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Chapter

Bioethanol Production from Lignocellulosic Biomass

Thi Tuong An Tran, Thi Kim Phung Le, Thanh Phong Mai and Dinh Quan Nguyen

Abstract

An overview of the basic technology to produce bioethanol from lignocellulosic biomass is presented in this context. The conventional process includes two main steps. First, lignocellulose must be pretreated in order to remove lignin and enhance the penetration of hydrolysis agents without chemically destruction of cellulose and hemicellulose. Second, the pretreated material is converted to bioethanol by hydrolysis and fermentation. Some typical published studies and popular processing methods in attempts to improve the biomass conversion to bioethanol and increase the cost-effectiveness are also introduced briefly. Herein, the refinery of the resulted raw bioethanol mixture to obtain higher concentrated solution is not regarded.

Keywords: bioethanol, lignocellulose, pretreatment, hydrolysis, fermentation

1. Background

For thousands of years in the human being history, production of alcohol from natural and agricultural products with high starch or sugar contents, such as fruits, grains, sugarcane, or corn, has been well known. Microorganisms are cultivated in these carbohydrate materials to convert sugars and starches to ethanol through metabolism. Since these polysaccharides are polymers of monosaccharides, also known as single sugars, such as glucose, fructose, xylose, etc., they can be first enzymatically hydrolyzed and then fermented to ethanol by the microorganisms. Through such bioprocesses, alcoholic mixtures are obtained [1].

Nowadays, biofuels attract intensive interests from over the world due to its environmental friendliness. Biofuels are renewable as a neutral carbon source, which does not break the balance in atmosphere's air contents leading to global warming. Biofuels are among the most feasible ways to free the human being from dependence on traditional fossil resources [2].

In the last 20 years, the crude oil crisis causes an increasingly high demand of renewable energy, especially biofuels. America and Brazil are the two leading countries in producing bioethanol from sugarcane and corn. A report in 2009 claimed that Brazil produced annually 12.5 billion liters of bioethanol from sugarcane as fuels for the industry, while America also produced 5 billion liters of bioethanol from corn with establishing 111 gas stations selling E85 gasoline (a gasoline mixture composed of 85 vol.% as bioethanol) [3]. However, the production of biofuels from starches and sugars vigorously violates against the world's food security for humanity.

	Starch	Cellulose	Glycogen
Existence in the nature	Popularly found in plants as carbohydrate storage	The main component of cell wall of plants	Found in fungi and animals as their energy sources
Monomer unit	Alpha glucose	Beta glucose	Alpha glucose
Chemical structure of the polymer	1,4-Glycosidic bonds in amylose; 1,4- and 1,6-glycosidic bond in amylopectin	1,4-Glycosidic bonds	1,4- and 1,6-Glycosidic bond
Molecular morphology	Amylose as unbranched, coiled chain; amylopectin as long branched chain, partially coiled	Straight, long, unbranched chain with H bonds among adjacent chains	Short, many branched chains, partially coiled
Average molecular weight	Variable	162.14 g/mol	666.6 g/mol

Table 1.
A comparison of starch vs. cellulose and glycogen.

In an attempt to find an alternative approaches to bioethanol production, lignocellulosic biomass raises an intensive attention as cellulose is similar to starch and sugar because it is also a polymer of glucose. A comparison between three popular polysaccharides, starch, cellulose, and glycogen, is presented in **Table 1**; the most difference between cellulose and starch is their glycosidic linkages and the complex form of cellulose in plants, where it strongly incorporates with lignin and hemicellulose. In contrast, lignocellulose is the most abundant biomass in the world, which can be found as leaves, peels, bodies, branches, etc. of almost all the existing plants. Therefore, lignocellulosic bioethanol production is definitely a strategy of energy supply, especially suitable for countries with agricultural and forestry wastes to be utilized as the input materials.

2. Lignocellulose

Cellulose and hemicellulose, like starch, are made up of sugars. However, most of the cellulose in the nature is in the form of lignocellulose. Lignocellulose is a complex structure of natural materials found in plants. It represents the most abundant source of renewable organic matter on the earth. Cheap lignocellulosic biomass resources can be forestry, agricultural, and agro-industrial wastes. A variety of such materials can be mentioned here including sawdust, poplar trees, sugarcane bagasse, brewer's residue, grasses and straws, stems, leaves, husks, shells, and peels from grains, corn, sorghum, and barley. In contrast to a desire of utilizing these materials to produce valuable products, lignocellulose wastes are still accumulated every year in large quantities, causing environmental problems [3].

