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Research review paper

Cellulosic ethanol production: Progress, challenges and strategies for solutions

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ABSTRACT

Lignocellulosic biomass is a sustainable feedstock for fuel ethanol production, but it is characterized by low mass and energy densities, and distributed production with relatively small scales is more suitable for cellulosic ethanol, which can better balance cost for the feedstock logistics. Lignocellulosic biomass is recalcitrant to degradation, and pretreatment is needed, but more efficient pretreatment technologies should be developed based on an in-depth understanding of its biosynthesis and regulation for engineering plant cell walls with less recalcitrance. Simultaneous saccharification and co-fermentation has been developed for cellulosic ethanol production, but the concept has been mistakenly defined, since the saccharification and co-fermentation are by no means simultaneous. Lignin is unreactive, which not only occupies reactor spaces during the enzymatic hydrolysis of the cellulose component and ethanol fermentation thereafter, but also requires extra mixing, making high solid loading difficult for lignocellulosic biomass and ethanol titers substantially compromised, which consequently increases energy consumption for ethanol distillation and stillage discharge, presenting another challenge for cellulosic ethanol production. Pentose sugars released from the hydrolysis of hemicelluloses are not fermentable with *Saccharomyces cerevisiae* used for ethanol production from sugar- and starch-based feedstocks, and engineering the brewing yeast and other ethanologenic species such as *Zymomonas mobilis* with pentose metabolism has been performed within the past decades. However strategies for the simultaneous co-fermentation of pentose and hexose sugars that have been pursued overwhelmingly for strain development might be modified for robust ethanol production. Finally, unit integration and system optimization are needed to maximize economic and environmental benefits for cellulosic ethanol production. In this article, we critically reviewed updated progress, and highlighted challenges and strategies for solutions.

1. Introduction

Fuel ethanol is the largest liquid biofuel in volume so far, which is produced predominantly from sugar- and starch-based feedstocks (1G fuel ethanol) for blending with gasoline. The US and Brazil are the largest fuel ethanol producers in the world. While the US produces fuel ethanol mainly from corn with a total production capacity of ~57.7 billion liters in 2016, Brazil produces fuel ethanol from sugarcane juice or molasses, with a total production capacity of ~27.6 billion liters in the same year (Mohanty and Swain, 2019). 1G fuel ethanol is unlikely to be sustainable, when its production capacity is expanded substantially to address concerns on the sustainable supply of petroleum-based transportation fuels as well as environmental issues associated with their consumption, taking into account of increased population and consequent demand for food supply, especially in developing countries such as China and India with large population. In fact, debates for the impact of 1G fuel ethanol on food security and biodiversity associated with deforestation for sugar and corn production to support the biofuel industry have never been mitigated within the past decade (Naylor et al., 2007; Ramos et al., 2016).

Lignocellulosic biomass, particularly agricultural residues, is non-food related and abundantly available without geographical limitation.

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The consumption of lignocellulosic biomass at large scales does not compete for food with people, directly or indirectly through diverting the use of arable land as that might occur in fuel ethanol production from sugar and grains, making it a sustainable feedstock for producing 2G fuel ethanol (Gupta and Verma, 2015). Major components in lignocellulosic biomass are cellulose, hemicelluloses and lignin, which are entangled together to form lignin-carbohydrate complexes (LCCs). LCCs are recalcitrant to degradation, and thus pretreatment is needed for their destruction to separate the cellulose component from hemicelluloses and lignin so that the enzymatic hydrolysis can be performed to release glucose for ethanol fermentation (Chundawat et al., 2011). Although inhibitors may be generated from lignin during the pretreatment (Jönsson and Martín, 2016), its presence is a limiting factor for high solid loading (Nguyen et al., 2017). As a result, high solid loading cannot be performed effectively for lignocellulosic biomass, and ethanol titers achieved for cellulosic ethanol production are much lower, about 50% of that achieved for ethanol fermentation from grains (Koppram et al., 2014). This disadvantage increases energy consumption for ethanol recovery by distillation, and subsequently discharges large amounts of stillage to be treated properly.

Moreover, significant amounts of pentose sugars such as xylose and arabinose are produced during the hydrolysis of hemicelluloses (Scheller and Ulvskov, 2010), but they are not fermentable with the brewing yeast *Saccharomyces cerevisiae* currently used for ethanol production from sugar and grains, which not only compromises ethanol yield on total sugars in lignocellulosic biomass, but also increases workload for stillage treatment. Although different strategies have been explored for utilizing those pentose sugars to produce other products, none of them is economically competitive, and recombinant strains engineered with pentose metabolism to produce ethanol are still needed for cellulosic ethanol production (Avanthi et al., 2017).

The characteristic of lignocellulosic biomass makes the production of 2G fuel ethanol substantially different from those established processes for 1G fuel ethanol production from sugar and grains. In this article, cutting-edge technologies for cellulosic ethanol production are critically reviewed, with a focus on the logistics of lignocellulosic biomass and its structure, leading pretreatment technologies, enzymatic hydrolysis of the cellulose component, co-fermentation of hexose and pentose sugars, stillage treatment and process development through unit integration and system optimization. Strategies for addressing challenges are also highlighted.

2. Lignocellulosic biomass

Lignocellulosic biomass is scattered on the ground (Fig. 1a), and characterized by low mass and energy densities for large volumes and high cost in logistics, particularly for agricultural residues such as corn stover, wheat and rice straw preferred for 2G fuel ethanol production. This not only increases costs in collecting, storage and transportation, but also determines the design and operation of cellulosic ethanol production: plants with relatively small processing capacities should be distributed and located closely to the feedstock supply to better balance the logistic cost (Marvin et al., 2012; Ebadian et al., 2013; Zhang et al., 2018). Compared to sugar- and starch-based feedstocks, lignocellulosic biomass is more complicated, and understanding of its structure, particularly at molecular levels, is a prerequisite for developing effective pretreatment technologies to destruct the LCCs with the following purposes: 1) producing less inhibitors to improve yields of sugars, particularly sugars released during the hydrolysis of hemicelluloses; 2) designing enzymatic hydrolysis of the cellulose component to further liberate glucose; 3) engineering microorganisms for robust ethanol production by utilizing major sugars more efficiently and tolerating the inhibitors better with less demand for detoxification.

Lignocellulosic biomass is mainly from plant cell walls, which compose mainly of structural carbohydrates of cellulose and hemicelluloses as well as phenolic polymer lignin. Although contents of these major components vary, depending on plant species and varieties, climate and soil conditions and fertilization practices, for agricultural residues such as corn stover and wheat and rice straw, they contain 30–40% cellulose, 20–30% hemicelluloses and 10–20% lignin (Zabed et al., 2016). The distinctive feature of plant cell walls is the two-part structure, which is highlighted in Fig. 1b (Zhao et al., 2012).

Primary cell walls are formed during plant growth. Cellulose synthesized within the cytoplasm from glucose by the cellulose synthase complex is transported across the membrane, which further develops as an amorphous structure to be embedded into the matrix composed mainly of hemicelluloses and lignin as the major part of the primary cell walls (Verbanćić et al., 2018). When the growth of plant cells is slowing or stopped, secondary cell walls are gradually developed between the plasma membranes and primary cell walls by incorporating more crystalline cellulose microfibrils into the xylem fibers, providing support for plants (Kumar et al., 2015). The formation of vascular tissue and deposition of cellulose microfibrils within the secondary cell walls are the results of evolution for land plants, which not only benefit the transport of water and many other nutrients absorbed through their roots, but also eventually support their upright growth (Yang and Wang, 2016; Li et al., 2016a). However, such a delicate evolution creates recalcitrance to degradation for the secondary cell walls to protect plants from invasion as well as the conversion of lignocellulosic biomass into fuels and chemicals (Gilna et al., 2017).

