Recent developments in pretreatment technologies on lignocellulosic biomass: effect of key parameters, technological improvements, and challenges

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Abstract

Lignocellulosic biomass is an inexpensive renewable source that can be used to produce biofuels and bioproducts. The recalcitrance nature of biomass hampers polysaccharide accessibility for enzymes and microbes. Several pretreatment methods have been developed for the conversion of lignocellulosic biomass into value-added products. However, these pretreatment methods also produce a wide range of secondary compounds, which are inhibitory to enzymes and microorganisms. The selection of an effective and efficient pretreatment method discussed in the review and its process optimization can significantly reduce the production of inhibitory compounds and may lead to enhanced production of fermentable sugars and biochemicals. Moreover, evolutionary and genetic engineering approaches are being used for the improvement of microbial tolerance towards inhibitors. Advancements in pretreatment and detoxification technologies may help to increase the productivity of lignocellulose-based biorefinery. In this review, we discuss the recent advancements in lignocellulosic biomass pretreatment technologies and strategies for the removal of inhibitors.

Keywords: Biomass; biorefinery; detoxification; inhibition; lignocellulose; pretreatment

1. Introduction

Lignocellulose is the most abundantly available, inexpensive and renewable raw material (Bhatia et al., 2019b; Jagtap et al., 2012). The production of commercially valuable chemicals and biofuels using lignocellulose-based processes has the potential to decrease greenhouse gas emissions, bring benefits to rural economies, and promote energy security (Patel et al., 2019). The composition of lignocellulosic biomass varies with the biomass source (i.e. hardwoods, softwoods, agricultural residues, and energy crops) and is affected by origin, age, climatic conditions, harvesting and storage processes. Lignocellulosic biomass is composed of three major interwoven polymeric components (cellulose, hemicellulose, and lignin) and exists as a natural resistant bio-composite (Bhatia et al., 2018; Saini et al., 2016).

The recalcitrant nature of lignocellulosic biomass presents a technical challenge for releasing fermentable sugars from the biomass, and a major hurdle in its use in biorefinery (Bhatia et al., 2019a; Sindhu et al., 2017). Pretreatment of lignocellulosic biomass is the initial step in exposing the cellulose and hemicellulose content for hydrolysis using enzymatic or chemical methods (Bhatia et al., 2017c). Lignin content, hemicellulose chemistry, and acetyl groups affect the enzymatic conversion of cellulose into glucose. Several pretreatment technologies have been developed for lignocellulosic biomass. These pretreatments include mechanical pretreatment, biological pretreatment, alkaline pretreatment, dilute acid hydrolysis, ammonia fiber explosion pretreatment, hydrothermal treatment (steam explosion and hot water pretreatment), and novel green processes (ionic liquids and sub/supercritical fluids) (Sankaran et al., 2019; Singh, 2018). Pretreatment methods increase the surface area and provide easily accessible binding sites for enzymes (Parthiba Karthikeyan et al., 2018). Pretreatment methods such as ionic liquids, supercritical fluid based, low temperature steep delignification (LTSD), and cosolvent enhanced lignocellulosic fractionation (CELF), which are considered to be the most advanced methods, result in higher sugar yield with a minimum amount of byproducts generated (Meng et al., 2018; Patinvoh et al., 2017; Sorn et al., 2019). The released pentose and hexose sugars can be used for the production of organic acids, polyols, fatty acids, alcohols, and bioplastics by multiple microbes (Jagtap et al., 2019; Kumar et al., 2019a).

Pretreatment processing results in the production of various byproducts such as furan aldehydes (furfural, 5-hydroxy methyl furfural (HMF)), phenolics (vanillin), weak acids (acetic acid and formic acid), along with fermentable sugars, which inhibit microbial growth by causing intracellular acidification, energy drainage, and accumulation of reactive oxygen species (Jönsson & Martín, 2016; Moreno et al., 2019). Biomass hydrolysate requires detoxification before it can be used in microbial fermentation to avoid negative effects during the fermentation process. Various physical (evaporation, membrane filtration), chemical (resin exchange, alkali detoxification, active carbon adsorption), and biological (microbial and enzymatic) detoxification methods have been reported to remove the inhibitors (Farmanbordar et al., 2018; Oliveira et al., 2017). Researchers are also working on other strategies, such as the improvement of plants for reduced lignin content and the engineering of microbes to tolerate various inhibitors (Bhatia et al., 2017b; Shafrin et al., 2017). Simultaneous detoxification and fermentation processing seems to be a more feasible and economical approach for the conversion of lignocellulosic biomass into biofuel and other commercially important products (Hazeena et al., 2019). Several review articles have been published on lignocellulosic biomass treatment technology. However, a review article describing recent advancements in pretreatment technologies along with their advantages and disadvantages, the role of the key factors in biomass recalcitrance, and the methods available for detoxification of hydrolysate is the need of the hour.

In this review, the focus is to provide an overview of the advancements in pretreatment methods and strategies developed for detoxification of inhibitors present in hydrolysate.

