Recent updates on lignocellulosic biomass derived ethanol - A review

Rajeev Kumar, Meisam Tabatabaei, Keikhosro Karimi, Ilona Sárvári Horváth

1 Center for Environmental Research and Technology (CE-CERT), Bourns College of Engineering, University of California, Riverside, California, USA.
2 Microbial Biotechnology Department, Agricultural Biotechnology Research Institute of Iran (ABRII), AREEO, Karaj, Iran.
3 Biofuel Research Team (BRTeam), Karaj, Iran.
4 Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran.
5 Microbial Industrial Biotechnology Group, Institute of Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan 84156-83111, Iran.
6 Swedish Centre for Resource Recovery, University of Borås, 501 90 Borås, Sweden.

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Lignocellulosic (or cellulosic) biomass derived ethanol is the most promising near/long term fuel candidate. In addition, cellulosic biomass derived ethanol may serve a precursor to other fuels and chemicals that are currently derived from unsustainable sources and/or are proposed to be derived from cellulosic biomass. However, the processing cost for second generation ethanol is still high to make the process commercially profitable and replicable. In this review, recent trends in cellulosic biomass ethanol derived via biochemical route are reviewed with main focus on current research efforts that are being undertaken to realize high product yields/titers and bring the overall cost down.

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Lignocellulosic biomass, otherwise termed as cellulosic biomass, is the only sustainable feedstock for biorefineries to meet the ever increasing energy demand (Wyman, 2007; Lynd et al., 2008). Cellulosic biomass conversion into biofuels and chemicals has several advantages including greenhouse gas mitigation, near carbon neutrality, lesser dependence on fossil fuels, and improvement in nations’ energy security (Wyman, 2007). Lignocellulosic biomass derived ethanol is often termed as “second generation” or “2G” as the “first generation” or “1G” ethanol is derived from sugar cane, corn, wheat, and other starchy feedstocks (Jordan et al., 2012). Studies suggest that the net energy return on 2G ethanol is much higher than ethanol derived from corn (Lynd et al., 2006; Schmer et al., 2008). In addition, 2G ethanol has much higher potential for greenhouse gas (GHG) emissions reduction than 1G ethanol (Hsu et al., 2010). The cost of energy in lignocellulosic biomass at $60/ton is roughly the same as $20/barrel oil; however, due to recalcitrant nature of cellulosic biomass (Lynd et al., 1999), the current processing cost of 2G ethanol is still high and is much higher than 1G ethanol (www.doe.gov). The reasons for high processing costs of cellulosic biomass to biofuels are several including inherent recalcitrant nature of cellulosic biomass than corn, energy and chemical intensive pretreatment, inefficient and expensive enzymes resulting in low conversion at high solids loadings required for commercial application, incomplete conversion of all sugars to fuels and chemicals, and distillation (Lynd et al., 2008). This review discusses the recent research efforts made in biological conversion of cellulosic biomass to ethanol and challenges that need to be addressed to bring the processing cost further down.

2. Why ethanol?

Among renewable fuels, ethanol due to its long history, use, and inherent characteristics, such as low toxicity to microbes and environment, low boiling point, high octane number, and comparable energy content, is considered to be a primary fuel candidate for near-term applications (Lynd et al., 1991; Lynd et al., 2008). Although ethanol’s energy content is roughly 2/3rd of gasoline and butanol, it has higher research octane number (RON; 107) than butanol (96) and gasoline (91-99) (Lynd, 1996). Research shows that ethanol can be used up to 85% (v/v) in vehicles without major modifications (Bala et al., 2008). Although, novel biochemical, thermo-catalytic, and hybrid routes are being developed to produce drop-in fuels and fuel additives to meet the infrastructure and other requirements (Huber et al., 2006; Anbarasan et al., 2012; Buijs et al., 2013; Caratzoulas et al., 2014; Harvey and Meylemans, 2014; Sreekumar et al., 2014). Figure 1 shows that ethanol derived from cellulosic biomass can also be used to produce other fuel candidates such as butanol, gasoline, hydrogen, diesel, and others (Whitecroft et al., 1983; Costa et al., 1985; Deluga et al., 2004; Nanula et al., 2015; Riittinen et al., 2015). Moreover, ethanol can also serve as a precursor for several other chemicals and intermediates that are currently derived from non-renewable resources (Angelici et al., 2013; Sun and Wang, 2014).