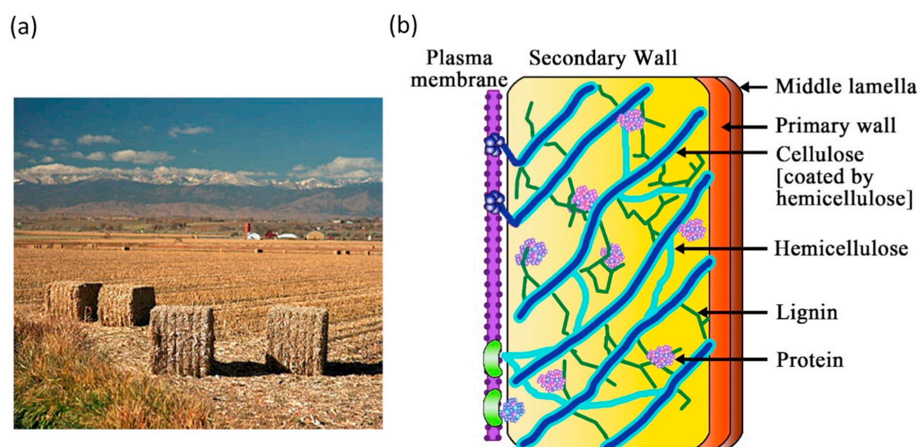


Fig. 1. Corn stover distributed in the field downloaded from public website with unknown resource (a) and the schematic diagram of plant cell walls (b) that was adapted with permission from Zhao et al., 2012

2.1. Cellulose

As the largest carbohydrate component in lignocellulosic biomass, cellulose is a homogeneous glucan formed by β-1,4-glycosidic bonds with cellobiose as the repeating unit, presenting with different degrees of polymerization (DP) (Klemm et al., 2005). Although polymorphs exist with cellulose, X-ray diffraction analysis indicates that native cellulose is with cellulose I composed of I_α and I_β (Poletto et al., 2014). While cellulose I_α is synthesized through the extension of the microfibrils, and seems prevailing in bacterial species and lower plants (Ruan et al., 2016), cellulose I_β is the major component of plant cell walls with higher plants for toughness and strength (Oehme et al., 2015). The elucidation on the molecular structures of cellulose, particularly its crystallinity, indicates that cellulose I_α is formed by the triclinic unit with only one chain, but there are two chains in the monoclinic unit of cellulose I_β for forming more intramolecular hydrogen bonds, making it more stable and recalcitrant to degradation. As a result, harsh reaction conditions are required for transforming cellulose I_β in lignocellulosic biomass into an amorphous polymorph characterized by a lower crystallinity index (CI) for more efficient hydrolysis by cellulases (Park et al., 2010).

2.2. Hemicelluloses

Hemicelluloses are the second largest carbohydrate in lignocellulosic biomass, but they are heterogeneous polysaccharides (Scheller and Ulvskov, 2010). Although several pentose and hexose sugars have been observed in hemicelluloses for different plant species, which are linked through β-1,4-glycosidic bonds as backbones, xyloglucan and xylan with glucose and xylose residues as monomers for their backbones are major components of hemicelluloses in all plants (Höfte and Voxeur, 2017). The structural similarity between hemicelluloses (Fig. 2) and cellulose benefits from a conformational homology, leading to a strong non-covalent connection between hemicelluloses and cellulose microfibrils for hemicelluloses to be embedded and interweaved with cellulose and lignin, providing strength and toughness for plant cell walls and recalcitrance to degradation as well. However, hemicelluloses are branched in nature through their side chains, creating an amorphous structure different from the linear and crystalline structure of cellulose. Therefore, hemicelluloses can be hydrolyzed into monomer sugars easily during the pretreatment of lignocellulosic biomass.

While different sugar residues are observed in hemicelluloses, xylose and arabinose are the most abundant pentose sugars. As a result, arabinose is the second largest pentose sugar only after xylose in the hydrolysate of lignocellulosic biomass, and its utilization should be targeted for strain development. On the other hand, acetylation frequently occurs during the biosynthesis of hemicelluloses, particularly on their galactose residues (Scheller and Ulvskov, 2010), and consequently acetic acid is a major byproduct of the hydrolysis of hemicelluloses, which inhibits microbial growth and ethanol fermentation. Therefore, understanding the structures of hemicelluloses and the products and byproducts of their hydrolysis is a prerequisite for developing robust strains for cellulosic ethanol production.

2.3. Lignin

Lignin is a non-sugar based polymer, which cannot be used as a feedstock for ethanol production through microbial fermentation, but it significantly affects the techno-economic performance of 2G fuel ethanol production (Nguyen et al., 2017). On the one hand, phenolic inhibitors of microbial growth and ethanol fermentation are produced mainly from the degradation of lignin during the pretreatment (Jönsson and Martín, 2016). On the other hand, lignin might deposit onto cellulose to make its surface inaccessible, or even adsorb cellulases, which inevitably compromises the effectiveness of the enzymatic hydrolysis of cellulose (Li et al., 2013; Martín-Sampedro et al., 2013).

From the viewpoint of bioprocess engineering, lignin is an unreactive component for cellulosic ethanol production, which not only unproductively occupies reactor spaces during the enzymatic hydrolysis of the cellulose component and ethanol fermentation thereafter, but also requires extra mixing with more energy input for homogeneous suspension of the fermentation broth. For 1G fuel ethanol production, energy consumption for mixing is negligible, since it is performed naturally by CO_2 produced during ethanol fermentation for relatively small fermenters equipped with internal cooling coils, but agitation through the circulation of the fermentation broth by pumps for cooling benefits the mixing for large fermenters equipped with external heat exchangers (Kelsall and Lyons, 2003a).

Therefore, it is difficult for cellulosic ethanol to be performed under high gravity (HG) conditions for high ethanol titers as that achieved in 1G fuel ethanol production, and consequently ethanol recovery by distillation is more energy-intensive with more stillage discharged. However, lignin yields more energy when it burns, and thus is an excellent fuel for heat and power production to drive cellulosic ethanol production (Lythcke-Jøgensen et al., 2014). In addition, lignin is a starting material for producing various products including transportation fuels and value-added chemicals through chemical catalysis to credit cellulosic ethanol production, if it can be valorized properly (Ragauskas et al., 2014).

Understanding of fundamentals underlying lignin biosynthesis is a basis for developing more efficient pretreatment process, enzymatic hydrolysis of the cellulose component and engineering microorganisms with improved tolerance to phenolic inhibitors. Monomers for lignin biosynthesis are monolignols such as coniferyl, sinapyl and *p*-coumaryl alcohols. These monolignols are synthesized as secondary metabolites in cytoplasm from phenylalanine through deamination to form cinnamic acid, which is further modified on the aromatic ring through hydroxylation and *O*-methylation and the reduction of the side chain to finally form an alcohol moiety (Bonawitz and Chapple, 2010). When the monolignols are exported out of the plasma membrane, their polymerization starts in the apoplast with the formation of the phenoxy radicals catalyzed by oxidative enzymes such as laccase and peroxidase, and the process proceeds through cross-coupling reactions with radicals formed on the free-phenolic ends (Fig. 3). The ratio of monolignols changes among plant species and tissues and subcellular organelles even for the same plant, which is also affected by stages with plant development and environmental conditions. In addition to coniferyl, sinapyl and *p*-coumaryl alcohols, other compounds such as ferulates, coniferaldehyde and acylated monolignols may also be involved in lignin biosynthesis, which could be liberated as inhibitors during biomass pretreatment (Tobimatsu and Schuetz, 2019).

2.4. Others components

Lignocellulosic biomass also contains proteins and ashes, which affect cellulosic ethanol production economically, but haven't been addressed adequately up till now, since no commercial production of 2G fuel ethanol has been operated stably. For example, extra nutrients are required for nourishing ethanologenic microorganisms, either *S. cerevisiae* or *Zymomonas mobilis* engineered for cellulosic ethanol production, due to insufficient nutrients in the feedstock, which consequently increases cost for 2G fuel ethanol production.

Nutritional components also vary in lignocellulosic biomass with species and varieties as well as environmental conditions. For major agricultural wastes such as corn stover, wheat and rice straw, the content of proteins is 3–5% (Chundawat et al., 2011), much lower than that in grains. Both *S. cerevisiae* and *Z. mobilis* cannot take up these proteins. Therefore, supplementation of proteases that has been practiced in fuel ethanol production from starch-based feedstock is also applicable for cellulosic ethanol production to hydrolyze the proteins as nitrogen sources for microbial growth during ethanol fermentation. In