2. Lignocellulose composition and role of various factors in its recalcitrance

Lignocellulose biomass is composed of carbohydrate polymers such as cellulose (40-50%), hemicellulose (20-30%), lignin 10-25%, small amounts of pectin, proteins, and extractives (chlorophyll, waxes, and nonstructural sugars). The composition of lignocellulosic biomass varies with plant species, age, stage of growth, and season. Cellulose is a linear polymer composed of D-glucose units linked together by β-(1-4) glycosidic bonds. The degree of polymerization is approximately 4000-6000 glucose in woody biomass. Polymers of cellulose are interlinked through hydrogen, and van der Walls bonds to form a microfibril, and present in crystalline and amorphous form. Microfibrils are covered by hemicellulose and lignin (Fig. 1). Crystalline cellulose fiber parts attached to each other by non-covalent hydrogen bonding, which provides 3-30 times lower degradability as compared to the amorphous part. Cellulase is readily able to hydrolyze more accessible amorphous cellulose but is not effective at degrading the less accessible crystalline portion (Taherzadeh & Karimi, 2008).

Hemicellulose, the second most abundant heterogeneously branched polymer, is composed of pentoses (D-xylose and L-arabinose), hexoses (D-glucose, D-mannose, D-galactose), acetyl groups, and uronic acids. The degree of polymerization is as high as 50–300 monosaccharide units. Hemicellulose lacks a crystalline structure owing to its branched structure and presence of the acetyl group and is easily degradable owing to its amorphous nature. The composition of hemicellulose varies with plant species. Softwood hemicellulose components are galactoglucomannan and arabinoglucuronoxylan, while glucuronoxylan is the main component of hemicellulose in hardwood (Schutyser et al., 2017). Hemicellulose acts as a physical barrier and restricts the accessibility of cellulase to cellulose. Removal of hemicellulose with pretreatment methods (acid or steam hydrolysis) and the addition of enzymes (hemicellulose) increases cellulose hydrolysis.

Lignin is the most complex amorphous polyphenolics polymer composed of three o-methoxylated *p*-hydroxyphenyl propanoid units (monolignols; i.e. *p*-coumaryl, coniferyl, and sinapyl alcohol, Fig. 1). These monomer units give rise to *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) subunits when incorporated into a lignin polymer (Bhatia et al., 2019a) Fig. 1). Depending on the biomass source, lignin composition varies with the change in the ratio of different monomer units. Gymnosperms (softwood) and fern lignin are generally composed of G as the main component, followed by a small H unit content. Contrastingly, in angiosperm (hardwood) plants, lignin is mainly composed of S units followed by G units. The main lignin components of herbaceous crops are G followed by H and S units. Various monomer units are linked through (β-O-4) aryl ether bonds. Lignin acts as glue around the cellulose and hemicellulose fibers and its main function is to provide mechanical strength and support for the formation of vascular tissue for the transport of nutrients and to promote resistance against microbial attack. Lignin makes biomass recalcitrant by restricting the accessibility of cellulase to cellulose and by preventing the deactivation of enzymes by various lignin-derived compounds (Bichot et al., 2018).

Lignocellulosic biomass recalcitrance is the natural resistance of plant cells against microbial degradation, animal attacks, and other environmental conditions. Along with the structural components (i.e. hemicellulose and lignin), there are other factors that influence recalcitrance, including the presence of acetyl groups and proteins, and the porosity of biomass (Table. 1). Acetyl groups bind hemicellulose via covalent ester bonds, and deacetylation of biomass may increase lignocellulose degradation by 5–7 times. This recalcitrance property is a bottleneck in industrial utilization of lignocellulosic biomass and various pretreatments are required to overcome this issue. Proteins also have negative and positive influences on recalcitrance. Some proteins help to break hydrogen bonds between polysaccharides, which improves degradation, while some proteins inhibit the activity of various hydrolases (Zhao et al., 2012). To overcome the inhibitory effect of various proteins usually dried lignocellulosic materials are used in the biorefinery, as drying and storage of biomass denature proteins. The physical structure (accessible surface area (ASA), particle size, and pore volume) of the material also plays an important role in biomass recalcitrance. Higher ASA provides more surface area for enzymes during hydrolysis. Ultrafine grinding leads to smaller particle sizes, leading to changes in polymerization and porosity, and enhances enzymatic hydrolysis (Zhang et al., 2016). The pore size of biomass also has an important role as enzymes can only enter the pores of a specific size.

3. Pretreatment methods

Different lignocellulosic biomass requires different pretreatments based on their composition to convert these into free sugars. Various pretreatment methods have been reported and each method has its advantages and disadvantages (Table 2). The ideal biomass pretreatment method should have the following characteristics: no need for biomass size reduction, preservation of the cellulosic and hemicellulose part, no toxic inhibitors generated, lower energy demands, low cost, and recycling of chemicals. Pretreatment methods have been broadly classified into biological (fungi, bacteria, and archaea) and non-biological. Non-biological pretreatment methods can be further divided into three categories including, physical (milling, microwave, ultrasound, and pyrolysis), chemical (acid, alkali, ozonolysis, and organosolvent, ionic liquids), and physicochemical (hot water, steam explosion, ammonia based, wet oxidation, and carbon dioxide (CO_2) explosion). Most of the traditional pretreatment methods (acids and alkali) have disadvantages including the release of the degradation products, lack of selectivity, low sugar yields, lower process efficiency, and higher processing costs. There are always open opportunities to develop a novel and green pretreatment method with low cost, lower energy consumption, lower waste and new product production (Jagtap & Rao, 2018b). A single method cannot be suitable for pretreatment of all types of biomass. Therefore, this review covers the advancements in recently developed pretreatment methods of lignocellulosic biomass.