3. Cellulosic biomass

Lignocellulosic biomass includes forestry residues (e.g., hard & softwood), agricultural residues (e.g., corn stover, wheat straw, rice straw), herbaceous (e.g., switchgrass, miscanthus), and plants that grow in arid regions (e.g., Agave) (Somerville et al., 2010). The 2011 report from the United States (US) Department of Energy (DOE) suggests that in the US alone more than a billion ton of lignocellulosic biomass is potentially available at ~$60/ton for conversion into >20 billion gallons of cellulosic biofuels (Perlack and Stokes, 2011). Whereas, a study published by Lal in 2005 estimated that total crop residue available is more than one billion ton in the US alone and more than 9 billion ton world-wide (Lal, 2005). Lignocellulosic biomass is primarily composed of cellulose (35-50 wt. %, dry basis), hemicelluloses (15-30%), pectin (2-5%), and lignin (12-35%). Cellulose and hemicelluloses that make more than 50% of total mass can be potentially converted to sugars for their conversion to ethanol. Lignin can be burned to meet the plants energy requirement and/or valorized to make fuels and chemicals (Ragauskas et al., 2014; Wyman and Ragauskas, 2015).

4. Cellulosic biomass to ethanol

Figure 2 shows the simple process flow diagram of converting cellulosic biomass to ethanol that is comprised of several steps: 1) biomass size reduction, 2) pretreatment, 3) enzymes production, 4) enzymatic hydrolysis of pretreated solids to fermentable sugars, 5) fermentation of sugars to ethanol, and 6) ethanol recovery. Steps 4 and 5 have several process configurations including separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF) of hexose and pentose sugars, and consolidated bioprocessing (CBP), that combines enzymes production, enzymatic saccharification, and fermentation in a single step. Most pretreatments require some sort of size reduction to achieve better efficiency in terms of sugar release in pretreatment and/or biological conversion (Zhu and Pan, 2010). Nonetheless, pretreatment and enzymes are the most expensive
contributing factors to the 2G ethanol processing cost (Lynd et al., 2008), and, thus, have drawn a lot of attention in recent years.

5. Biomass recalcitrance

The inherent resistance of cellulosic biomass to pathogens, enzymes/microbes, and/or chemicals is called recalcitrance (Lynd et al., 1999), and is perceived to be majorly contributed by lignin including its amount, location, and type (syringyl vs. guaiacyl) (Studer et al., 2011; Ding et al., 2012). Other components such as pectin, hemicellulose, cellulose characteristics, and other biomass features are also believed to contribute to the plant’s recalcitrance (Mohnen, 2008; Kumar et al., 2009b; Foston et al., 2011; Urbanowicz et al., 2012; Kumar and Wyman, 2013). However, it appears that all these features directly or indirectly contribute to the enzymes accessibility to plant’s carbohydrates and/or enzymes effectiveness (Kumar and Wyman, 2010). Accessibility can further be divided into macro and micro-accessibility in that lignin, hemicellulose, and other components removal/relocation enhances macro-accessibility; whereas, changes in cellulose characteristics such as crystallinity and/or change in allomorph type (e.g., cellulose I to cellulose II and III) enhance micro-accessibility (Kumar and Wyman, 2013). However, for high sugar yields at low enzyme loadings, it is vital to enhance both macro and micro-accessibility and increase enzymes effectiveness (Kumar and Wyman, 2013). Nonetheless, with genetically engineered plants, the question of their performance in field trials in terms of their growth, resistance to pathogens, and sugars yields often arises as most plant engineering studies are performed on model plants, such as Arabidopsis thaliana, grown in greenhouses. However, a recent study by researchers at the BioEnergy Science Center (BESC), one of the bioenergy research centers funded by the United States Department of Energy, showed that the field trials of switchgrass transgenic lines resulted in similar sugar and ethanol yields to those grown in greenhouses. In addition, the switchgrass grown in the fields was not susceptible to rust (Baxter et al., 2014).