Lignocellulose consists of cellulose, hemicellulose, and lignin and always exists beside other extracts and mineral traces. The general composition of lignocellulose is presented in **Table 2**. In lignocellulose, cellulose fiber strands are formed by cellulose linking to each other via hydrogen bonding. The cellulose structure within the polymer is not homogenous. Crystalline regions are where cellulose nano-fibrils are organized in order and compact, while amorphous regions are disordered and easier to be hydrolyzed [1]. Cellulose fibers are like skeletons surrounded by hemicellulose and lignin (**Figure 1**). This structure naturally protects the polysaccharides from hydrolysis by enzymes and chemicals, thus raising a difficulty in both chemical and bioconversion of lignocellulose to other products, i.e., ethanol.

In lignocellulose, besides cellulose, hemicellulose is also a noticeable polysaccharide. Hemicellulose is a linear and branched heterogeneous polymer typically made up of five different sugars—L-arabinose, D-galactose, D-glucose, D-mannose, and D-xylose. The backbone of the chains of hemicelluloses can be either a homopolymer or a heteropolymer (mixture of different sugars). Hemicelluloses differ from cellulose not only by the different sugar units but also by their molecular morphology of being amorphous, where shorter chains are branching from the main chain molecules. As a result of this chemical characteristic, hemicellulose is easier to be hydrolyzed than cellulose [3].

Coating the fibrils and playing the role as an adhesive bunching the fibers in lignocellulose, lignins are high-molecular-weight, hydrophobic heteropolymer with complex and variable structures. Lignins are composed of phenylpropanoid alcohols, mainly coniferyl, sinapyl, and coumaryl alcohols with hydroxyl, methoxyl, and carbonyl functional groups (**Figure 2**). The ratio of these three monomers in lignins varies between angiosperms and gymnosperms and

Raw material	Lignin (wt.%)	Cellulose (wt.%)	Hemicellulose (wt.%)
Hardwoods	18–25	45–55	24–40
Softwoods	25–35	45–50	25–35
Grasses	10–30	25–40	25–50

Table 2.
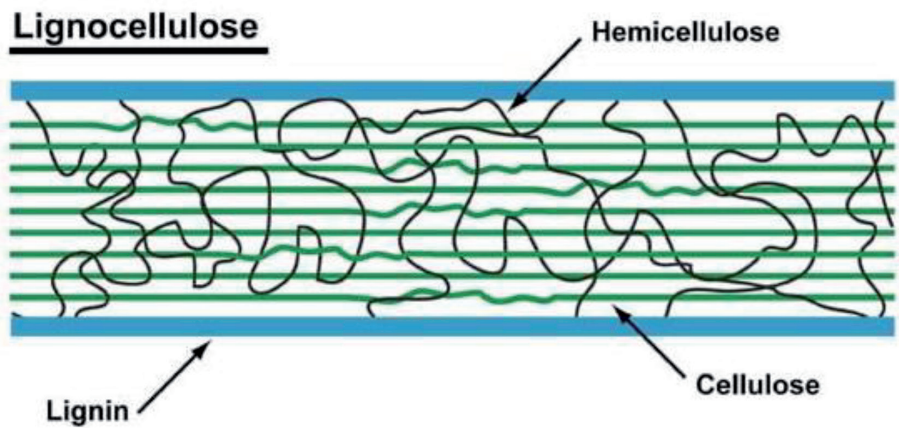*Typical chemical compositions of lignocellulosic biomass [4, 5].*

Figure 1.
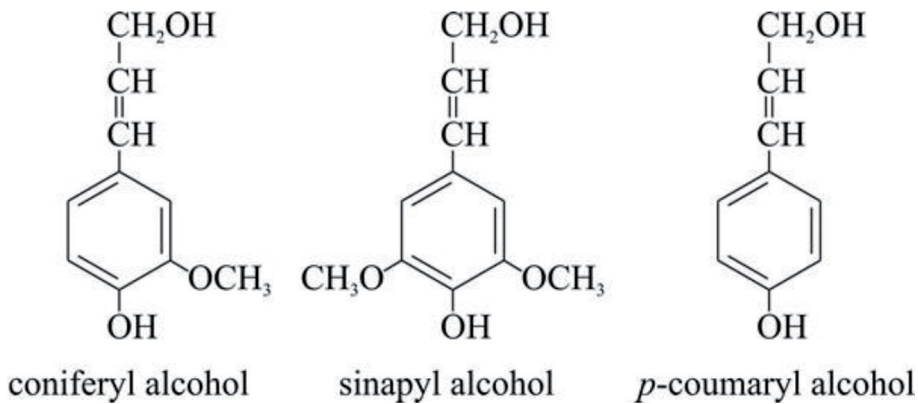*Lignocellulose and its components (Image: USDA Agricultural Research Service).*

Figure 2.
The typical aromatic alcohols as monomers of lignins.

between different plants. Lignins play a role as cross-linking cellulose and hemi-cellulose in the matrix. Lignins can be dissolved in acidic and alkaline solutions with the solubility depending on their precursors. These properties of lignins make the lignocellulose structure more robust and hard in nature, resisting hydrolysis agents. Therefore, pretreatment to remove lignins from lignocellulose and enhance the penetration of hydrolysis agents is a vital step in the process of converting biomass to bioethanol [2].