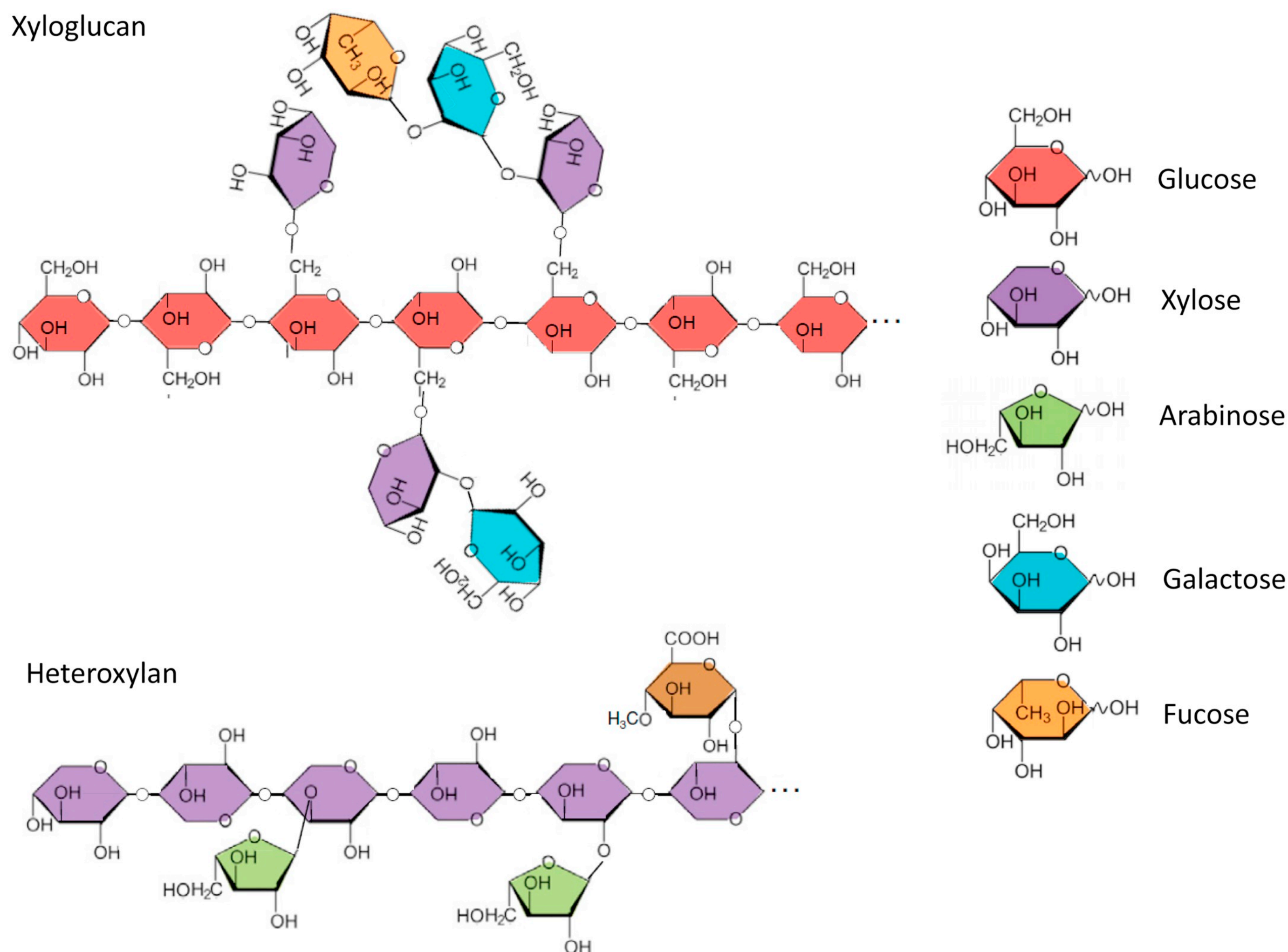


Fig. 2. Diagrams for xyloglucan and heteroxylan in hemicelluloses that were adapted with permission from Höfte and Voxeur (2017).

addition, nitrogen supplementation from ammonia or urea is also needed for cellulosic ethanol production, but corn steep liquor (CSL), a by-product of the corn wet-milling process, is a cost-effective nutrient, not only for assimilable nitrogen sources but also for trace minerals and vitamins, particular for ethanol production from corn stover, since CSL is conveniently available. As for mineral components, they are usually enough due to high ash contents in lignocellulosic biomass.

2.5. Engineering plant cell walls

Reduced recalcitrance of plant cell walls whilst increasing their carbohydrate contents are preferred for producing cellulosic ethanol, other biofuels and bio-based products, if these modifications do not significantly affect normal growth and development of the plants for grain production. Recently, major advances in the understanding of fundamentals underlying the biosynthesis of various cell wall polymers and its regulation have enabled the development of strategies for altering their composition, making them less recalcitrant to degradation, such as the modification of LCCs to enhance polysaccharide accessibility, reduction of polymer derived inhibitors and increase in polysaccharides with a high ratio of hexose over pentose sugars, which were reviewed respectively by Loqué et al. (2015) and Johnson et al. (2018). Research progress in this regard is highlighted through the work performed by Li et al. (2017), in which a mutation on the conserved site of cellulose synthase led to enhanced biomass enzymatic saccharification by reducing the cellulose DP and crystallinity in rice. Alternatively, the overproduction of native *endo*-β-1,4-glucanases in transgenic rice for specific modifications of the cellulose structure in the cell walls enhanced saccharification of the rice straw and bioethanol production (Huang et al., 2019). Although these progress could benefit cellulosic ethanol production, risks with genetically modified organisms in food production and consumption should be fully taken into account.

3. Pretreatment

Crystalline cellulose microfibrils entangled and interacted with hemicelluloses and lignin creates LCCs in plant cell walls (Chundawat et al., 2011), making cellulose inaccessible for cellulases to bind onto its surface for the enzymatic hydrolysis. After a preliminary size reduction, pretreatment is required for destructing LCCs so that the cellulose component can be exposed for enzymatic hydrolysis by cellulases.

3.1. Physicochemical pretreatment

Pure physical pretreatment does not use chemicals. The size reduction by mechanical methods such as chopping is one of them, through which the surface of lignocellulosic biomass is increased, and the DP and the crystallinity of cellulose may also decrease to some extent under ultra-fine milling conditions. However, energy consumption for reducing the feedstock from the size of millimeters to fine particles of micrometers is extremely high, which is unacceptable from the viewpoint of engineering design and process operation. Radiations

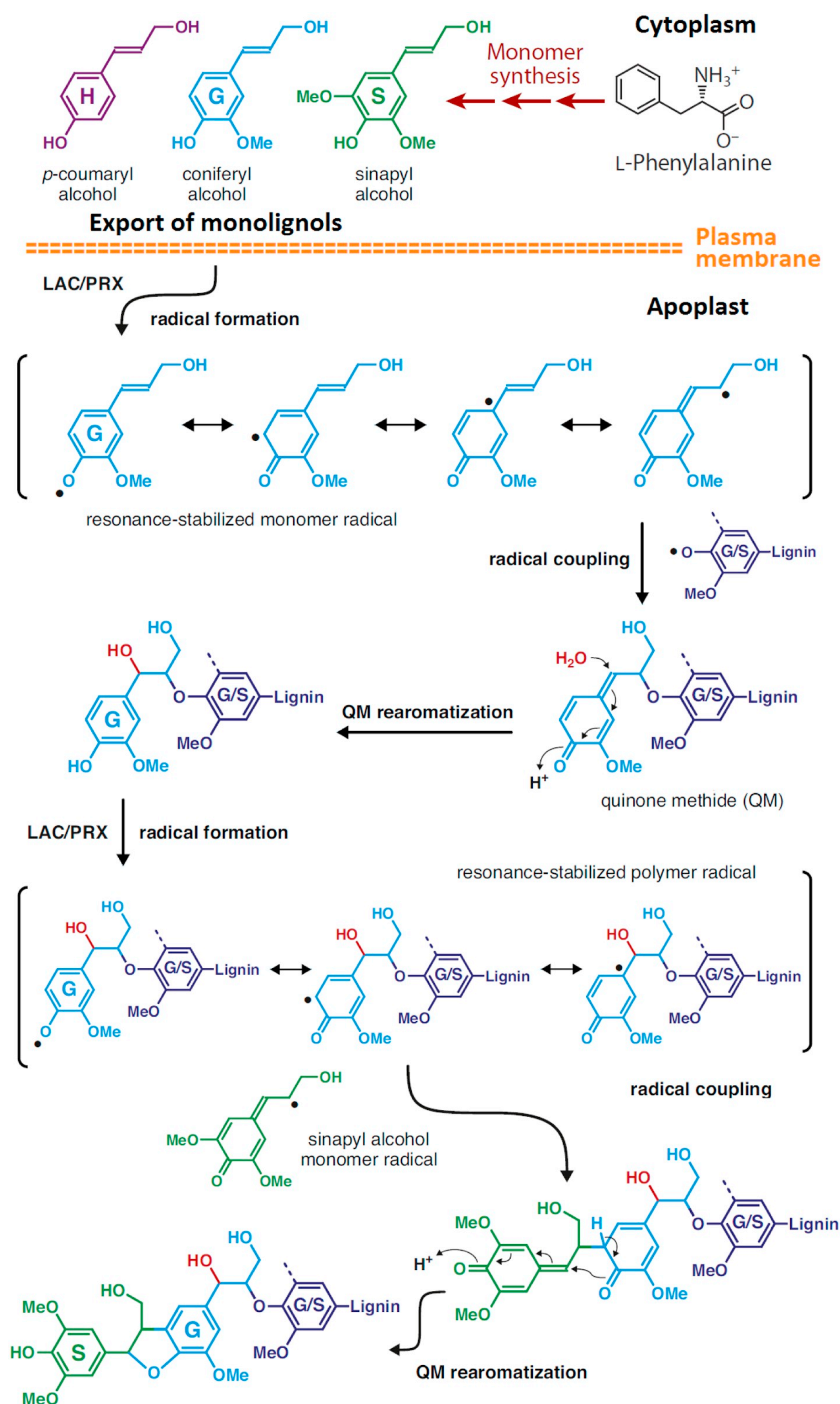


Fig. 3. Diagram for lignin biosynthesis that was adapted with permissions from Bonawitz and Chapple (2010) and Tobimatsu and Schuetz (2019). LAC: laccase, PRX: peroxidase, H: *p*-hydroxyphenyl alcohol, G: guaiacyl alcohol, and S: syringyl alcohol.

such as microwave that can penetrate and heat lignocellulosic biomass quickly have also been investigated (Puligundla et al., 2016; Aguilar-Reynosa et al., 2017), but they are impractical for processing the feedstock at mass quantity for cellulosic ethanol production, due to high capital investment on the facilities for generating the radiations as well as protecting workers from the radiation risks. Therefore, more attention has been focused on hydrothermal pretreatment, during which physicochemical reactions occur to destruct LCCs through autocatalysis, particularly the hydrolysis of hemicelluloses to create pores and expose cellulose for enzymatic hydrolysis.