6. Pretreatment

Pretreatment is a processing step to make lignocellulosic biomass more amenable to biological conversion at high yields that otherwise suffers from low yields and high processing costs (Wyman et al., 2013). The details on the type of earlier pretreatment technologies including liquid hot water or hydrothermal (Bobleter et al., 1976), dilute acid (Grethlein et al., 1980), and others are described in previous publications (Cao et al., 2006; Ding et al., 2012; Foston et al., 2011; Urbanowicz et al., 2012; Doblin et al., 2014). Pretreatment is a critical step for lignocellulosic biomass conversion to biofuels as it facilitates the conversion of lignocellulosic biomass into sugars that can be fermented into ethanol. Pretreatment technologies are designed to (1) remove inhibitors (e.g., pentoses and furfural) that can inhibit fermentation; (2) increase the fraction of cellulose available for enzymatic hydrolysis; and (3) reduce the energy consumption required for biomass conversion. Pretreatment technologies can be divided into two main categories: physical and chemical pretreatments. Physical pretreatments include processes such as steam explosion, dilute acid pretreatment, and dry grinding, while chemical pretreatments include processes such as aqueous acid hydrolysis, ammonia fiber expansion (AFEX), and ionic liquids. The choice of pretreatment technology depends on the type of biomass, the desired products, and the available infrastructure.
and Converse, 1991; Yang and Wyman, 2009; Trajano and Wyman, 2013), (non) aqueous and (near) critical ammonia (Dale and Moreira, 1982; Chundawat et al., 2013), ammonia recycled percolation (ARP), and soaking in aqueous ammonia (SAA) (Yoon et al., 1995; Kim et al., 2003), lime (Chang et al., 1997; Vincent et al., 1998), and others and their impact on biomass features and biological digestibility, and their economic viability are available in several previous and recent reviews (Millett et al., 1975; Lin et al., 1981; Ladisch et al., 1983; Knauf and Moniruzzaman, 2004; Mosier et al., 2005; Yang and Wyman, 2008; da Costa Sousa et al., 2009; Kumar et al., 2009a; Karimi et al., 2013). It is worth mentioning a few new promising pretreatments that have recently been developed including co-solvent enhanced lignocellulosic fractionation (CELF) (Nguyen et al., 2015a; Nguyen et al., 2015b), co-solvent based lignocellulosic fractionation (COSLIF) (Zhang et al., 2007), γ-valerolactone (GVL) pretreatment (Shuai et al., 2016; Wu et al., 2016), pretreatment applying ionic liquid(s) (Swatloski et al., 2002; Dadi et al., 2006; Seema et al., 2009; Li et al., 2010; Cheng et al., 2011; Perez-Pimienta et al., 2013; Singh and Simmons, 2013; Konda et al., 2014), sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) (Zhu et al., 2009), and switchable butadiene sulfone pretreatment (de Frias and Feng, 2013).