3.1 Ionic liquids

Ionic liquids (ILs) are considered green solvents owing to their unique solvation properties (Table. 3). ILs shows high thermal stabilities and low toxicity, and require low vapor pressure (Morais et al., 2015). These ILs selectively remove the lignin and hemicellulose part of biomass to provide pure cellulose for further hydrolysis. The IL pretreatment process can be operated more efficiently in continuous mode with high biomass input (Brandt et al., 2011). However, the main challenges are ILs toxicity, pH compatibility, costliness, and process complexity (Singh, 2018). Cheap and environment-friendly ILs have been synthesized using lignin and hemicellulose derived compounds (Socha et al., 2014). The reduced amination of lignin monomers furfural, vanillin, and *p*-anisaldehyde followed by treatment with phosphoric acid has generated ILs including, $[FurEt_2NH][H_2PO_4]$, $[VanEt_2NH][H_2PO_4]$, and [p-AnisEt2NH]$[H_2PO_4]$, respectively. Comparable sugar yields were obtained from enzymatic hydrolysis of pretreated biomass using newly synthesized three ionic liquid and [C2mim][OAc].

ILs have applications in the dissolution of cellulose due to good solubilities (5-20%). The dissolution of cellulose is attributed to the strong hydrogen bonding between equatorial hydroxyl groups of cellulose and anions of ILs. The two approaches have been widely used for the solubilization of the entire biomass. In the first approach, acidic or acidified lignocellulose dissolving ILs are used. In the second approach, ILs dissolve the lignin and partial hemicellulose are used, while the cellulose part remains intact. The 15-92% lignin removal from biomass has been achieved in several studies using IL treatment (Arora et al., 2010; Brandt et al., 2011). An IL mixture of 1,3-dimethylimidazolium methyl sulfate $[C_4C_1im][MeSO_4]$, 1-butyl-3-methylimidazolium hydrogen sulfate $[C_4C_1im][HSO_4]$, 1-

butylimidazolium hydrogen sulfate $[C_4Him][HSO_4]$, and 1-butyl-3-methylimidazolium chloride $[C_4C_1im]Cl$, 1-Butyl-3-methylimidazolium acetate $[C_4C_1im]MeCO_2]$ with water were used for the pretreatment of biomass, including miscanthus, pine, willow, maple wood, switchgrass, and oak. The lignin part was removed (15-92%) and the cellulose enriched fraction was used for enzymatic hydrolysis. The 25% of hemicellulose and 90% of glucose content of biomass were released after IL and enzyme hydrolysis treatment. Rice straw, corncobs, barley straw, and wheat bran were pretreated with conventional acid and alkali methods. The yield of reducing sugars was in the range 34-49% for acid pretreated biomass and 46- 65% for alkali treated biomass (Kucharska et al., 2018). In another study, the yields of glucose and xylose from ILs pretreated corn stover were higher as compared to AFEX pretreatment (Xu et al., 2012).

Effective pretreatment of biomass has been demonstrated using ILs, including 1-ethyl-3-methylimidazolium acetate ($[C_2C_1Im][OAc]$), 1-butyl-3-methylimidazolium chloride ($[C_4C_1Im]Cl$, cholinium lysinate ([Ch][Lys]), and triethylammonium hydrogensulfate ($[TEA][HSO_4]$) (Sun et al., 2017b) Table. 3). The reusability of ILs following biomass pretreatment has been demonstrated (Sun et al., 2017b). In addition to structural differences, [Ch][Lys], [TEA][HSO4], and $[C_2C_1Im][OAc]$ are basic, acidic, and near neutral, respectively. IL pretreatment on Kraft lignin revealed that it mainly begins with depolymerization, dehydration, and recondensation pathways (Dutta et al., 2017).

The separation of the liquid and solid portions after pretreatment is associated with a loss of biomass and sugars. Therefore, a combination of deconstruction, saccharification, and fermentation is ideal for reducing operational costs. Consolidation of pretreatment using IL tolerant enzymes used for saccharification of biomass. Recently, cholinium lysinate ([Ch][Lys]) and enzyme cocktails, Cellic CTec2 and HTec2, were used for deconstruction and saccharification of milled sorghum. The hydrolysate is directly fermented by the oleaginous yeast

Rhodosporidium toruloides for the production of bisaboline (Dinh et al., 2019; Sundstrom et al., 2018). In another study, one pot integrated ethanol production was demonstrated using low cost ILs (Sun et al., 2017a).

The cost of ILs is an important consideration for the industrial application. The recovery and reuse of ILs are very crucial for the practical employment of ILs based technology. Almost a full recovery of ILs was achieved by adding glycerol to carboxylate ILs (Clough et al., 2016). The high cost of imidazolium cations is a major obstacle limiting the industrial application of ILs for biomass pretreatment. Therefore, novel ionic liquids based on cations have been developed from renewable sources. ILs are synthesized using vanillin, *p*-anisaldehyde and furfural derived from lignin and hemicellulose as starting material (Socha et al., 2014). The technoeconomic analysis suggested the raw materials cost to 13.71$/kg for the biomass-derived ILs (Socha et al., 2014). The modification of the reductive amination step in the synthesis of ILs could significantly lower the raw material cost to 4$/kg.