3. Conventional process of converting lignocellulose to bioethanol

For a general chemical expression of converting natural glucose-based carbohydrates to bioethanol, it can be expressed as the following:

$$(C_6H_{10}O_5)_n + n\,H_2O \rightarrow n\,C_6H_{12}O_6 \rightarrow 2n\,C2\,H_5OH + 2n\,CO_2$$

The input materials can be fruit juices, brewery residues, soya residue, peanut residue, rice, wheat, and especially lignocellulose types like straw, woods, grasses, etc. **Figure 3** presents a conventional flowchart of the process.

As mentioned above, pretreatment is a vital step followed by hydrolysis and fermentation of the pretreated lignocellulose. To hydrolyze lignocellulose, in principles, enzymes and chemicals can be employed. However, in this chapter, only enzymatic hydrolysis is the mean due to its feasibility, while the other leads to solutions with high concentration of chemicals (acid, alkaline, or salt), which is not friendly to the growth of fermentation microorganisms.
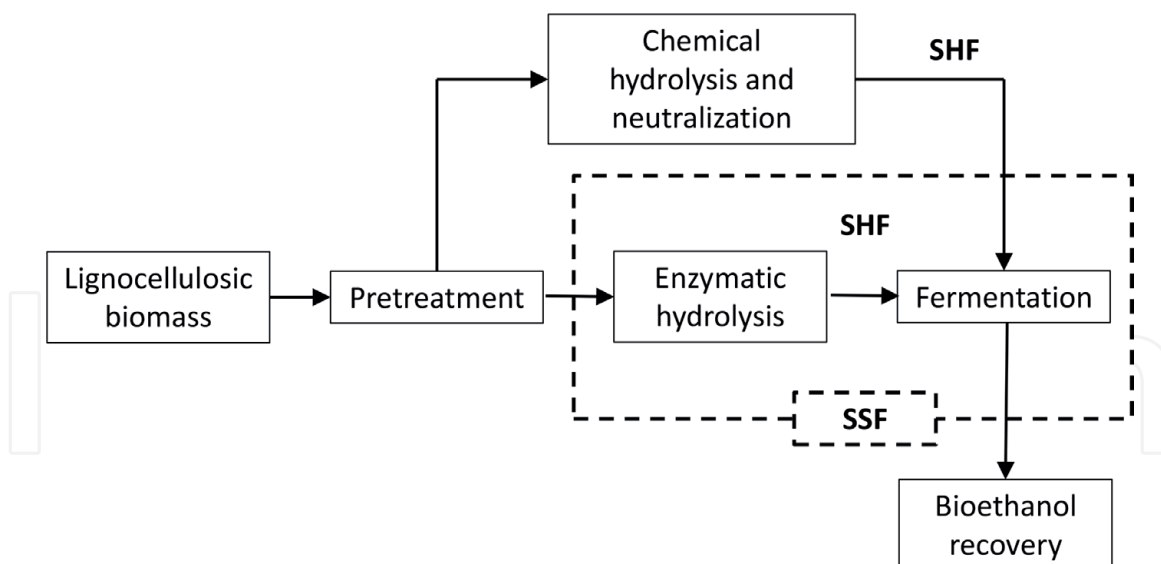

Figure 3.
Scheme of lignocellulosic ethanol production in general principle.

4. Pretreatment of lignocellulosic biomass

Pretreatment processes are applied to lignocellulose prior to hydrolysis and fermentation in order to:

• Increase the amorphous regions, which is easier to be hydrolyzed than crystalline cellulose.

- Increase the porosity of the fiber matrixes to promote the penetration of chemicals and enzymes into the structure.

- Liberate cellulose from the surroundings of lignins and hemicelluloses.

Physical, chemical, and biological pretreatment methods are used in pretreating lignocellulose. For a classification of pretreatment methods, the following are a brief summary:

4.1 Physicochemical pretreatment methods

- Mechanical processes reduce the size of the biomass and thus enhance the contact surface. Mechanical processes do not change the chemical properties of the materials. Therefore, they just can be a step to process raw materials before other steps of the pretreatment. Cutting, crushing, milling, and grinding can be carried out with specific equipment.

