



Review Paper

Recent updates on lignocellulosic biomass derived ethanol - A review

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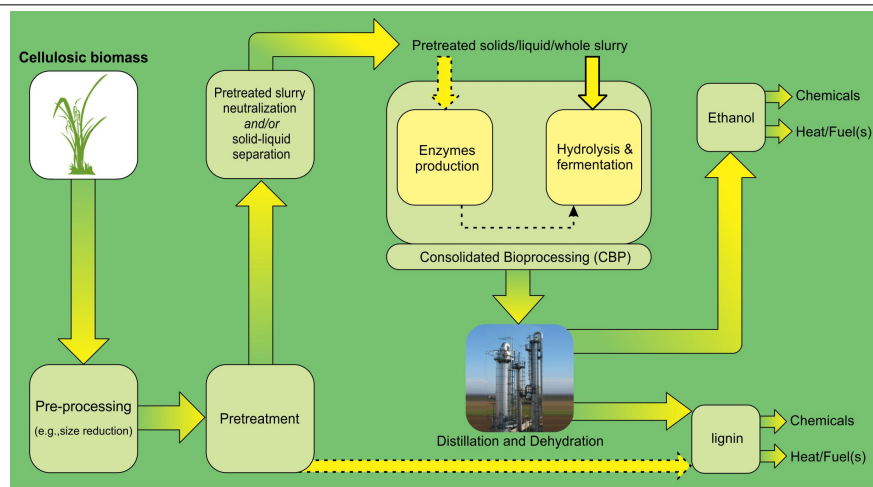
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HIGHLIGHTS

- Cellulosic biomass is the only source for sustainable fuels.
- Ethanol is a promising fuel candidate for near/long term applications.
- Ethanol can also serve as a precursor for other fuels and chemicals.
- However, processing cost for 2G ethanol is still high.
- Thus, urgent research efforts are needed to bring the cost down.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 12 December 2015
 Received in revised form 11 January 2016
 Accepted 12 January 2016
 Available online 1 March 2016

Keywords:

Pretreatment
 Cellulase
 Fermentation
 Consolidated bioprocessing

ABSTRACT

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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1. Background

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 esu 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/long 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 (Balat 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 (Whitcraft et al., 1983; Costa et al., 1985; Deluga et al., 2004; Narula et al., 2015; Riitonen 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),

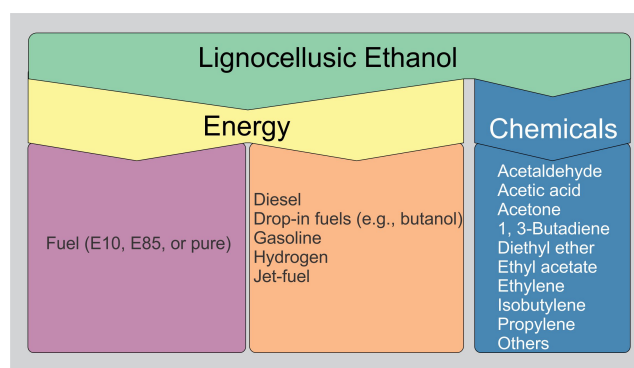


Fig.1. Promising applications of lignocellulosic ethanol for sustainable energy and chemicals.