3.2 Supercritical fluids-based pretreatments

Supercritical fluids (SCFs) have unique properties like gas and liquid, including diffusivity and viscosities similar to gases and densities similar to liquids (Mani Rathnam & Madras, 2019). The solvation power of SCFs is lower than that of other fluids and tunable by slight changes in temperature and pressure. CO_2 is listed as supercritical CO_2 ($scCO_2$) because it has a lower critical temperature (31°C), pressure (73.8 bar), and solubility (7.118 $(cal/cm^3)^{0.5}$) (Morais et al., 2015). CO_2 has a lower critical temperature and pressure than many other compounds including, ammonia (132.3°C, 112.8 bar), methanol (240°C, 79.6 bar), and water (374.2°C, 221.2 bar). These characteristics make CO_2 an excellent solvent for monomers and nonpolar materials, and a poor solvent for polymers and polar compounds. These characteristics are useful for the easy separation of biomass components following pretreatment, compared to other solvents. At high pressures, CO_2 easily penetrates the surface of biomass to reduce recalcitrance and increase the permeability of cellulose. This leads to increased accessibility to hemicellulose and cellulose for enzymatic hydrolysis to reach maximum reducing sugar production (Daza Serna et al., 2016).

Enzymes are green catalysts which are used to hydrolyze polysaccharides under milder conditions. However, higher cost and low enzyme loading limit the quick and efficient hydrolysis process (Jagtap et al., 2014). Enzymatic hydrolysis, coupled with high-pressure CO_2/H_2O process, provides green conditions for biomass conversion without the need to adjust the pH of the medium. Several research groups are studying green solvent methods (ionic liquids and CO_2 based) for the conversion of biomass. In a previous study, the maximum sugar yield from the untreated pine sawdust sample was only 5.2%. The conversion of pine sawdust after CO_2 pretreatment increased to 32.5 g of reducing sugars/100 g of substrate, in comparison to 26 g of reducing sugars/100 g for steam explosion (Hohlberg et al., 1989).

Eucalyptus chip pretreatment with CO_2 at subcritical conditions (at 180°C and 50 bar for 80 min) and subsequent enzymatic hydrolysis leads to maximal glucan conversions of 92% (Zhang & Wu, 2015). The effect of changes in pressure (80 to 120 bar) on the wheat straw at 190°C for 30 min resulted in a higher yield of glucose (Relvas et al., 2015). In another study, rice straw was treated with $scCO_2$ at 10–30 MPa, and 40–110°C for 15–45 min (Cha et al., 2014; Gao et al., 2010). Pretreated rice straw generated glucose yields of 32%, compared to the glucose yield of 27% from untreated rice straw (Gao et al., 2010) (Table 4).

Increased glucan recovery and enzymatic conversion of sugars have been achieved using combined pretreatments because of the synergistic action on biomass treatment (Cha et al., 2014; Zhang & Wu, 2015). For example, high-pressure CO_2 accelerates the swelling effect on biomass in the presence of water leading to high glucose yields from enzymatic hydrolysis. In one study, aspen and yellow pine were treated at different temperatures (112–165°C), reaction pressures (200-275 bar), and moisture contents (0–73% (w/w)) for 10-60 min (Kim & Hong, 2001). The increased moisture content significantly increased the final sugar yield from $scCO_2$ treated aspen biomass (Table 4). The untreated aspen and yellow pine biomass produced less reducing sugar (14% and 12%, respectively). Sugar yields of $scCO_2$ aspen and yellow pine biomass were 84% and 27% at 3100 psi and 165°C for 30 min. The optimum temperature (165°C) showed a better effect on the production of glucose from aspen compared to yellow pine.

There is still a room for process improvements using CO_2 for pretreatment. CO_2 has not yet been explored in several other areas including, in the use of CO_2 in direct hydrolysis of untreated lignocellulosic biomass, search for synergistic solvents with CO_2, and one pot process development of value-added products from biomass.

3.3 Low temperature steep delignification

Existing delignification processes have several cons including that all processes are handled at a higher temperature and higher pressure, chemical and enzyme recovery is costly, and toxic inhibitors are produced (Kumar & Sharma, 2017). Therefore, additional work is required to develop an efficient pretreatment process that operates at low temperature, rapidly removes lignin, easily recovers chemicals, and generates sugars for the production of value-added products using renewable biomass.

LTSD is an efficient process that utilizes low concentrations of non-toxic chemicals, oxygen, and bases for lignocellulosic biomass conversion. The mild operation conditions separated biomass to lignin, hemicellulose, and cellulose. It removes and recovers 90% of the lignin from biomass. LTSD has several benefits including no production of toxic inhibitors or chemicals, low operational costs, environmental and ecological sustainability, and recycling of chemicals. LTSD was developed by the Bioprocess Innovation Company working with agricultural residues (wheat straw), softwood biomass (switchgrass and miscanthus), and woody biomass (forestry residues) (Inc, 2015). The LTSD process allows the integration of a pretreatment process with enzymatic hydrolysis, fermentation, and distillation processes at a pilot level.