- Thermophysical methods: milling, steam exploding, high-pressure steaming, etc. improve the contact surface, decrease the polymerization degree of cellulose, decrease the crystallinity of cellulose, and somewhat crack the lignin cross-linking. In 1925, WH Mason invented steam explosion method to pretreat woody materials. The method was quickly applied in other industries, such as animal feedings, producing wood powder from hardwoods, veneer production, etc. [6–8]. In the 1980s, Iotech Corporation researched about steam explosion's effects on the hydrolysis of the puffed lignocellulose biomass. According to Iotech, the optimal conditions for steam explosion of natural lignocellulose were 500–550 psi with retention time of 40 seconds [9]. Shultz et al. also investigated the efficiency of steam explosion pretreatment on a variety of lignocellulosic biomass, such as hardwood chips, rice husk, corn straw, and sugar cane [10]. Effects of time, temperature, and pH during the steam explosion of poplar wood were studied with the aim of optimizing both pentose recovery and enzymatic hydrolysis efficiency [11, 12]. As a common conclusion, steam explosion pretreatment can puff the lignocellulose to enhance significantly the contact surface of the substrate, which yields much higher hydrolysis degree in the following step.

- Critical CO_2 extraction method: at the first glance, this method was expected to make a winding turn in lignocellulose pretreatment [13]. However, the system cost was too high with high-pressure equipment, which makes it not feasible for industrial production. Moreover, other similar studies showed that this method was not effective to enhance the conversion yield of the biomass to bioethanol [14, 15].

- Ionic liquid methods: ionic liquids are also known as melting organic salts with several special properties, such as nonvolatile, highly thermally durable, and, especially, cellulose dissolvable. Therefore, ionic liquid was studied to pretreat lignocellulose as a green solvent. Marzieh Shafiei et al. used 1-ethyl-3-methyl-imidazolium to pretreat hardwood for bioethanol conversion. The final conversion yield was 81.5%, very positive in terms of academy [16]. Unfortunately, this method is also not feasible for industrial scales due to the very high cost of ionic liquids.

Pretreatment by using organic solvents and surfactants to remove lignin is also in some interests, but the results have been not rather than just academic investigation or only for lignin extraction [17, 18].

4.2 Chemical methods

Aqueous acidic and alkaline solutions are known for their dissolvability of lignin. Acidic and alkaline pretreatments of lignocellulose have been so far the most effective and feasible for lignocellulosic bioethanol production technology [19, 20]:

- Acidic pretreatments: H_2SO_4 and HCl are popularly employed in pretreating lignocellulose. High-concentrated acidic solutions are not preferred because of toxicity and hydrolysis of cellulose and hemicellulose during pretreatment. Moreover, the corrosion of equipment and safety for human operators when using high-concentrated acids are the issues. Diluted acids are also not very recommended because of the generation of furfural compounds during the pretreatment process, which inhibits the growth of microorganism in fermentation process. Despite that fact, some studies still showed that acidic pretreatments with the acidic solution's concentration lower than 4.0 wt.% were still efficient and economic [21]. Lu et al. used diluted aqueous H_2SO_4 with concentrations of 2.0, 4.0, and 6.0 wt.% at 80, 60, and 120°C to pretreat corn stalks prior to fermenting the material to bioethanol. They found that an optimal concentration of acid was 2.0 wt.% to pretreat the corn stalk in 43 minutes at 120°C [22]. Cara et al. also suggested using 1.0 wt.% aqueous H_2SO_4 to pretreat dried olive tree at 170–210°C [23].

- Alkaline pretreatments: comparing to acids, pretreatment of lignocellulose with alkaline solutions minimizes the loss of carbohydrates due to hydrolysis. Alkali also helps to remove acetyl groups, promoting the later hydrolysis, and inhibit furfural formation [24]. Sodium hydroxide is the most popular alkali to pretreat lignocellulose due to its low cost and high efficiency. Calcium hydroxide is also considered because it is cheap despite low efficiency and precipitation [25].

Figure 4a–c shows the scanning electron microscopic (SEM) photos of pretreated rubber wood (*Hevea brasiliensis*) by acidic and alkaline solutions (Dr. Nguyen et al.). As seen from **Figure 4a**, before the pretreatment, the structure of rubber wood was well intact and recognized in order. After alkaline pretreatment (**Figure 4b**), the fibril structure was flaked due to loss of adhesive lignin and disorder. In addition, an alkaline pretreatment followed by acidic solution soaking like that at **Figure 4c** yielded an over-destruction of the material, where delignified cellulose was strongly hydrolyzed by the acid.