Pretreatment with liquid hot water (LHW) is one of this kind of physicochemical processes, and both batch and continuous reactors have been developed (Fig. 4). For batch operation, standard Parr reactors have been widely employed for lab research, but solid loading is

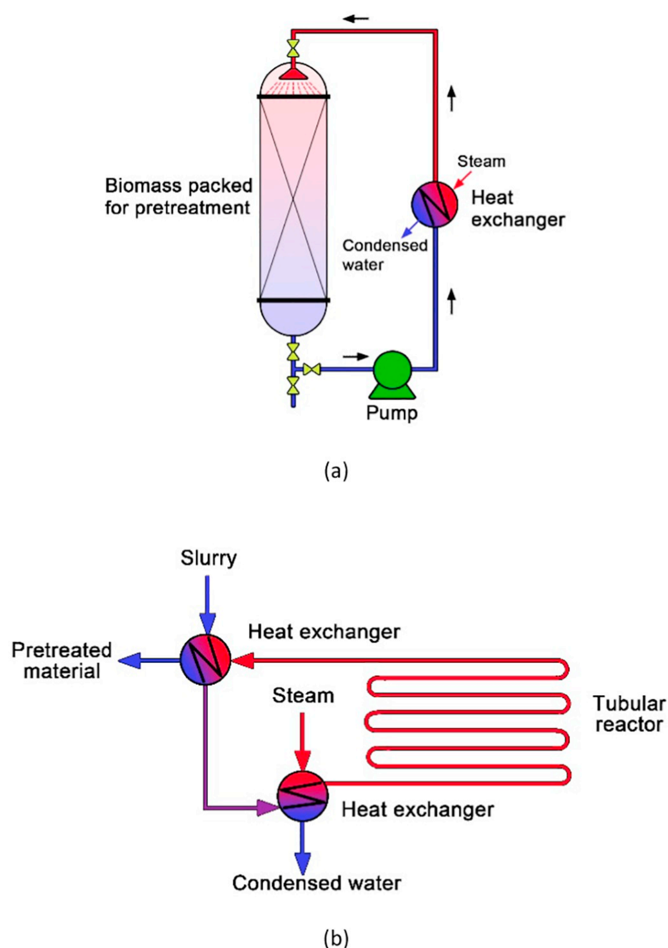


Fig. 4. Liquid hot water pretreatment at batch (a) and continuous operation (b) that was adapted with permission from Zhao et al., 2012.

limited due to the difficulty in mixing. Alternatively, packed bed reactors developed for chemical catalytic reactions can be used for the LHW pretreatment by packing lignocellulosic biomass within the reactors at high solid loading, and then circulating LHW for complete impregnation (Yan et al., 2016). However, tubular reactors are needed for continuous LHW pretreatment, and the problem for such a process is much lower solid loading, even lower than that with stirring reactors for batch LHW pretreatment, to guarantee the flow within the reactors, particularly at relatively high speed to make their mixing performance close to a plug flow. The underlying mechanism of the LHW pretreatment is assumed to be the partial degradation of LCCs catalyzed by organic acids such as acetic acid released from the hydrolysis of acetylated hemicelluloses, making the process autohydrolytic in nature (Jiang et al., 2016). As a result, the pH of the slurry could drop below 4, resulting in the formation of inhibitors due to the degradation of sugars under acidic conditions, and a pH control strategy can be applied to maintain the pH properly by adding base (Li et al., 2014).

Another physicochemical pretreatment of lignocellulosic biomass is steam explosion (SE), during which the feedstock is heated by saturated steam, maintained for a short period of time at designated temperature and pressure, and then followed by depressurizing instantly to disrupt LCCs by explosion (Liu et al., 2014). In addition to the autohydrolysis of hemicelluloses by weak acids as that occurs similar to LHW pretreatment, lignocellulosic biomass is torn up by forces created during the depressurizing process, and hydrolyzed products are released efficiently for cellulose to be exposed more completely (Rodríguez et al., 2017; Auxenfans et al., 2017). Due to its advantages of lower investment in facilities, less impact on the environment and simple process design and operation, SE has been intensively studied and tested at pilot scales around the world, which is highlighted in the technical report: *Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol*, which was released and updated irregularly by National Renewable Energy Laboratory (NREL) (https://www.nrel.gov/docs/fy11osti/47764.pdf), USA. Acid (H_2SO_4) can be supplemented into the SE process at low concentrations so that it can be operated at less severe conditions to improve sugar yield, making the process a hybrid of physicochemical and chemical reactions in nature. In general, batch operation is preferred for SE, since continuous operation makes engineering design more complicated with substantially higher capital investment on the facilities, and multi-tanks can be used alternatively.

3.2. Chemical pretreatment using acids and alkalis

High temperatures used in the LHW and SE processes dehydrate sugars and produce inhibitors (Jönsson and Martín, 2016), but acid or alkali can be used to destruct LCCs under less severe conditions. Although concentrated acid can hydrolyze cellulose near completely at lower temperature, the process not only generates more byproducts, but also is not green, since acid recovery presents a big risk to the environment. Therefore, dilute acid is preferred for the pretreatment of lignocellulosic biomass through hydrolyzing hemicelluloses, and delignification by alkaline is another strategy (Loow et al., 2016).

Dilute acid pretreatment has been intensively studied for various lignocellulosic biomass feedstocks (Sievers et al., 2017; Tizazu and Moholkar, 2018; Djioleu and Carrier, 2016). Although research progress is still trickling in, most detailed data for dilute acid pretreatment are available in the technical report released by NREL: *Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol* (https://www.nrel.gov/docs/fy11osti/47764.pdf). Dilute acid pretreatment is effective for hemicelluloses hydrolysis to destruct the LCCs, but corrosion that requires expensive acid-resistant stainless steel or coating is intrinsic to the process. Although less inhibitors are produced, they still inhibit microbial fermentation. Another issue is that lignin cannot be separated and removed during dilute acid pretreatment, and high solid loading for enzymatic hydrolysis of the cellulose component is problematic from the viewpoint of bioprocess engineering due to the non-Newtonian fluid behaviors of the mash and its poor mixing performance.

Alkaline pretreatment can address challenges observed in dilute acid pretreatment, and various alkalis including NaOH, $Ca(OH)_2$ and NH_3 have been explored for this purpose. Principally, alkaline pretreatment is a delignification process with a long history in paper making, which has been termed as the kraft process. Compared to dilute acid pretreatment, alkaline pretreatment is performed at lower temperature and pressure. The underlying mechanism is the de-esterification or saponification of intermolecular ester bonds among lignin, cellulose and hemicelluloses, together with the solvation of lignin through dissolution and degradation, to destruct LCCs and reduce the DP of the cellulose component (Kim et al., 2016). The effectiveness of alkaline pretreatment depends on the characteristic of lignocellulosic biomass, alkali used and reaction conditions applied to the process.

NaOH is one of the strongest bases, which has been intensively studied for the pretreatment of lignocellulosic biomass, and its effectiveness to various feedstocks including corn stover, sugarcane bagasse and Switchgrass is evident due to its strong reactivity to lignin (Zhao et al., 2018; Wang et al., 2016a; Jung et al., 2018). However, significant amounts of salts produced during the process are a big problem, since they inhibits microbial growth and ethanol fermentation if not washed away properly, producing more wastewater. Moreover, the black liquor with lignin dissolved raises similar environmental concerns as those challenging the pulping industry. An alternative solution for these problems is to replace sodium hydroxide with ammonia, but ammonia is a weak base, which is less effective for the delignification of lignocellulosic biomass. In general, pressurized reactors for ammonia to

penetrate into lignocellulosic biomass more effectively are preferred (Jin et al., 2016).