The LTSD process was used for the conversion of mixed hardwood (HW) chips to sugars (Park et al., 2015). The effect of delignification on biomass was investigated using oxygen, hydrogen peroxide, and sodium chlorite treatments. Sodium chlorite delignification (SCD) method was the most effective to remove lignin in mixed hardwood samples as compared to oxygen delignification (OD) and hydrogen peroxide delignification (HPD) methods. Mixed hardwood samples subjected to repeated oxygen delignifying treatments. It showed a complete conversion of xylan and 78% yield for glucan after enzymatic hydrolysis (Park et al., 2015). The untreated mixed hardwood samples showed only 2.5% conversion of carbohydrates. The HPD method was more significant in enzymatic hydrolysis of hemicellulose. The SCD and OD methods showed an increase in conversion for glucan and xylan. Among the three delignification methods, SCD was the most effective method to remove lignin and achieve high conversion yield.

3.4 Cosolvent enhanced lignocellulosic fractionation

Cosolvent enhanced lignocellulosic fractionation (CELF) is a process that uses a combination of the organic compound, tetrahydrofuran water, and dilute sulfuric acid for fractionation of lignin from lignocellulosic biomass (Keating et al., 2014). In the organosolvent pretreatment process, organic solvents including ethanol, methanol, and acetone are used for fractionation of lignin from biomass (Meng et al., 2018). THF has been used as a multifunctional renewable solvent to dissolve acetylated lignin from biomass and promotes hydrolysis of cellulose in water to obtain the highest sugar yields at low enzyme dosage (Nguyen et al., 2017). The polar co-solvents such as γ-valerolactone, 1,4-dioxane, dimethyl sulfoxide, and acetone are also used for biomass delignification (Petridis & Smith, 2018). An effective solvent need to disrupt hydrogen bonds and interfere with hydrophobic stacking interactions in cellulose (Petridis & Smith, 2018).

The CELF pretreatment of biomass operates at high temperatures and produces additional byproducts, including furfural (FF), 5-hydroxymethylfurfural, and levulinic acid. These precursors can be used for the production of value-added chemicals. Under milder conditions, CELF pretreatment can achieve more than 95% recovery of fermentable sugars after enzyme hydrolysis compared to traditional dilute acid treatment (Nguyen et al., 2015). In one study, poplar biomass was treated at different temperatures (160–180ºC), for different lengths of time (15–60 min), with different catalysts (0.1 M H_2SO_4), and different THF:water ratios (1:1 or 7:1 (v:v)). In the same study, a new type of lignin, CELF lignin, was recovered. In a one-pot reaction, THF promoted hydrolysis of maple wood to fuel precursors furfural, HMF, and levulinic acid. The yields were 86% furfural, 21% HMF, and 40% levulinic acid in a liquid fraction, while more than 90% of the lignin recovered was in the solid fraction (Cai et al., 2013). The pretreatment process also produces various side products which affects microbial fermentation.

4. Composition of inhibitors and their effects

Different types of byproducts are produced during the pretreatment of lignocellulosic biomass. Most byproducts have an inhibitory effect on microbes which necessitates a detoxification step before subjecting hydrolysate to fermentation (Table. 5).

4.1 Lignin and hemicellulose derived inhibitors

Phenolic compounds and aromatics are generally produced by most pretreatment methods involving agricultural residues, softwood, or hardwood. The concentration and type of phenolic compounds are based on biomass species and loading of substrate (Qin et al., 2016). The pretreatment usually converts the lignin to guaiacyl and syringyl moieties found in phenolic compounds (Yang et al., 2020). Polyphenolic compounds such as hydroxycinamic acid derivatives (cinnamic acid, *p*-coumaric acid, ferulic acid, caffeic acid, chlorgenic acid, and rosmarinic acid), tannins, and gallic acid are also released during biomass pretreatment. Furan aldehydes and aliphatic acids are produced from pentose and hexose sugars present in the biomass. Additionally, 5-hydroxymethylfurfural is a degradation product of hexose sugars (glucose, galactose, and mannose) in the hemicellulose content of biomass (Fig. 1). HMF is further converted to formic acid and levulinic acid (Fig. 1) (Kumar et al., 2019b). Furfural is generated from pentose sugars such as xylose and arabinose. Furfural can also be converted to formic acid. Weak acids such as acetic acid, benzoic acid, vanillic acid, and syringic acid have also been identified in pretreated hydrolysates.

Although the formation of inhibitors is less during IL pretreatment, the minor concentration of ILs remaining in pretreatment process is toxic to microorganisms and enzymes. The 18 aromatic compounds were generated from kraft lignin after common ILs pretreatment. Among the 18 monomeric lignin derived products, uaiacol, vanillin, guaiacylacetone, and acetovanillone were the major products (Dutta et al., 2017). The acidity of IL liquor generates furfural during IL pretreatment. The high salinity of ionic liquids inactivates the enzyme. Levulinic acid and HMF were generated in one-pot hydrolysis of four different lignocellulosic biomass with ILs (Halder et al., 2019). HMF and furfural was produced from Eucalyptus chips during subcritical CO_2 pretreatment process. The higher temperature decreased the HMF and furfural yields because of their degradation (Zhang & Wu, 2015). The 4-5% of HMF and 10-23% of furfural was produced from switchgrass, corn stover, big bluestem, and mixed perennial grasses pretreated using biphasic CO_2-H_2O pretreatment (Luterbacher et al., 2010). The CELF pretreatment of biomass produces inhibitors including, furfural, HMF, and levulinic acid.