Nonetheless, an ideal pretreatment should be feedstock agnostic, should be less energy and chemical intensive, and should generate highly reactive solids by enhancing their both macro and micro-accessibility (Kumar and Wyman, 2013) for their high yield conversion at low enzyme (biocatalyst) loadings with minimal sugars degradation (Yang and Wyman, 2008) and water demand (Kumar and Moriarty, 2011). Although, as shown in Figure 3, some of the previously (and newly) developed pretreatment technologies meet some of these criteria (Dale and Ong, 2012), a rigorous techno-economic and life cycle analyses are necessary to show their viability for commercial applications (Mosier et al., 2005). For example, COSLIF pretreatment fractionates biomass at low temperatures (~50°C) and has been shown to be highly effective for variety of biomass types in terms of high sugar (especially, glucan to glucose) yields at low enzyme loadings (as low as 1 filter paper unit (FPU)/g glucan, i.e., ~ 2 mg of protein) (Rollin et al., 2011; Zhang et al., 2007). However, COSLIF requires concentrated phosphoric acid (>80 wt.%), which poses a recovery and recycling challenge, and doesn’t appear to be highly effective for softwoods (Zhang et al., 2007). Pretreatments applying ionic liquids also appear highly promising and feedstock agnostic; however, the current cost of ionic liquids (>53 per kg) makes this approach less commercially attractive (Klein-Marcuschamer et al., 2011). However, research efforts are underway at the Joint BioEnergy Institute, USA to develop ionic liquids from cellulosic biomass components (termed as bionic liquid) to drive the cost down (Socha et al., 2014). On the other hand, recently developed CELF uses a low boiling and renewable tetrahydrofuran (THF) as a co-solvent (boiling point-66°C), and fractionates all biomass types into three pure streams: highly reactive glucan enriched solids, xylose and other hemicellulose components in the liquid stream at near theoretical yields, and an ultra-pure stream of lignin, with >80% original lignin removed and recovered (Cai et al., 2013; Cai et al., 2014; Nguyen et al., 2015a). In addition, unlike most other pretreatment/fractionation technologies, CELF can be tuned to produce fuel precursors furfural, hydroxymethylfurfural, and levulinic acid at high yields for their catalytic conversion to drop-in fuels (Cai et al., 2013; Cai et al., 2014). CELF as a pretreatment defeats biomass recalcitrance and achieves high ethanol yields and titers at enzyme loadings as low as 2 mg protein/g glucan (Nguyen et al., 2015a; Nguyen et al., 2015b); however, recovery and recycling of THF is the key to the commercial scalability and feasibility of the technology.

![Fig.3. Comparison of various pretreatments for their characteristics and applicability in cellulosic biomass conversion to ethanol and other fuels and bio-based chemicals (taken with permission from Dale and Ong (2012)).](https://example.com/fig3.png)
7. Enzymes

High cost of cellulase and other accessory enzymes required for biological conversion of pretreated lignocellulosic biomass into sugars is another major impediment in the commercialization of lignocellulosic biomass to fuels and chemicals (Calhoun et al., 2013; Hong et al., 2013). Although enhancement in enzymes stability, activity, and several fold decrease in cost have been reported in recent years, enzymes available at about $1.5-$2.0 per kg are still expensive (Stephen et al., 2012). Nonetheless, the enzymes cost per gallon would vary with the pretreatment applied, the extent of anhydrous polymers (cellulose and hemicellulose) conversion to sugars, and sugars conversion to ethanol (Klein-Marcuschamer et al., 2012).

In addition to enzymes high accessibility (he(ni) cellulose), their strong inhibition by components generated during pretreatment (e.g., phenols) (Ximenes et al., 2010; Kim et al., 2011) and enzymatic saccharification (Mandels and Reese, 1965; Halliwell and Griffin, 1973; Kumar and Wyman, 2008; Kumar and Wyman, 2009b; Qing et al., 2010; Kumar and Wyman, 2014) is one of the main reasons for high loading of enzymes required for commercially viable sugar yields. In addition, enzymes unproductive binding to lignin (Yang and Wyman, 2006; Selig et al., 2007; Kumar and Wyman, 2009a; Kumar and Wyman, 2009c; Kumar et al., 2012; Li et al., 2013) and solids loadings (Kristensen et al., 2009; Di Risio et al., 2011) often applied enzymes available and affects their effectiveness. The rates and yields are also substantially lower at industrially relevant high solids loading than with low solids loadings (Kristensen et al., 2009; Di Risio et al., 2011) and applied and studied in laboratory settings. Although cellulase end-product inhibition by sugars can be alleviated on a process scale by saccharification and fermentation (SSF), and inhibition by cellulose and hemicellulose oligomers can be alleviated by supplementing cellulase with accessory enzymes, low reaction rates at fermentation temperatures (32-37°C) (Alfani et al., 2000; Elia et al., 2008) and inhibition by ethanol still pose a challenge to high yields and titers at low enzyme loadings (Podkaminer et al., 2011; Podkaminer et al., 2012).