4.3 Biological methods

Employing microorganism to pretreat lignocellulose is an attractive way, promising low cost in both equipment and operation expenses. *Pleurotus*, *Pycnoporus*, *Ischnoderma*, *Phlebia*, etc. have been cultivated on lignocellulose with expectation of utilizing the metabolism of the microorganism to destruct the crystallinity of cellulose and remove lignin. However, most studies were based on laboratory scales and showed limited results [26–29].
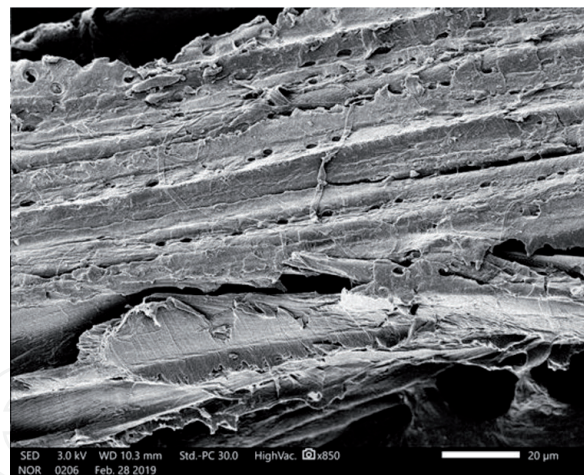
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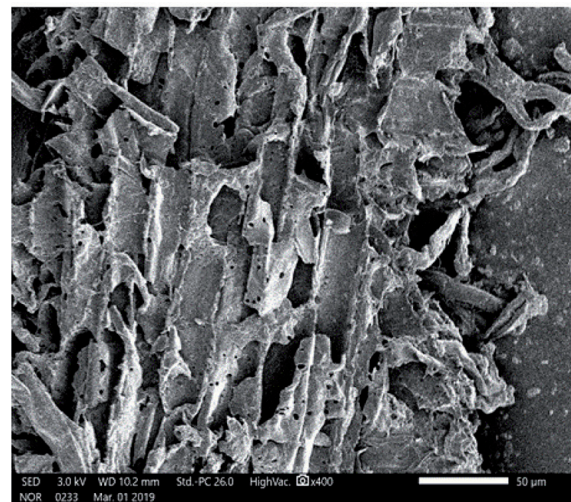
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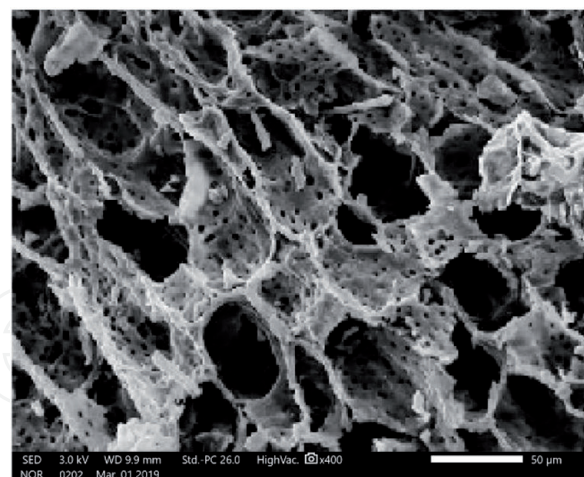
(c)

Figure 4.
(a) SEM photo of rubber wood as starting material. (b) SEM photo of rubber wood pretreated by aqueous alkaline solution (2.0 wt.% NaOH for 24 h, the ratio of dry-biomass/solution was of 1/10 wt./wt.). (c) SEM photo of alkaline-pretreated rubber wood soaked in acidic solution for 24 h (2.0 wt.% H_2SO_4, the ratio of dry-biomass/solution was of 1/10 wt./wt.).

4.4 Combination of pretreatment methods

It seems that a single pretreatment method cannot yield an expected result. The combination of pretreatment methods has been a conventional procedure

in producing bioethanol from lignocellulose. First, the biomass needs to be size-reduced by a mechanical process, such as milling, cutting, and crushing, optionally followed by steam explosion before being soaked in alkaline or acidic solution (alkaline pretreatment is more preferred). Second, the pretreated material is washed and neutralized. The final product of this step is always a puffing material, which is ready for further fermentation [30, 31]. In addition, the reusability of internal waste was also discussed to enhance the sustainability of the lignocellulosic bioethanol production and reduce chemical cost [32].