Among various technologies that have been developed for the pretreatment of lignocellulosic biomass by ammonia, ammonia fiber explosion/expansion (AFEX), a combination of ammonia pretreatment and explosion, seems most promising due to its relatively high productivity (Jin et al., 2016). Lignocellulosic biomass is pretreated with ammonia at mild temperature and high pressure within the AFEX reactor. When it is discharged with pressure released, ammonia gas is expanded instantly, causing a swell of the pretreated feedstock to disrupt LCCs for more accessible surfaces of the cellulose component, and in the meantime releases ammonia for recovery. Since temperature for AFEX is much lower than that applied to SE, energy consumption for heating can be saved, and less inhibitors are produced. Moreover, washing is not needed for biomass pretreated by ammonia so that high solid loading can be applied to enzymatic hydrolysis of the cellulose component. Furthermore, ammonia remaining with the pretreated biomass can be used as nitrogen source for microbial growth during ethanol fermentation. Based on the AFEX process, extractive ammonia was developed, through which lignin was extracted more efficiently, not only making the cellulose component more accessible for enzymatic hydrolysis to save the enzyme dosage, but also separating lignin for valorization (Da Costa-Sousa et al., 2016). Recently, a hybrid process was configured for the pretreatment of corn stover, in which ammonia impregnation was followed by the treatment with dilute alkali of 0.1 M NaOH to minimize salt production, which enhanced the lignin fractionation (Mittal et al., 2017).

3.3. Solvent extraction

Solvent extraction is a fractioning process, and an organic solvent is usually used to destruct LCCs (Zhang et al., 2016). Among various solvents, alcohols such as ethanol with low boiling points are favored because of their easy recovery at low cost, but high boiling point alcohols including butanol for the process to be operated at relatively high temperatures are more effective (Lancefield et al., 2017). Technically, either cellulose or lignin can be targeted for solvent extraction. Compared with physicochemical and chemical pretreatment, relatively mild temperature and pressure and a neutral pH environment applied to the solvent pretreatment of lignocellulosic biomass reduce carbohydrate degradation into undesired byproducts. Another advantage of solvent extraction is that a pure lignin fragment could be recovered for valorization.

Ionic liquids (ILs) and IL-based solvent systems have been developed for biomass pretreatment through selective dissolution of cellulose or lignin (Hou et al., 2017). ILs are molten salts composed completely of paired ions, which are in liquid state at low temperatures below 100 °C in general, particularly at room temperature. Recently, they were used for simultaneous pretreatment of lignocellulosic biomass and enzymatic hydrolysis of the cellulose component by cellulases (Elgharbawy et al., 2016). With the understanding of the chemistry of the anions and cations and their interactions, various ILs can be designed to dissolve either cellulose or lignin from lignocellulosic biomass, and consequently destruct LCCs for enzymatic hydrolysis (George et al., 2015).

Compared to conventional solvents, IL-based solvents are more environmentally friendly. Furthermore, the recovery of ILs is less energy-intensive compared to distillation or evaporation for recovering conventional solvents, since dissolved cellulose or lignin can be precipitated by adding specially designed anti-solvents. Vapor pressure of ILs is also very low, and thus less hazards are released into workshops and the environment. However, there are still many challenges for ILs to be economically competitive for pretreating lignocellulosic biomass to produce bulk commodities like fuel ethanol, and reagents derived from lignocellulosic biomass for synthesizing ILs at low cost might be a solution (Socha et al., 2014).

3.4. Biological pretreatment

Compared to major pretreatment technologies reviewed previously, biological pretreatment through solid fermentation employs microorganisms, which selectively degrade lignin in lignocellulosic biomass, exposing the cellulose component for enzymatic hydrolysis at mild conditions without special requirements for instruments (Sindhu et al., 2016). Both bacteria and fungi have been explored for such a purpose, but rot fungi associated with wood rot are predominant species of lignin degradation (García-Torreiro et al., 2016).

Although biological pretreatment is energy-saving and environmentally friendly, its disadvantages are apparent. Firstly, extremely low degradation rate requires time as long as weeks for a significant change in the structure of LCCs, making the process unmatchable with the subsequent hydrolysis of the cellulose component and ethanol fermentation. Secondly, significant amounts of biomass components are lost during the process. Not only is lignin mineralized into low-molecular weight fragments which would be further catabolized into useless final product CO_2, but also sugars released from hemicelluloses, even cellulose by hydrolytic enzymes (simultaneous decay with lignin degradation) are consumed to support microbial growth (Asina et al., 2016). Finally, it is unreliable to control microbial growth and metabolism under open and solid fermentation conditions with mixed species, which inevitably increases contamination risks for subsequent cellulose hydrolysis and ethanol fermentation. Unless these problems are well addressed, biological pretreatment would not be practical for 2G fuel ethanol production at commercial scales from the viewpoint of engineering design and process operation.

4. Enzymatic hydrolysis of the cellulose component

After pretreatment, enzymatic hydrolysis is succeeded to hydrolyze the cellulose component into glucose for ethanol fermentation. Although intensive R & D efforts have been performed worldwide for decades, two barriers still remain to be overcome for developing a viable process to make the sugar platform economically competitive.

Unlike amylases and glucoamylases that are available at low prices for starch hydrolysis, cellulases for cellulose hydrolysis are much more expensive. *Trichoderma reesei* has been acknowledged as the best species for cellulase production, and studied for more than 70 years (Bischof et al., 2016). However, the biosynthesis efficiency of cellulases by the fungal species under induction conditions is still low, and energy consumption associated with the submerged culture for the large scale production of cellulases is extremely high due to the non-Newtonian fluid behaviors developed as the mycelia grow (Li et al., 2016). Since cellulose hydrolysis is a synergetic process coordinated through different cellulolytic enzymes, engineering *T. reesei* with improved performance for individual enzymes and optimized ratio of those cellulolytic enzymes to hydrolyze cellulose more efficiently is an ultimate solution, which was reviewed recently by Druzhinina and Kubicek (2017). In addition, the enzymatic hydrolysis is heterogeneous in nature, which substantially compromises the reaction rate, and high enzyme dosage is needed for cellulose hydrolysis (Jeoh et al., 2017). As a result, glucose released from lignocellulosic biomass is by no means cheaper, although the feedstock is not expensive.

4.1. Separate hydrolysis and co-fermentation

For separate hydrolysis and co-fermentation (SHCF), the cellulose component is completely hydrolyzed into glucose by cellulases under optimal conditions, particularly temperature around 50 °C optimal for the enzymatic hydrolysis catalyzed with cellulases produced by *T. reesei* (Bischof et al., 2016). However, such a high temperature cannot be tolerated by *S. cerevisiae* or *Z. mobilis*, which performs ethanol fermentation at temperatures generally below 35 °C (Bai et al., 2008). After complete cellulose hydrolysis, lignin is left, which can be

separated from the mash to obtain hydrolysate without solid residues for HG fermentation to increase ethanol titers.

During the SHCF process, glucose accumulated during the hydrolysis inhibits the activity of β-glucosidases, which consequently results in the accumulation of cellobiose to further inhibit the activity of cellobiohydrolases (Wang et al., 2012). The supplementation of β-glucosidases could be a solution to address this problem if their cost is not too high, for example, β-glucosidases commercially produced by *Aspergillus niger* (Abdella et al., 2016). However, microbial contamination during the hydrolysis of the cellulose component would be another concern with SHCF. Currently, SHCF is being explored by researchers and industry. For example, the Switzerland-based company Clariant has developed and employed SHCF for its demo plant in Straubing, Germany (Rarbach and Söltl, 2013).

4.2. Saccharification coupled with co-fermentation

For ethanol fermentation from starch-based feedstock, mash is heated to 110–120 °C and held for 10–20 min, which is then flashed to 85–90 °C and maintained for 60–90 min for amylases to hydrolyze starch completely into dextrin. This two-step process is termed cooking or liquefaction (Kelsall and Lyons, 2003b), which also sterilizes the mash to control contamination thereafter. After the liquefaction, mash is cooled down to 60–65 °C for glucoamylases to be supplemented to hydrolyze the dextrin into glucose and other fermentable sugars, but the process is maintained only for 20–30 min to release fermentable sugars for yeast propagation. The mash is then further cooled down to 30–34 °C for inoculation to start ethanol fermentation (Devantier et al., 2005). If enough sugars are released during the liquefaction, the presaccharification can be removed. Since most dextrin is hydrolyzed into sugars during ethanol fermentation, the process is termed simultaneous saccharification and fermentation (SSF), which has been practiced for fuel ethanol production from starch-based feedstock for a long time (Power, 2006).