The discovery of novel non-hydrolytic enzymes like polysaccharide monoxygenases (LPMOs), appears to be highly promising in reducing cellulase and ultimately overall processing costs (Vaaje-Kolstad et al., 2010; Horn et al., 2012; Agger et al., 2014). Although the mechanism is not clear yet, these LPMOs are believed to oxidize the highly recalcitrant crystalline regions of cellulose and create more reducing/reducing ends for cellulase components to attack (Horn et al., 2012). In fact, a recent study with current generation of cellulase enzymes containing LPMOs (e.g., Cellic® Cex2 from Novozymes) showed that it is possible to achieve higher rates and yields in SHF than SSF (Cannella and Jørgensen, 2014), which with older generation of enzymes was the other way around (Alfani et al., 2000; Lynd et al., 2002). This may be due to the fact that LPMOs require an electron donor, e.g., oxygen, for their effective action (Hu et al., 2014; Müller et al., 2015). Nonetheless, in addition to loss of some of the carbohydrates and requirement of different process configurations, the aldonic acids resulting from polysaccharide oxidation by LPMOs can be inhibitory to enzymes as well as microbes (Cannella et al., 2012). In addition, it was recently shown that LPMOs can make cellulase cocktails less stable (Scott et al., 2015). Thus, it is still to be seen whether these new non-hydrolytic enzymes would be advantageous in the long run.

8. Fermentation

Incomplete utilization of all the sugars including hexoses (C6: glucose, galactose, and mannose) and pentoses (C5 sugars; xylose and arabinose) is another factor for high cost of 2G ethanol. In recent years, however, a lot more progress has been made in modifying various microbes including yeast (e.g., Saccharomyces cerevisiae, Scheffersomyces (Pichia) stipites, Kluyveromyces marxianus) and bacteria (e.g., Zymomonas mobilis, Escherichia coli, Klebsiella oxytoca) to make them capable of fermenting both hexoses and pentoses at comparatively high yields (metabolic (g ethanol/g sugar consumed) as well productive yield (g ethanol/g of total potential) (Hahn-Hagerdal et al., 1986; Jeffries and Jin, 2004; Jeffries, 2005; Kuhad et al., 2011; Fox et al., 2012; Laluce et al., 2012; Kim et al., 2013; Wang et al., 2013). The exhaustive details on the research efforts in making microbes capable of fermenting pentoses can be found in several recent reviews (Kuhad et al., 2011; Kim et al., 2012; Laluce et al., 2012; Balan, 2014; He et al., 2014). It is worth noting that in addition to making (mesophilic/thermophilic) microbes capable of fermenting pentoses together with hexoses, research efforts are also underway to make microbes metabolize cellulose and higher cellodextrins directly to ethanol and other valuable metabolites. Although the concept is not new, as it was shown by (Spindler et al., 1989) that by directly fermenting cellulose, it is possible to achieve higher conversion and ethanol yields, Galazka et al. (2010) recently reported a much higher conversion and yields by reconstituting the Neurospora crassa cellodextrins transporters system into S.cerevisiae. In another study, Ha et al. (2010) engineered a yeast strain to co-ferment cellulose, glucose, and xylose together; however, high glucose concentrations expected after enzymatic saccharification of pretreated solids at high solids loading suppressed the metabolism of xylose. Although some of the engineered strains show great promises in metabolizing both hexas as well as pentose sugars, the incomplete pentose sugars utilization, low metabolic and productive yields and rates, low ethanol titers (<5wt% ethanol) than yeasts >10wt%, and inhibition by process-generated inhibitors (e.g., acetic acid, furfural) are still some of the challenges that must be overcome.