5. Conversion of pretreated lignocellulose to bioethanol

Pretreated biomass can be converted to bioethanol by both direct microbial conversion (DMC) and hydrolysis along with fermentation [33]. In fact, DMC method requires much time, while the conversion yields were rather low with high risk of contamination [34, 35]. In contrast, enzymatic hydrolysis combining microorganism fermentation is a more preferable method with proven much better performance [33].

5.1 Saccharification of lignocellulose

After lignocellulose being pretreated, the polysaccharide-enriched material is hydrolyzed to single sugars (hexoses and pentoses) with enzymes. The commercialized enzyme to hydrolyze cellulose and hemicellulose is in fact a mixture of some different kinds of enzymes, commonly called cellulase, extracted from microorganism. These enzymes cleave glycosidic linkages in carbohydrates, typically via inverting or retaining mechanisms, the latter of which proceeds via a two-step mechanism that includes formation of a glycosyl-enzyme intermediate [36].

In contrast with hemicellulose, which can be hydrolyzed more easily, cellulose is somewhat stable with crystalline structure, resisting depolymerization. To enzymatically hydrolyze cellulose effectively, the following three cellulose enzymes are popularly together employed [37]:

- Endo-1,4-β-glucanases (EG) or 1,4-β-D-glucan 4-glucanohydrolases (EC3.2.1.4). This enzyme randomly breaks the 1,4-β-glucan bonds.

- Exo-1,4-β-D-glucanases including 1,4-β-D-glucan glucohydrolase (EC 3.2.1.74), to free D-glucose from 1,4-β-D-glucan and slowly hydrolyze D-cellobiose, and enzyme 1,4-β-D-glucan cellobiohydrolase (EC 3.2.1.91) (CBH) to free cellobiose from 1,4-β-glucan.

- β-D-Glucosidase or β-D-glucoside glucohydrolase (EC 3.2.1.21) to form D-glucose from cellobiose, as well as other oligomers of glucose.

The cellulase enzyme activity can be measured by a standard method described in Technical Report NREL/TP-510-42628 (National Laboratory of the US Department of Energy Office of Energy Efficiency and Renewable Energy). Enzymatic hydrolysis gives an advantage to the growth of microorganism in fermentation although there is a little difference in optimal temperature for the two processes.

5.2 Fermentation

Microorganisms are employed to metabolize the liberated single sugars from enzymatic hydrolysis to convert them to bioethanol. There are two approaches:

- Separate hydrolysis and fermentation (SHF): the hydrolysis is carried out until finish, and then microorganisms are added to the mixture to ferment the sugars. This method has some inherent weak points, including contamination, formation of inhibitors, and requirement of more time and extra equipment.

- Simultaneous saccharification and fermentation (SSF): the enzymatic hydrolysis and microorganism fermentation are carried out in the same equipment at the same time. Both enzymes and microorganisms are loaded to the mixture. This method is proven much better than the SHF above with shorter time, less equipment, and minimized risk of contamination.

SSF is currently considered the optimal method to convert lignocellulose to bioethanol. The process is reported with high conversion yield [21]. However, there are still some small backwards of this method. The optimal temperature for enzymatic hydrolysis is 45–50°C, while fermentation is at its highest efficiency at 28–35°C. Moreover, some intermediate products also resist the growth of microorganisms [25, 38].

Different microorganisms can be employed to enhance the fermentation. **Table 3** presents popular microorganisms, which can metabolize sugars and excrete ethanol.

From **Table 3**, it is easy to understand why *Saccharomyces cerevisiae* is a favorable choice of yeast to ferment sugar solution to bioethanol. Thanks to its tolerance to high ethanol concentration and material's inhibitors, *Saccharomyces cerevisiae* is known not only a traditional but also the most popular yeast in bioethanol production.

In fermentation process, an additional nutrient is necessarily added to provide organic nitrogen source for the growth of microorganisms. Peptone, corn steep liquor (CSL), urea, and even the distillation residue of bioethanol production process have been employed and investigated [32].

Production of lignocellulosic ethanol is still cost-inefficient. In attempts to improve bioethanol fermentation yield, more than one microorganism strain can

Characteristic	Microorganism			
	E. coli	*Z. mobilis*	*Saccharomyces cerevisiae*	*Pichia stipitis*
D-Glucose fermentation	+	+	+	+
Other hexose utilization (D-galactose and D-mannose)	+	—	+	+
Pentose utilization (D-xylose and L-arabinose)	+	—	—	+
Direct hemicellulose utilization	—	—	—	w
Anaerobic fermentation	—	+	+	w
Mixed-product formation	+	w	w	w
High ethanol productivity (from glucose)	—	+	+	w
Ethanol tolerance	w	w	+	w
Tolerance to lignocellulose-derived inhibitors	w	w	+	w
Acidic pH range	—	—	+	w

+, positive; −, negative; w, weak.

