7	66.8 [76]
Douglas-fir	32.0	44.0	11.0	4.7	2.8	2.7	65.2 [76]
Hardwood							
Red maple	24.0	46.0	2.4	0.6	19.0	0.5	68.5 [76]
Aspen	23.0	45.9	1.2	0.0	16.7	0.0	63.8 [76]
Yellow poplar	23.3	42.1	2.4	1.0	15.1	0.5	61.1 [76]
Poplar	N/A	39.8	2.4	0.0	14.8	1.2	58.2 [77]
Poplar stem	N/A	40.3	3.1	0.7	17.6	0.6	62.3 [68]
Poplar DN34	23.9	43.7	2.9	0.6	17.4	0.6	65.2 [76]
Euclyptus saligna	26.9	48.1	1.3	0.7	10.4	0.3	60.8 [76]
Salix	26.4	41.4	3.2	2.3	15.0	1.2	63.1 [76]

S. bagasse = sugarcane bagasse.

Table 3.
Hexose, pentose and lignin contents in different types of biomass.

There is a strong incentive to develop a process to perform simultaneous saccharification and fermentation (SSF) as it reduces investment costs by reducing the number of vessels and has the potential to become the preferred approach. In SSF, the problem of end-product feedback inhibition is largely eliminated because glucose molecules are fermented immediately by the fermentative microbes as it is produced from hydrolysis of cellulose [85]. However, the benefits come with a major downside which is an inherent mismatch between the optimal temperatures for the enzymes (fungal cellulases and hemicellulose's) on the one hand, and yeast biocatalysts on the other. The temperature optima for saccharifying enzymes (50–55°C for cellulase) are higher than those for fermenting mesophilic culture.

The optimal temperature for yeasts is below 35°C. Mesophilic yeasts (that thrive best in a moderate temperature) exhibit slower growth rates at higher temperatures. Currently, SSF must run at temperatures between the optimum temperature for cellulase and the optimum temperature for fermentative organisms. The compromise results in higher cellulase loading and an increase in enzyme costs. Efficient bioethanol production by SSF requires the use of thermotolerant ethanologenic yeast. It is a hot topic for research to genetically modify microorganisms with the ability to ferment at higher temperatures [43]. Some isolated yeasts, including *Pichia*, *Candida*, *Saccharomyces* and *Wickerhamomyces*, are found to grow at temperatures of 40°C and ferment sugars at higher temperatures [41]. To make SSF process highly efficient in ethanol production, the pentose metabolic pathway is been engineered into microorganisms to enables the use of C5 sugars by microbes that do not ferment them earlier [86].

Reduction in enzyme cost is been sought by searching for new organisms with cellulolytic and hemicellulytic activities [87], lowering the enzyme dosage through protein engineering [86, 88], and improving cellulase thermostability for performing hydrolysis at elevated temperatures to increase the efficiency of cellulose hydrolysis [89]. Cellulase enzyme cost reductions are challenging as cellulase costs need to be significantly lower than those of amylase enzymes on a unit-of-protein basis. The high price of the enzymes encouraged research into solutions to the problem of glucose inhibition and to the deactivation caused by lignin by-products [90].

Further integration of enzyme production with SSF leads to a new technology of consolidated bioprocessing (CBP). One area of research is aimed at engineering all three capabilities (saccharification, hexose fermentation and pentose fermentation) into a single strain for the CBP process [91, 92]. Cellulase-encoding genes may be introduced into specific species during recombination [63] to eliminate the need for exogenous cellulases in the process of SSF and decrease the capital costs of processing. CBP technology promises to eliminate costs associated with enzyme production and additional infrastructure/vessels [93].

9.4 Other opportunities for cost reduction

Working with a high dry matter (DM) concentration is also potentially an effective way to reduce the hydrolytic enzyme costs. However, high DM content causes an increase in viscosity, inadequate mass and heat transfer within the bioreactor, and, consequently, a strong reduction in the conversion of cellulose/hemicellulose to fermentable sugars. This problem could be overcome by adopting various fed-batch strategies or coprocessing substrates with different degrees of porosity [94].

A variation of SSF, simultaneous saccharification and co-fermentation (SSCF), in which a starch material is co-fermented, has been adopted to address low ethanol concentration issue in lignocellulosic ethanol production. SSCF can reduce ethanol production cost by increasing ethanol concentration and thus reducing distillation cost [95].

Recycling yeasts and enzymes is also an effective way to reduce the cost of ethanol production. The remaining unhydrolyzed solids with some enzymes adsorbed are collected by filtration or centrifuge and are recycled to the next cycle for further hydrolysis. In one study, the enzyme loading was reduced from 36 to 22.3 and 25.8 mg protein per gram glucan, respectively, for separate hydrolysis and fermentation (SHF) and for SSCF on AFEX™ pretreated corn stover [96]. Enzyme adsorption to the residual solids is probably inhibited at high sugar concentrations in the fast SHF process [97] and hence affected enzyme recycling. The fast SSCF process removed most of the sugars by fermentation but produced ethanol whose effect on enzyme adsorption is unclear.

10. Conclusion

Cost effect renewable fuel generation from lignocellulosic materials is one of the few options the human beings have to slow down/eliminate global warming and achieve energy independence from fossil fuels. Second generation bioethanol is a promising path in the roadmap to the future world of renewable energy. The cellulosic ethanol industry is still in its infancy and its survival is relying on heavy policy support. Major technological advances at every stage of the cellulosic ethanol production are critically needed to lower the ethanol production cost to a level comparable to the corn ethanol. The key problems that remain to be solved include: (1) Effective and low-cost biomass pretreatment method that exposes polysaccharides to enzymes for efficient saccharification, (2) efficient fermentation of all sugars (pentoses and hexoses) released during the pretreatment and hydrolysis steps into ethanol, (3) development of enzymes that tolerate various inhibitors including monosaccharides (mainly glucose), and ethanol accumulation, and (4) heat-tolerant fermentation microbes and enzymes for efficient simultaneous saccharification and fermentation.

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Conflict of interest

There is no conflict of interest involved in this work.

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