9. Consolidated bioprocessing

As shown in Figure 4, three main steps in lignocellulosic biomass conversion- enzymes production, biological hydrolysis of biomass to sugars and oligomers, and fermentative metabolites (e.g., ethanol) production) in a process combined into a single bioprocessing system “Direct Microbial Conversion (DMC)” (Viljoen et al., 1926; Cooney et al., 1979; Demain et al., 2005) or lately known as “Consolidated Bioprocessing (CBP)” (Lynd, 1996). Studies have shown that CBP system combining three processing steps into one can save capital as well as operating costs (Lynd et al., 2008).

There are several cellulytic/non-cellulytic and thermophilic/thermophilic candidate microorganisms for CBP including bacteria, e.g., Clostridium thermocellum (Lynd et al., 1989; Argyros et al., 2011; Shao et al., 2011), Thermoaerobacterium saccharolyticum (Shaw et al., 2008), Clostridium phytofermentans (Jin et al., 2012), Cellulolysinivoror bescii (Yang et al., 2009; Chung et al., 2014), and yeasts, e.g., S. cerevisiae and thermotolerant K. marxianus (Yamada et al., 2013). Thermophiles have an added advantage of higher hydrolysis rates and less probability of contaminations at fermentation temperatures of >60°C than mesophiles that usually operate at temperatures <50°C (Olson et al., 2012). However, most CBP organisms identified and developed, wild or genetically engineered, to date suffer from either low ethanol titer (<3wt %), low growth, or low metabolic yield and/or productive yield.

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Among thermophiles, \textit{C. thermocellum}- an anaerobe- is the most promising candidate due to its much faster degradation rates of crystalline cellulose than possible with free fungal enzymes (Shao et al., 2011), but it lacks the ability to metabolize pentoses. Another problem with \textit{C. thermocellum} is its low metabolic yield ($<0.30 \text{ g ethanol/ g sugar}$) due to waste of carbon to undetectable and undesired products (Argyros et al., 2011; Deng et al., 2013; Yee et al., 2014) and low ethanol tolerance ($<30 \text{ g/L}$) (Deng et al., 2013). A recent report, however, has shown that a titter of 38 g/L ethanol can be produced with \textit{C. thermocellum} in a co-culture with \textit{T. saccharolyticum} (Argyros et al., 2011). \textit{T. saccharolyticum}- a thermophilic anaerobe- has been engineered to produce a high titer of ethanol (33-37 g/L) (Shaw et al., 2008), but it lacks the ability to hydrolyze cellulose and needs exogenous supplementation of cellulase.

\textit{C. bescii} has recently been engineered to produce ethanol at high metabolic yield; however, the productive yields are too low for commercial application yet (Chung et al., 2014). It is important to note here that although all these microbes perform greatly with pure (heml) cellulose compounds, their performance is comparatively not that great with real (unpretreated/pretreated) lignocellulosic biomass solids, most possibly due to inhibition of their free/cell-bound enzymes by lignin, hemicellulose, and/or other compounds in the unpretreated/pretreated solids (Shao et al., 2011; Brunecky et al., 2013; Resch et al., 2013). In addition to thermophilic and other bacteria, research is also underway in modifying yeasts to convert them into CBP organisms (Hasunuma and Kondo, 2012; Yamada et al., 2013). However, most of these genetically engineered strains still need some supplementation of exogenous enzymes for high ethanol yields.

10. Concluding remarks

In summary, a lot of progress has been made in recent years in terms of engineering plants, to make them less recalcitrant to breakdown, engineering microorganisms, to enhance their metabolic/productive yields and products/inhibitors tolerance, and developing novel pretreatments and improved enzyme cocktails to make lignocellulosic biomass derived ethanol commercially viable and profitable. Although several cellulolytic ethanol plants are up and in operation around the globe; however, continued research efforts are still needed to bring the cost further down to make cellulolytic ethanol plants profitable and replicable.

References


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