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

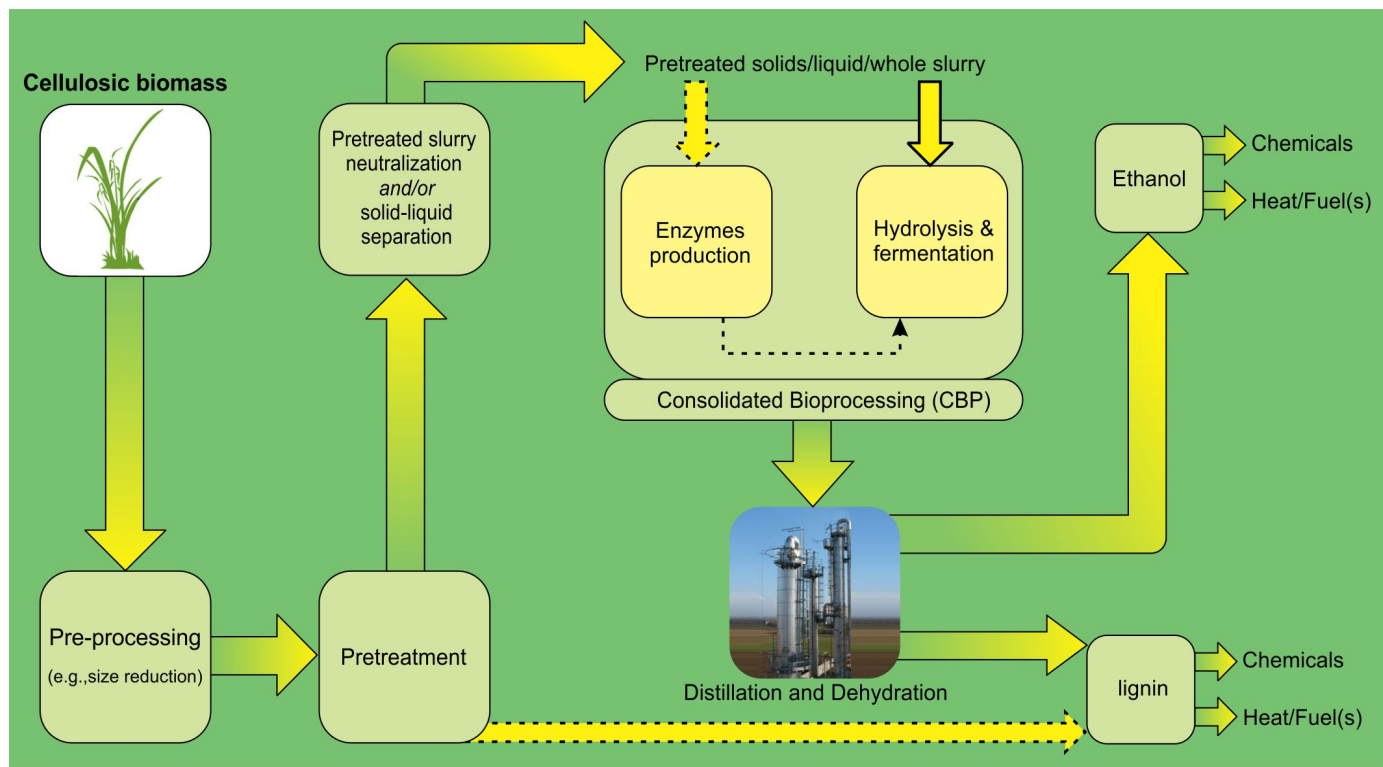


Fig.2. Schematic of second generation ethanol production process.

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. guacyl) (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 and/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), as enzymes are prone to deactivation and inhibition by their own end products and other components (Mandels and Reese, 1965; Reese and Mandels, 1980; Holtzapple et al., 1990; Kumar and Wyman, 2009b; Andrić et al., 2010; Ximenes et al., 2010; Kumar and Wyman, 2014).

Plants are being engineered to make them less resistant to break down, consequently requiring less harsher pretreatments and low enzyme loadings for high product yields (Funaoka et al., 1995; Chen and Dixon, 2007; Grabber et al., 2008; Sticklen, 2008; Fu et al., 2011). Since lignin is believed to be one of major impediments in low cost conversion of lignocellulosic biomass, the focus of most plant engineering studies is to alter the content, location, and type of lignin (syringyl: guaiacyl or S:G ratio) (Ding et al., 2012; Ragauskas et al., 2014; Wilkerson et al., 2014; Wagner et al., 2015). In this direction,

an investigation showed that down regulating the hydroxycinnamoyl transferase (HCT) and caffeic acid 3-*O*-methyltransferase (COMT) genes in alfalfa (one of the energy grasses) resulted in decreased lignin content and enhanced sugar release (Chen and Dixon, 2007). However, in contrast to other studies (Davison et al., 2006; Studer et al., 2011), the changes in S: G ratios for these transgenic lines had no correlation with sugar release. Fu and coworkers recently showed that down regulating COMT gene in switchgrass resulted in a decrease in lignin content, reduction in the S:G lignin monomer ratio, improved forage quality, and an increase in the ethanol yield by up to 38% (Fu et al., 2011). On the other hand, changes in hemicelluloses, pectins, and other components in terms of backbone composition, chain length, branching, and content have also shown promise for reduction in plants recalcitrance (Daniel et al., 2006; Bindschedler et al., 2007; Dhugga, 2007; Mohnen, 2008; Foston et al., 2011; Cook et al., 2012; Urbanowicz et al., 2012; Doblin et al., 2014). However, more research efforts need to be directed to investigate the effect of hemicelluloses genetic engineering on plants recalcitrance (Pauly et al., 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 (Gretlein

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), extractive ammonia (EA) pretreatment (Chundawat et al., 2013), 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 Murthy, 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 (>\$3 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.