4.2 Effect of inhibitors on cell growth and fermentation

Inhibitors reduce microbial growth and increase the cost of the target products (Table 5). The small size of these compounds allows them to penetrate cell membranes and damage the internal structures of the cell, changing the morphology of cells, and inhibiting RNA and protein synthesis (Jönsson & Martín, 2016). The presence of vanillin, *p*-courmaric acid, and ferulic acid were found to reduce cellulose conversion by 26%, 30%, and 16%, respectively. Weak acids penetrate the cell membrane to disrupt sugar and ion transport, resulting in the inhibition of cell growth. Weak acids have also been reported to act as glycolytic enzymes and ATP regeneration inhibitors, which results in an energy drain (Cunha et al., 2019). In yeast cells, an increase in reactive oxygen species concentration, and a reduction in the glutathione pool occurs in response to increased hydrogen ion (H^+) concentration inside the cell (Guo & Olsson, 2014).

These compounds negatively affect cell growth and microbial fermentation, reduce the rate of sugar uptake leading to a subsequent decrease in the production of the target product (Jönsson et al., 2013; Jönsson & Martín, 2016). Furfural toxicity is higher than that of HMF owing to the inhibitory action on enzymes involved in central carbon metabolism (Liu et al., 2019a). Furan derivatives also affect amino acid biosynthesis and damage cell organelles (Allen et al., 2010). Furfural has shown negative effects on the activity of cellulase at 4 g/L. The combined action of furans, phenols, and other lignin derivatives are detrimental to cell growth. Xylose contains acetyl groups that lead to the formation of furfural after acid hydrolysis.

The cell growth of xylose fermenting yeasts such as *Candida shehatae* and *Pichia stipitis* have been shown to be inhibited by furfural concentrations of 2–4 g/L (Palmqvist & Hahn-Hägerdal, 2000). Phenolic compounds generated from pretreated hardwood decrease conversion yield by 50% (Kim et al., 2011). Ding et al. (2011) studied the interactive effect of phenol, furfural, and acetic acid on *Saccharomyces cerevisiae* and found that, in combination with other inhibitors, acetic acid exerts a more severe effect on cells by causing loss in membrane integrity and inhibiting metabolism (Ding et al., 2011). Keating et al. (2014) studied effects of ammonia-pretreated lignocellulose-derived aromatic inhibitors on *Escherichia coli* ethanologenesis by studying transcriptomics and proteomics during fermentation (Keating et al., 2014). Four major regulators MarA/SoxS/Rob, FrmR, AaeR, and YqhC were identified, and induction of these regulons was linked to reduced ethanol production, pyruvate accumulation, ATP and NAD(P)H depletion, and inhibition of xylose conversion (Keating et al., 2014). *E. coli* cells cultured in the presence of phenol showed reduced lipid-to-protein ratio in their cytoplasmic and outer cell membrane to counter the inhibitory effect of phenol (Keweloh et al., 1990). The effect of various inhibitors on white rot fungi *Trametes versicolor* was studied and it was reported that this microorganism is able to utilize most of the inhibitors up to 0.2–0.6 g/L and levulinic acid was found to inhibit xylose and mannose utilization (Nilsson et al., 2016). Various inhibitors affect microbial growth and secondary metabolite production. A separate study investigated the effect of various inhibitors on *Streptomyces coelicolor* for growth and reported toxicity of various inhibitors as vanillin > furfural > HMF > acetate (Bhatia et al., 2016a; Bhatia et al., 2016b). Delayed cell growth is related to slowed carbon source utilization, and various inhibitors have different effect on *Saccharomyces cerevisiae* glucose fermentation, as follows: vanillin > phenol > syringaldehyde > 5-HMF > furfural > levulinic acid > acetic acid > formic acid (Li et al., 2017). Liu et al. (2019) studied effect of different inhibitors on *Clostridium acetobutylicum* and reported downregulation of enzymes/proteins involved in glycolysis, reductive tricarboxylic acid cycle (TCA), acetone-butanol synthesis, and upregulation of gluconeogenesis and oxidative TCA cycle (Liu et al., 2019a).