When such a similar strategy was employed to ethanol production from lignocellulosic biomass, SSCF was adapted for simultaneous saccharification and co-fermentation, taking into account of the unique characteristic of the hydrolysate with both pentose and hexose sugars. This acronym might be reported for the first time by Dr. James R. Hettenhaus as the NREL Chief Executive Assistance in the technical report: *Ethanol fermentation strains: Present and future requirements for biomass to ethanol commercialization*, which was released on December 16, 1998. However, saccharification of pretreated cellulose and co-fermentation of pentose and hexose sugars are by no means simultaneous, and they are sequential in nature. Therefore, SSCF should be corrected as saccharification coupled with co-fermentation (SCCF), which can accurately interpret their correlation: the fermentation consumes glucose to alleviate its inhibition in the activities of cellulases, and the saccharification is thus facilitated to hydrolyze the cellulose more efficiently and completely for ethanol production.

The SCCF process is simple and easy to operate. Most importantly, high ethanol yield could be obtained because of the alleviation of glucose inhibition in the activities of cellulases for more complete cellulose hydrolysis (Hahn-Hägerdal et al., 2006). However, temperatures for cellulose hydrolysis by cellulases and ethanol fermentation by microorganisms are significantly different, and both of the two processes cannot be optimized simultaneously within same bioreactors/fermenters. Therefore, SCCF must be operated at lower temperature for microbial growth and ethanol fermentation, normally below 35 °C, and the rate for the enzymatic hydrolysis of the cellulose component is substantially compromised. Moreover, lignin cannot be separated before ethanol fermentation under SCCF conditions, and extra mixing is thus needed for the homogenous suspension of the fermentation broth. As a result, it is difficult for SCCF to be operated under HG conditions for high ethanol titers, and energy consumption is high for ethanol recovery by distillation due to low ethanol titers achieved during the fermentation, and in the meantime large amounts of stillage are discharged from the distillation. Fed and multi-fed batch can address this problem to some extent, but ethanol titers achieved are still low. For example, fed and multi-fed batch processes were reported for SCCF with the solid loadings of 11.7% and 20% (*w*/w), respectively, producing 37.5 g/L and 57.0 g/L ethanol from steam pretreated wheat straw at 48 and 72 h (Bondesson and Galbe, 2016; Wang et al., 2016b).

A hybrid process much like that used for ethanol production from starch-based feedstock can be developed for cellulosic ethanol production, in which a pre-hydrolysis under optimal temperature is applied to the enzymatic hydrolysis of the cellulose component, followed by the SCCF process to shorten time required by the saccharification and ethanol fermentation (Cassells et al., 2017), but the impact of lignin on the rheology of the mash still cannot be overcome.

4.3. Consolidated bioprocessing

Cellulases are produced and supplemented to hydrolyze the cellulose component for ethanol production in SHCF and SCCF. This is one of bottlenecks for cost reduction due to the high cost of cellulases produced predominantly so far by strains from *T. reesei* through submerged fermentation (Bischof et al., 2016). In nature, many organisms, particularly some microbes, can synthesize and excrete cellulases to hydrolyze cellulose as carbon and energy sources to support their growth and metabolism. This inspires scientists to engineer mimic systems, using either individual microorganisms through pure culture or microbial communities under mixed culture conditions for ethanol production directly from lignocellulosic biomass, a process known as consolidated bioprocessing (CBP), which combines the production of cellulases, enzymatic hydrolysis of cellulose and ethanol fermentation of the resulting sugars (Den Haan et al., 2015; Brethauer and studer, 2014).

However, no natural microorganisms are available for cellulosic ethanol production through such a CBP strategy, and developing CBP strains is a prerequisite. At present, two strategies have been proposed: 1) engineering cellulase producers with ethanol production (Ali et al., 2016; Xiong et al., 2018), and 2) engineering ethanologens with cellulase production (Liu et al., 2016; Liu et al., 2018). So far, more attention has been focused on engineering *S. cerevisiae* with genes encoding glycoside hydrolases including cellulases and hemicellulases (Den Haan et al., 2015), but their expression is generally poor. In theory, CBP can completely eliminate the supplementation of cellulases. However, more fundamentals are to be elucidated to make it practical for cellulosic ethanol production. For example, the production of cellulolytic enzymes, hydrolysis of cellulose and hemicelluloses and fermentation of released sugars need to be well coordinated through engineered strains. Furthermore, kinetic models of the heterogeneous enzymatic hydrolysis with mass transfer limitation and ethanol production need to be developed for the process optimization.

5. Strain development

In addition to hexose sugars, the hydrolysate of lignocellulosic biomass contains large amounts of pentose sugars released from the hydrolysis of hemocelluloses (Scheller and Ulvskov, 2010). However, all *S. cerevisiae* strains currently used for 1G fuel ethanol production and strains from *Z. mobilis*, another candidate for ethanol production, cannot metabolize pentose sugars unless they are engineered properly. Therefore, engineering ethanol-producers with the co-fermentation of pentose and hexose sugars for cellulosic ethanol production has been performed endlessly within the past four decades (Jin and Cate, 2017; Zhang et al., 2019). However, it is worth noting that simultaneous utilization of pentose and hexose sugars that has been overwhelmingly pursued in academia since the beginning of this work should be critically reviewed from the viewpoint of bioprocess engineering, since converting sugars into ethanol at maximized yield and productivity is

the sole purpose for commercial production, and sequential utilization of different sugars may be another strategy for more robust cellulosic ethanol production.

5.1. Strategies for engineering S. cerevisiae

1G Fuel ethanol is solely produced by *S. cerevisiae*, which is superior to other species (Russel, 2003). *S. cerevisiae* is more tolerant to ethanol, and high ethanol titers can be achieved to save energy consumption not only for ethanol recovery by distillation, but also for stillage treatment due to the substantial reduction of the discharge. Currently, 13% (*v*/v) ethanol is produced routinely from grains in the industry (Mohanty and Swain, 2019). Moreover, *S. cerevisiae* prefers an acidic environment with pH values below 4.5, which effectively prevents microbial contamination (Russel, 2003). Fuel ethanol, as a bulk commodity, is marketed at low prices, and ethanol fermentation cannot be operated under sterilization conditions for big fermenters with working volumes of thousands of cubic meters and large amounts of fermentation broth due to high energy consumption. Therefore, *S. cerevisiae* stands firmly for cellulosic ethanol production once it is engineered with pentose metabolism. All pilot and demo plants established so far use engineered *S. cerevisiae* strains except the former DuPont Facility in Nevada, Iowa, USA (Lynd et al., 2017). Since xylose and arabinose are the most abundant pentose sugars in the hydrolysate of lignocellulosic biomass (Scheller and Ulvskov, 2010), they have been targeted in engineering *S. cerevisiae* through different strategies, which are highlighted in Fig. 5.

Some xylose-metabolizing yeast such as *Pichia stipitis* (*Scheffersomyces stipitis*) can metabolize xylose to xylulose through the two-step reduction/oxidation pathway catalyzed by xylose reductase (XR) and xylitol dehydrogenase (XDH), respectively (Hilliard et al., 2018). Xylulose can be phosphorylated to xylulose-5-phosphate in *S. cerevisiae* by xylulokinase (XK) to enter the non-oxidative pentose phosphate (PP) pathway. Dr. Ho at Purdue University, USA employed this strategy for developing xylose-utilizing yeast from the industrial strain *S. cerevisiae* 1400 (Ho et al., 1998). However, the preference for cofactors NADPH/$NADP^+$ by XR and NAD^+/NADH by XDH led to redox imbalance and xylitol accumulation, which substantially compromised ethanol yield. Increasing the NADPH pool could address this issue. Therefore, glyceraldehyde-3-phosphate dehydrogenase from *Kluyveromyces lactis* was co-expressed with XR and XDH in another industrial strain *S. cerevisiae* 424A (LNH-ST), and the engineered strain decreased xylitol production by more than 40% (Bera et al., 2011). In order to relieve metabolic burden exerted on *S. cerevisiae* by expressing the cassette with multi-genes, a scattered integration of xylose assimilation genes was developed (Zuo et al., 2013).

Another strategy was applied by Prof. Hahn-Hägerdal at Lund University, Sweden, in which the gene encoding xylose isomerase (XI) was cloned from *Thermus thermophiles*, which was expressed in *S. cerevisiae* to isomerize xylose directly to xylulose (Walfridsson et al., 1996), but the activity of XI from the bacterial species was not enough for xylose metabolism, and its mutation was thus carried out to address this problem (Lönn et al., 2003). Similar strategies have also been applied to engineering *S. cerevisiae* with arabinose metabolism (Bera et al., 2010; Wisselink et al., 2007). Both advantages and disadvantages are clear with *S. cerevisiae* strains engineered through these strategies. As Van Vleet and Jeffries (2009) pointed out ten years ago, it is not entirely clear which one is better, since strains for cellulosic ethanol production have been developed through both strategies by different researchers in academia and industry as well, and all of them claimed their strains had commercial significance, but details are not available unless cellulosic ethanol is produced at large scales for commercial applications.