Table 3.
Some popular microorganisms for bioethanol production [39].

be loaded to the fermentation mixture as simultaneous saccharification and cofermentation (SSCF) method [39]. Hereby, both hexose and pentose can be utilized to produce bioethanol.

6. Conclusion

Renewable fuels and energy are a vital demand of the human being when fossil resources are exhausted and the global warming is at the red alarming level. The production of lignocellulosic bioethanol can meet the requirement of food security and the sustainable vision of a green world. The process includes pretreatment, enzymatic hydrolysis, and fermentation stages. Intensive studies are being carried out in over the world, in order to increase the cost-effectiveness of ethanol production and to make the transition from the laboratory to the industrial/commercial scale. This brief background was written in hope to spot out some noticing information for the readers about lignocellulose-based bioethanol's technology, which currently attracts a lot of studies to shorten the gap between research and commercialization.

Acknowledgements

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References

[1] Perez J, Munoz-Dorado J, de la Rubia R, Martinez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview. International Microbiology. 2002;**5**:53-63

[2] Palmqvist E, Hahn-Hägerdal B. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanism of inhibition. Bioresource Technology. 2000;**74**:25-33

[3] Sheehan J. The road to bioethanol: A strategic perspective of the US Department of Energy's national ethanol program. In: Himmel M, Baker J, Saddler J, editors. Glycosyl Hydrolases for Biomass Conversion. Washington, D.C: American Chemical Society; 2001. pp. 2-25

[4] Betts RA, Cox PM, Lee SE, Wooward FL. Contrasting physiological and structural vegetation feedbacks in climate change simulations. Nature. 1997;**387**:796-799

[5] Malherbe A, Cloete TE. Lignocellulose biodegradation: Fundamentals and applications. Reviews in Environmental Science and Biotechnology. 2002;**1**:105-114

[6] Koullas DP, Christakopoulos P, Kekos D, Macris BJ, Koukios EG. Correlating the effect of pretreatment on the enzymatic hydrolysis of straw. Biotechnology and Bioengineering. 1992;**39**:113-l 16

[7] Azuma J, Asai T, Isaka M, Koshijima T. Effects of microwave irradiation on enzymatic susceptibility of crystalline cellulose. Journal of Fermentation Technology. 1985;**63**:529-536

[8] Ramos LP, Nazhad MM, Saddler JN. Effect of enzymatic hydrolysis on the morphology and fine structure of pretreated cellulosic residues. Enzyme and Microbial Technology. 1993;**15**:821-831

[9] Fernandes MC, Ferro MD, Paulino AF, Mendes JA, Gravitis J, Evtuguin DV, et al. Enzymatic saccharification and bioethanol production from *Cynara cardunculus* pretreated by steam explosion. Bioresource Technology. 2015;**186**:309

[10] Hernández D, Riaño B, Coca M, García-González MC. Saccharification of carbohydrates in microalgal biomass by physical, chemical and enzymatic pretreatments as a previous step for bioethanol production. Chemical Engineering Journal. 2015;**262**:939

[11] Excoffier G, Toussaint B, Vignon MR. Saccharification of steam-exploded poplar wood. Biotechnology and Bioengineering. 1991;**38**:1308-1317

[12] Puri VP, Pearce GR. Alkali-explosion pretreatment of straw and bagasse for enzymic hydrolysis. Biotechnology and Bioengineering. 1986;**28**:480-485

[13] Narayanan S et al. Pretreatment of Guayule biomass using supercritical CO_2-based method for use as fermentation feedstock. Bioresource Technology. 2010;**101**:9785

[14] Chen W-C et al. Producing bioethanol from pretreated-wood dust by simultaneous saccharification and co-fermentation process. Journal of the Taiwan Institute of Chemical Engineers. 2017;**79**:43

[15] Jeong H et al. Sugar and ethanol production from woody biomass via supercritical water hydrolysis in a continuous pilot-scale system using acid catalyst. Bioresource Technology. 2017;**245**:351

[16] Shafiei M et al. Enhancement of ethanol production from spruce wood

chips by ionic liquid pretreatment. Applied Energy. 2013;**102**:163

[17] Zhang K, Pei Z, Wang D. Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review. Bioresource Technology. 2016;**199**:21-33