4.3 Effect of inhibitors on enzymes during hydrolysis

A variety of enzymes are required for the hydrolysis of various components of lignocellulose (i.e. cellulose (cellobiohydrolase, endoglucanase, *β*-glucosidase), hemicellulose (endo-xylanase, esterase, endo-mannanase, *β*-mannosidase, *α*-glucuronidase, *α*-galactosidase), lignin (laccase, manganese peroxidase, lignin peroxidase; (Jagtap & Rao, 2018a; Van Dyk & Pletschke, 2012). Inhibitors also reduced the efficiency of enzyme hydrolysis by inducing protein precipitation (Jönsson et al., 2013). Data also shows that phenolics with a smaller molecular weight (<1 kDa) and higher carbonyl group content showed the strongest inhibitory effect on cellulase hydrolysis activity (Zhai et al., 2018). The strength of inhibition and deactivation also depends on the type and source of enzymes. Ximens et al. (2011) studied effect of various inhibitors (tannic, gallic, vanillin, hydroxy cinnamic and 4-hydroxybenzoic) and found all the inhibitors were able to cause 20-80% *β*-glucosidase inhibition. *β*-glucosidase from *Aspergillus niger* was more resistant and required 5-10 times higher inhibitor concentration compared to *Trichoderma reesei β*-glucosidase (Ximenes et al., 2011). Various phenolic compounds (gallic, chlorogenic, caffeic, coumaric cinnamic acid) are produced during the sugarcane saccharification and able to inhibit carboxymethylcellulase and xylanase activity by 87 and 47% respectively (González-Bautista et al., 2017). The product of the enzymatic reactions also inhibited enzyme activity. Cellulase is mostly inhibited by cellobiose and *β*-glucosidase by glucose, to overcome this excess *β*-glucosidase is added to cellulase reaction system (Van Dyk & Pletschke, 2012). Li et al. (2017) studied the effect of vanillin on cellulase activity and reported an IC_{50} value of 30 g/L and demonstrated that aldehyde and phenolic hydroxyl groups play the main role in enzyme inhibition (Li et al., 2017). Phenolic and organic acids at a concentration of 1 g/L are able to inhibit exo-cellulase activity by 92% and 87%, respectively (Rajan & Carrier, 2016). Lignin also affects the enzymes hydrolytic efficiency, and different types of lignin follow different mechanism. Organosolv lignin adsorbs cellulose and reduces the availability of enzymes, which ultimately results in decreased sugar yield. Kraft lignin precipitated on the surface of cellulose and inhibit its contact with the enzymes (Li et al., 2018).

5. Strategies to mitigate inhibitor effects

To reduce the inhibitory effects of various compounds on microbial fermentation, different strategies can be applied. These strategies involve detoxification of hydrolysate using various physicochemical methods, engineering of microbes to induce their inhibitor tolerance, and improvement of plants to reduce their lignin content (Table. 6).

5.1 Detoxification of lignocellulosic hydrolysate

5.1.1 Physical methods

Evaporation and membrane filtration are two physical methods reported for inhibitor removal from lignocellulosic hydrolysate. Various volatile inhibitors (acetic acid, furfural, vanillin, etc.) can be removed using the evaporation method under vacuum conditions. The evaporation method has been used for the detoxification of dilute acid treated lignocellulosic hydrolysate of spruce and results in the complete removal of furfural and HMF by 4% at evaporation of 90% of the initial volume (Larsson et al., 1999). The adsorptive microporous membrane is a special type of membrane that has functional groups attached to the surface of internal pores. Wickramasinghe and Grzenia used adsorptive membrane (Sartobind Q) for the removal of acetic acid from the biomass hydrolysate (Wickramasinghe & Grzenia, 2008). Nanofiltration is a pressure driven filtration process, which uses membranes with a molecular weight cut off ranging from 100 to 1000 g/mol. The nanofiltration method has also been used to detoxify hot water treated wood hydrolysate and is able to remove acetic acid, formic acid, furfural, and HMF using a molecular weight cut off of 100 g/mol (Liu et al., 2008). Fayet et al. (2018) compared several membranes for detoxification of wheat straw hydrolysate and found DK membranes to be the most suitable for inhibitor removal with high rejection of sugars (> 99%). DK membranes showed different rejection capability to various inhibitors (HMF < coumaric acid<levulinic acid< vanillin< ferulic acid< syringaldehyde) and are able to remove acetic acid and furfural by 92% with minimum removal of syringaldehyde (25%) (Fayet et al., 2018). For the simultaneous concentration and detoxification of lignocellulosic hydrolysate Pan et al. (2019) used a nanofiltration and reverse osmosis hybrid system to concentrate sugar by 3.8 fold with maximum removal of inhibitors (Pan et al., 2019). Qi et al. (2011) studied the effect of different processing parameters (pH, temperature, permeation flux) on filtration and rejection of glucose-xylose-furfural solution. Results showed that the rejection of three solutes decreased as the pH and temperature increased, and increases as the permeation flux increased (Qi et al., 2011).

5.1.2 Chemical methods

Different chemical methods, including alkaline detoxification, ion exchange, and biochar adsorption, have been reported for the detoxification of biomass hydrolysate. Biomass hydrolysate is acidic in nature and its neutralization is a necessary step before using it for fermentation. Commonly used alkali solutions are $Ca(OH)_2$, NaOH, and NH_4OH, which are used to maintain the pH. During this process, furfural and HMF are removed by precipitation up to a certain extent. Guo et al. (2013) compared different alkali for the detoxification of spruce hydrolysate and reported $Ca(OH)_2$ to be the most effective for the removal of furfural and HMF (Guo et al., 2013). Cavka & Jönsson (2013) explored sodium borohydride for detoxification of Norway spruce and found that it was able to effectively remove coniferyl aldehyde, *p*-benzoquinone, 2,6-dimethoxybenzoquinone, and furfural under mild reaction conditions (pH 6 and 20°C) (Cavka & Jönsson, 2013). To avoid the problems related to handling and phenolics compound generation, Ahmed et al. (2019) used dry calcium carbonate as an acid neutralizing agent. Dry detoxification helps to simplify the process by reducing the time, cost, and labor, when compared to wet detoxification (Ahmed et al., 2019).