In addition to intracellular metabolism, xylose transport is another rate-limiting step for its utilization, since hexose transporters in *S. cerevisiae* exhibit low affinity to xylose. Cloning of genes encoding xylose transporters from pentose-utilizing microorganisms such as *P. stipitis*, *Neurospora crassa* and *Meyerozyma guilliermondii* and their heterologous expression in *S. cerevisiae* have been performed (De Sales et al., 2015; Wang et al., 2015a; Wang et al., 2015b). With the progress of protein engineering, hexose transporters can also be engineered through directed evolution for the co-transport of glucose and xylose (Farwick et al., 2014; Li et al., 2016b). Moreover, various inhibitors are generated during biomass pretreatment, and engineering *S. cerevisiae* with tolerance to those inhibitors is also urgently needed for cellulosic ethanol production (Zhao and Bai, 2012; Wang et al., 2018a).

5.2. Potential of Z. mobilis

Compared to *S. cerevisiae* that metabolizes glucose through the

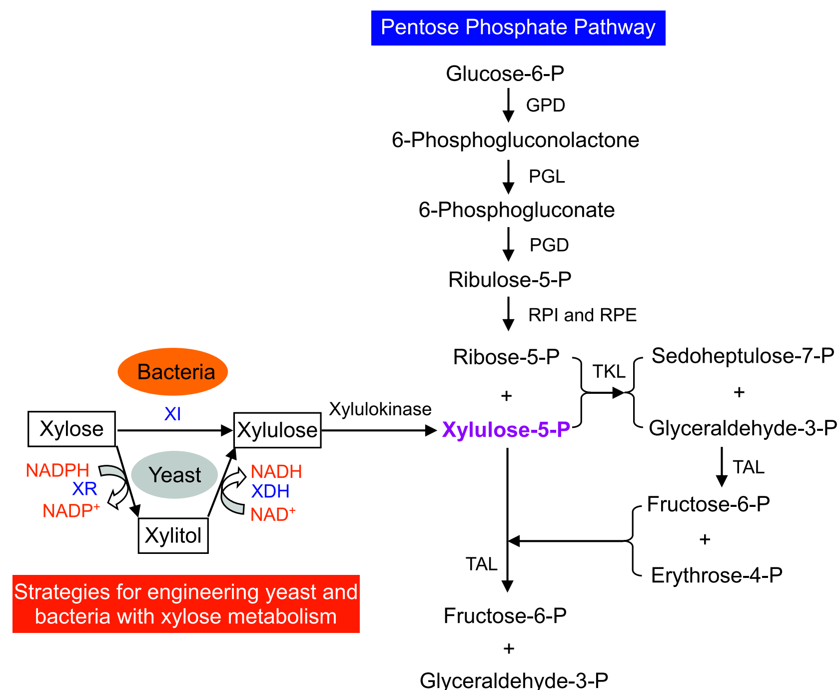


Fig. 5. Engineering *S. cerevisiae* and *Z. mobilis* with xylose metabolism for ethanol production. XI: xylose isomerase, XR: xylose reductase, XDH: xylitol dehydrogenase, GPD: glucose-6-phosphate dehydrogenase, PGL: 6-phosphogluconolactonase, PGD: 6-phosphogluconate dehydrogenase, RPI: ribulose-5-phosphate isomerase, RPE: ribulose-5-phosphate epimerase, TKL: transketolase, TAL: transaldolase.

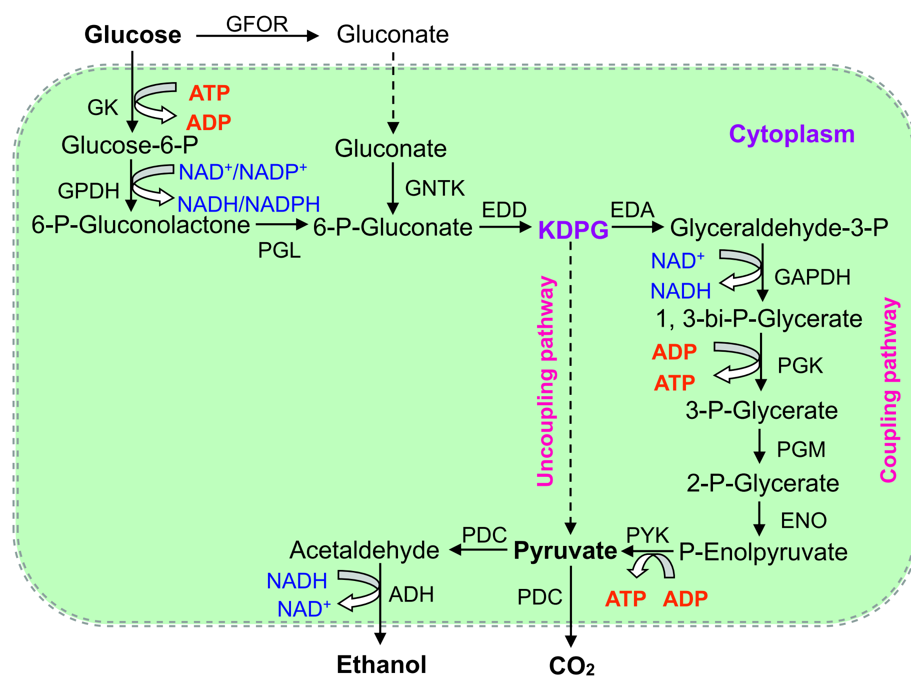


Fig. 6. Entner-Doudoroff (ED) pathway for ethanol fermentation in *Z. mobilis*. GK: glucokinase, ATP: Adenosine triphosphate, ADP: adenosine diphosphate, GFOR: glucose-fructose oxidoreductase, GPDH: glucose-6-phosphate dehydrogenase, PGL: phosphogluconolactonase, GNTK: gluconate kinase, EDD: 6-phosphogluconate dehydratase, KDPG: 2-keto-3-deoxy-6-phosphogluconate, EDA: 2-keto-3-deoxy-gluconate aldolase, GAPDH: glyceraldehydes-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, PGM: phosphoglyceromutase, ENO: enolase, PYK: pyruvate kinase, PDC: pyruvate decarboxylase, ADH: alcohol dehydrogenase.

Embden-Meyerhof-Parnas (EMP) pathway with 2 mol of ATP produced from 1 mol of glucose metabolized, *Z. mobilis* metabolizes glucose into ethanol and CO_2 through the Entner-Doudoroff (ED) pathway with only 1 mol of ATP produced from the same amount of glucose consumed (Fig. 6), and less biomass is thus accumulated during ethanol fermentation by the bacterium (Kalnenieks, 2007). As a result, ethanol yield, the most important factor for fuel ethanol production, could be improved. In addition, cells of *Z. mobilis* are much smaller in size than *S. cerevisiae* to provide more surfaces for glucose uptake to produce ethanol quickly, giving it the nickname of catabolic highway (Sprenger, 1996). Moreover, unlike the EMP pathway in which the growth of *S. cerevisiae* is tightly coupled with ethanol fermentation, the ED pathway from 2-keto-3-deoxy-6-phosphogluconate to pyruvate decouples the growth of *Z. mobilis* partially from energy/ethanol production, and consequently ethanol can be produced even without significant growth of the bacterial cells.

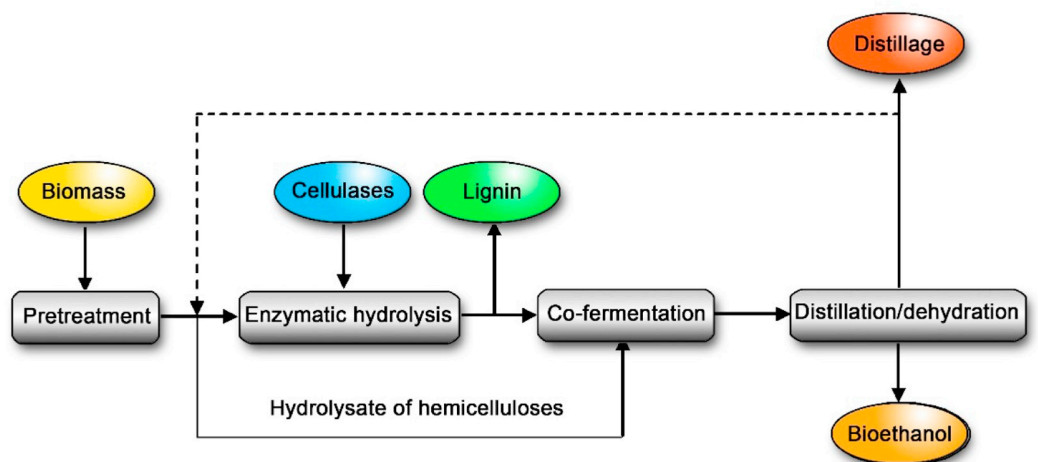
Albeit with the aforementioned advantages for ethanol fermentation, *Z. mobilis* has never been commercially used for ethanol production from sugar- and starch-based feedstocks. The reasons for this phenomenon are: 1) *Z. mobilis* can metabolize only glucose, fructose and sucrose (Sprenger, 1996), and the narrow substrate spectrum makes it unsuitable for ethanol fermentation from starch-based feedstock, since many other sugars are released during the hydrolysis of starch dextrin by glucoamylases, and 2) when sucrose is used as substrate, ethanol yield is substantially compromised due to the formation of a large amount of levan, making *Z. mobilis* not suitable for ethanol production from sugar-based feedstock such as sugarcane juice or molasses. However, these disadvantages for ethanol production by *Z. mobilis* from sugar- and starch-based feedstocks are not problematic for cellulosic ethanol production, since the only sugar released from the enzymatic hydrolysis of the cellulose component is glucose. Moreover, *Z. mobilis* can be engineered with pentose metabolism through the XI pathway more efficiently to overcome the intrinsic imbalance of cofactors associated with engineering *S. cerevisiae* through the oxido-reductase pathway.