[18] Chen Y, Zhou Y, Qin Y, Liu D, Zhao X. Evaluation of the action of Tween 20 non-ionic surfactant during enzymatic hydrolysis of lignocellulose: Pretreatment, hydrolysis conditions and lignin structure. Bioresource Technology. 2018;**269**:329-338

[19] Szczodrak J, Ilczuk Z, Rogalski J, Leonowicz A. Intensification of oak sawdust enzymatic hydrolysis by chemical or hydrothermal pretreatment. Biotechnology and Bioengineering. 1986;**28**:504510

[20] Farid MA, Shaker HM, El-Diwany AI. Effect of peracetic acid, sodium hydroxide and phosphoric acid on cellulosic materials as a pretreatment for enzymatic hydrolysis. Enzyme and Microbial Technology. 1983;**5**:421424

[21] Root DF, Saeman JF, Harris JF. Kinetics of the acid catalyzed conversion of xylose to furfural. Forest Products Journal. 1959;**158**:165

[22] Lu XB, Zhang YM, Yang J, Liang Y. Enzymatic hydrolysis of corn stover after pretreatment with dilute sulfuric acid. Chemical Engineering and Technology. 2007;**30**:938-944

[23] Cara C, Ruiz C, Oliva JM, Saez F, Castro E. Production of fuel ethanol from steam-explosion pretreated olive tree pruning. Bioresource Technology. 2008;**99**:1869-1876

[24] Chang VS, Holtzapple MT. Fundamental factors affecting biomass enzymatic reactivity. Applied Biochemistry and Biotechnology. 2000;**84-86**:5-37

[25] Kim S, Holtzapple MT. Effect of structural features on enzyme digestibility of corn stover. Bioresource Technology. 2006;**97**:583-591

[26] Rolz C, De Leon R, De Arriola MC, De Cabrera S. Biodelignification of lemon grass and citronella bagasse by white-rot fungi. Applied and Environmental Microbiology. 1986;**52**:607611

[27] Mes-Hartree M, Yu EKC, Reid ID, Saddler JN. Suitability of aspenwood biologically delignified with Phlebia rremellosus for fermentation to ethanol or butanediol. Applied Microbiology and Biotechnology. 1987;**26**:120-125

[28] Itoh H et al. Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi. Journal of Biotechnology. 2003;**103**:273

[29] Nazarpour FL et al. Biological pretreatment of rubberwood with Ceriporiopsis subvermispora for enzymatic hydrolysis and bioethanol production. BioMed Research International. 2013;**9**:9

[30] Tran UPN, Vu KLV, Nguyen QD, Le PTK, Phan TD, Mochidzuki K, et al. Energy balance of small-scale biorefinery system. Environmental Sciences. 2013;**26**:489-496

[31] Nguyen QD, Le TKP, Tran TAT. A technique to smartly-reuse alkaline solution in lignocellulose pre-treatment. Chemical Engineering Transactions. 2018;**63**:157-162

[32] Nguyen DQ, Vu LVK, Le TKP, Phan DT, Mochizuki K, Sakoda A. Self-reuse of distillation residue as a nitrogen source for simultaneous saccharification and fermentation in a bioethanol production process from rice straw. Environmental Sciences. 2015;**28**:335

[33] Wyman CE. Ethanol from lignocellulosic biomass: Technology, economics, and opportunities. Bioresource Technology. 1994;**50**:3-15

[34] Herrero AA, Gomez RF. Development of ethanol tolerance in *Clostridium thermocellum*: Effect of growth temperature. Applied and Environmental Microbiology. 1980;**40**:571-577

[35] Zertuche L, Zall RR. A study of producing ethanol from cellulose using Clostridium thermocellum. Biotechnology and Bioengineering. 1982;**24**:57-68

[36] Knott BC, Momeni MH, Crowley MF, Mackenzie LF, Götz AW, Sandgren M, et al. The mechanism of cellulose hydrolysis by a two-step, retaining cellobiohydrolase elucidated by structural and transition path sampling studies. Journal of the American Chemical Society. 2014;**136**:321-329

[37] Chosdu R, Hilmy NE, Erlinda TB, Abbas B. Radiation and chemical pretreatment of cellulosic waste. Radiation Physics and Chemistry. 1993;**42**:695-698

[38] Kong F, Engler CR, Soltes EJ. Effects of cell-wall acetate, xylan backbone, and lignin on enzymatic hydrolysis of aspen wood. Applied Biochemistry and Biotechnology. 1992;**34**:23-35

[39] Gírio FM et al. Hemicelluloses for fuel ethanol: A review. Bioresource Technology. 2010;**101**:4775-4800