COMPARISON CRITERIA	PRETREATMENT													Green	Yellow	Red										
	Concentrated Acid	Dilute Acid	Hydrofluoric Acid	Peracetic Acid	Steam Explosion (Acid)	Liquid Hot Water	Ammonia Recycle Percolation	Dilute Ammonia	Alkaline Recycle Percolation	Alkaline Wet Oxidation	Ammonia Peroxide	Lime	Concentrated Alkali					Ionic Liquids	Chelating Metal/Alkali	Supercritical/Alkaline	Organosolv	Biological	Grinding/Size Reduction	Gamma Irradiation	Microwave	
Sager Yield	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	> 90%	80-90%	70-80%	Total process glucan+xylan yield
Sugar Concentration	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	> 20%	10-20%	< 10%	Hydrolysis concentration (w/v)
Pretreatment Time	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Minutes	Hours	Days	Pretreatment time
Hemicellulase Needed?	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	No	Maybe	Yes	Are hemicellulase needed?
Distributed Processing	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	High	Medium	Low	Potential for distributed processing
Potential for Co-products	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	High	Medium	Low	Potential for co-products
Fermentation Compatibility	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Yes	Maybe	No	Fermentation compatibility
Expected Scalability	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Good	Medium	Low	Ease of scaling pretreatment
Pretreatment Energy	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	<5%	5-10%	>10%	Energy as %LHV of biomass
Pretreatment Capital Cost	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Low	Medium	High	Reactor capital costs
Water Use	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	< 1	1-5	> 5	Liter of water per liter of ethanol
Chemical Costs	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	High	Medium	Low	Cost of pretreatment chemicals
Process Control	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Simple	Moderate	Difficult	Expected ease of control
Is Lignin Preserved?	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Yes	Some	No	Lignin quantity/quality preserved?
Temperature/ Pressure	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	1 atm or Ambient T	< 20 atm or < 200°C	> 20 atm or > 200°C	Pretreatment temp./ press.
Toxic / Hazardous	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	No	Maybe	Yes	Toxic or hazardous chemicals?
Waste Disposal	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	No	Some	Yes	Pretreatment generates waste?
Feedstock Flexibility	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Large	Moderate	Limited	Effective range of feedstocks

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)).

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 (Culbertson 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 low accessibility to (hemi) 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 pseudo-lignin (Hu et al., 2012; Kumar et al., 2013) also lowers the amount of 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) often applied and studied in laboratory settings. Although cellulase end-product inhibition by glucose can be alleviated in a process configuration called simultaneous saccharification and fermentation (SSF), and inhibition by cellobiose 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/non-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® Ctec2 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 polysaccharides oxidation by LPMOs can be inhibitory to enzymes as well 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; Laluece 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; Laluece 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 cellobiose 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 cellobiose, 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 cellobiose, 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 hexose 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- can be 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).

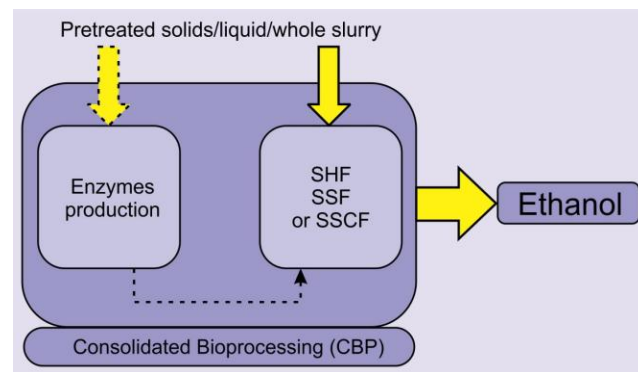


Fig.4. A simplified schematic of consolidated bioprocessing (CBP) system integrating three steps in lignocellulosic biomass conversion: enzymes production, hydrolysis (saccharification) of glucan and hemicelluloses in pretreated solids, and fermentation of sugars to ethanol (adapted from Lynd et al. (2002)). SHF, separate hydrolysis and fermentation; SSF, simultaneous saccharification and fermentation; and SSCF, simultaneous saccharification and co-fermentation.

There are several cellulolytic/non-cellulolytic and thermophilic/mesophilic candidate microorganisms for CBP including bacteria, e.g., *Clostridium thermocellum* (Lynd et al., 1989; Argyros et al., 2011; Shao et al., 2011), *Thermoanaerobacterium saccharolyticum* (Shaw et al., 2008), *Clostridium phytofermentans* (Jin et al., 2012), *Caldicellulosiruptor 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.

Among thermophiles, *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 *C. thermocellum* is its low metabolic yield (<0.30 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 g/L)(Deng et al., 2013). A recent report, however, has shown that a titer of 38 g/L ethanol can be produced with *C. thermocellum* in a co-culture with *T. saccharolyticum* (Argyros et al., 2011). *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.

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 (hemi) 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 cellulosic 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 cellulosic ethanol plants profitable and replicable.

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