The pioneer work for engineering *Z. mobilis* with pentose metabolism for ethanol production was performed at NREL in the middle of the 1990s, and genes isolated from *E. coli* for xylose assimilation and the PP pathway were engineered into *Z. mobilis* CP4 for ethanol production from xylose (Zhang et al., 1995). Shortly, arabinose utilization was further engineered into the same *Z. mobilis* strain for ethanol production from arabinose (Deanda et al., 1996). Finally, all those genes were engineered into the *Z. mobilis* strain through genomic DNA-integration for stable expression to ferment xylose, arabinose and glucose (Mohagheghi et al., 2002). In order to facilitate pentose metabolism, transporter engineering has also been performed for *Z. mobilis* (Dunn and Rao, 2014).

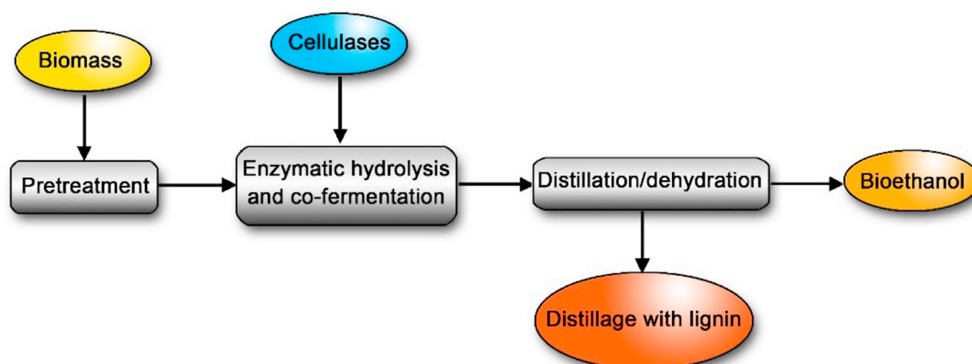
As an ethanologen, *Z. mobilis* can ferment medium containing ~200 g/L glucose with more than 90 g/L ethanol produced (Zhao et al., 2014), which is sufficient for cellulosic ethanol production, since hydrolysate with total sugars up to 200 g/L cannot be prepared from lignocellulosic biomass so far. However, the bacterial species may be less tolerant to inhibitors released during the pretreatment of lignocellulosic biomass due to its relatively small genome (Seo et al., 2005). Therefore, a complete understanding of its physiological and metabolic response to the inhibitors is needed for engineering *Z. mobilis* strains with robustness. While quantitative proteomics and transcriptomics analysis could help identify targets for genetic engineering of the species (Yang et al., 2014; Shui et al., 2015), the progress in sequencing and functional annotation of *Z. mobilis* genome would lay a foundation for engineering more robust *Z. mobilis* strains for cellulosic ethanol production (Seo et al., 2005; Yang et al., 2009; Wang et al., 2018b).

In addition to regular morphology characterized by unicellular cells, *Z. mobilis* can self-flocculate to form flocs, and such a morphological change endows the bacterial species with advantages in physiology and metabolism for stress tolerance due to potentially enhanced quorum sensing as well as bioprocess engineering for high cell density culture and fermentation that is preferred for cellulosic ethanol production (Zhao et al., 2014). Most recently, the mechanism underlying the self-flocculation of *Z. mobilis* was partly revealed (Xia et al., 2018), which provides an alternative host to be engineered with pentose metabolism for cellulosic ethanol production.

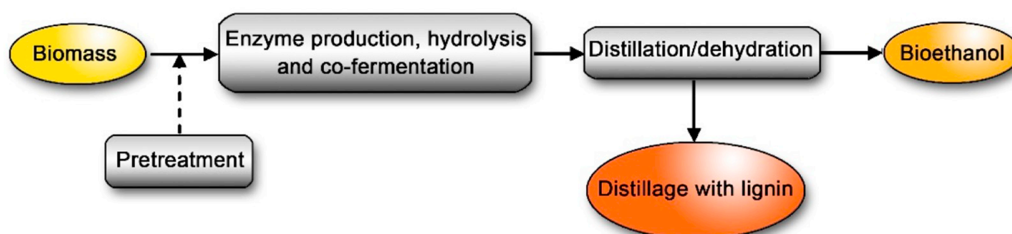
Although significant progress has been made in engineering *Z. mobilis* for cellulosic ethanol production, no stable production at commercial scales has been reported up till now. The most notable progress in cellulosic ethanol production by engineered *Z. mobilis* strains initially developed by NREL was the former DuPont Cellulosic Ethanol Plant established in Nevada, Iowa, USA with a projected production capacity of 30 million US gallons of fuel ethanol from corn stover (Lynd et al.,



(a) Separate hydrolysis and co-fermentation



(b) Saccharification coupled with co-fermentation



(c) Consolidated bioprocessing

Fig. 7. Diagram for the unit optimization and system optimization of cellulosic ethanol production through the precesses of SHCF (a), SCCF (b) and CBP (c) which were partly adapted with permission from Zhao et al., 2012

2017), which was open in October 2015, but unfortunately closed shortly in late 2017 for selling to be refit for biogas production.

6. Unit integration and system optimization

Unit operations have been developed for pretreatment, enzymatic hydrolysis of the cellulosic component and co-fermentation of pentose and hexose sugars for cellulosic ethanol production, and how to integrate these unit operations for optimization at system levels present another challenge, which is highlighted respectively for SHCF, SCCF and CBP processes in Fig. 7. Most significant work in this regard was highlighted in the technical report released by NREL: *Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol* (https://www.nrel.gov/docs/fy11osti/47764.pdf), in which detailed techno-economic analysis was performed for cellulosic ethanol production from corn stover.

At present, ethanol titers achieved for cellulosic ethanol production are 5–7% (*v*/v), only 50% of that achieved during 1G fuel ethanol production. The reasons for this phenomenon are with the inhibition of toxic byproducts released during the pretreatment of lignocellulosic biomass in microbial growth and ethanol fermentation, as well as extremely high viscosity of the mash under high solid loading conditions. As a result, more water needs to be supplemented into the production system, which not only compromises productivity of the whole process,

but also consumes more energy in ethanol distillation, with large amounts stillage discharged for treatment properly to minimize its impact on the environment. Therefore, one of the most important considerations for the unit integration and process optimization is to minimize water usage for the whole process without significant impact on the enzymatic hydrolysis of the cellulose component and ethanol fermentation.

7. Conclusions

Cellulosic ethanol is one of the solutions for current issues with the reliable supply of transportation fuels and environmental challenges. Although significant progress has been made over the past decades on fundamentals and technological innovations, cellulosic ethanol is still not economically competitive compared to fuel ethanol produced from sugar- and starch-based feedstocks, needless to say comparing to petroleum-based transportation fuels, making cost reduction a top priority. Taking into account of the multi-disciplinary nature of cellulosic ethanol production, a portfolio should be developed collaboratively by scientists, researchers and engineers to incorporate plant science for genetic modifications of lignocellulosic biomass to render its recalcitrance to degradation, even modify its composition properly to fit downstream bioconversion, an in-depth understanding on physiological and metabolic responses of microbial strains under industrial production conditions to engineer them with robustness, and unit integration and system optimization to save energy and water consumption. That will be an ultimate solution for de-bottling the whole process of cellulosic ethanol production, making it economically competitive. Many pilot and demo plants have been established worldwide for cellulosic ethanol production, but none of them has be operated with projected revenues, and most was closed temporarily, even permanently like the former DuPont facility. Therefore, there is no reason to be over-optimistic and in a hurry for commercial production of cellulosic ethanol, particularly at large scales.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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