Chemical adsorption is usually performed using ion exchange resin and wood charcoal. This process involves adsorption of inhibitors from the liquid phase to an adsorbent solid phase involving weak chemical bonds. Different ion exchange resins (anion, cation, and neutral) were evaluated by Nilvebrant et al. (2001) for the detoxification of dilute acid hydrolysate of spruce and their detoxification efficiency was recorded as anion> uncharged> cation (Nilvebrant et al., 2001). An anion exchanger has positively charged ammonium groups with hydroxyl ions as counter groups that exchange ionized carboxylic groups at pH 5.5 and phenolic groups at pH 10. An uncharged exchanger is able to remove only furan and phenolic compounds by weak hydrophobic interactions while cationic resins have a negative charge and show repulsion against anionic inhibitors and, are thus, unable to remove them.

Activated charcoal is a widely explored adsorbent for the detoxification of biomass hydrolysate that contains compounds of higher hydrophobicity (i.e. furan and phenolics compounds) than sugars. Lee et al. (2016) used activated charcoal for acid pretreated biomass detoxification in a continuous fixed bed column and affinity of biochar for different components was reported in following order vanillic acid, 4-hydroxybenzoic acid, furfural, acetic acid, sulfuric acid and xylose (Lee & Park, 2016). Monlau et al. (2015) prepared pyrochar from the solid waste of an anaerobic digester and analyzed them for the detoxification of lignocellulosic hydrolysate of corn stalks and found that it successfully removed 100% of the HMF and 91% of furfural without affecting the sugar concentration (Monlau et al., 2015). Coconut shell activated carbon (CSAC) has a high affinity for vanillin, tannic acid, and phenolic compounds and sugarcane biomass pretreated with 2% (w/v) CSAC leads to a 30% increase in sugar yield (Freitas et al., 2019). Use of torrefied biomass has also reported in the detoxification process, Doddapaneni et al. (2018) reported 60% of furfural removal in 50 min from torrefaction condensate (Doddapaneni et al., 2018).

Complex extraction is an ion-associated extraction process in which extraction is accomplished by ion exchange and differential solubility. Trialkylamine, n-octanol, and kerosene have been used to detoxify corn stover hydrolysate and were able to remove 100% furfural with 73.3% acetic acid and 45.7% HMF (Zhu et al., 2011). The main problems associated with this process are cost, toxicity, and extra steps required for the recycling of solvents.

5.2 Biological detoxification methods

Biological detoxification using microbes and enzymes is ecofriendly and more specific in nature. It can be performed using microbes or enzymes.

5.2.1 Microbial detoxification

Microorganisms can also be used to detoxify and remove the inhibitors. Singh et al. (2017b) isolated *Bordetella spp.* which was able to remove furfural (100%), HMF (94%), and acetic acid (82%) from sugarcane biomass hydrolysate in 16 h without affecting the sugar concentration. Biodetoxification conditions also affect the detoxification rate (Sun et al., 2017b). By optimizing aeration and mixing conditions He et al. (2016) was able to decrease the detoxification time of dilute acid treated corn stover hydrolysate from 96 h to 36 h using *Amorphothecaresinae* ZN1 (He et al., 2016). To achieve effective conversion of sugars (glucose and xylose) present in the hydrolysate in the presence of inhibitors Zhu et.al. (2016) adopted a co-culture approach using engineered xylose utilizing *S. cerevisiae* and an inhibitor tolerant *S. cerevisiae* strain which resulted in 41% increased ethanol production (Zhu et al., 2016). Due to regulatory mechanisms, microbes metabolize multiple substrates sequentially. To overcome this bottleneck, Singh et. al. (2019) engineered strains of *Acinetobacter baylyi* ADP1 to prepare a consortium able to simultaneously degrade benzoate and 4-hydroxybenzoate from biomass hydrolysate. Detoxified hydrolysate further inoculated with *Kluyveromyces marxianus* for ethanol production (Singh et al., 2019). Detoxification before fermentation increases cost and processing time. The use of simultaneous detoxification and product production may further improve the economics of the process and avoid the additional treatment step. Panda et al. (2019) used a feeding strategy to culture *Trichoderma reesei* using rice straw hydrolysate as raw material and they were able to increase carboxymethyl cellulase production 10-fold with simultaneous detoxification of hydrolysate (Panda & Maiti, 2019). *In situ* detoxification using microbial consortia seems a promising approach for ethanol production from biomass hydrolysate. Zhang et. al. (2017) co-cultured ferulic acid degrading *E. coli* with *T. reesei* to increase ethanol production by 7.8% using rice straw hydrolysate as raw material (Zhang et al., 2017). Using *in situ* detoxification, there was an increase in the stability and activity of different cellulases in a co-culture system compared to the single strain system.

5.2.2 Enzymatic detoxification

Enzyme mediated detoxification has generated significant interest owing to their broad substrate specificity and ecofriendly nature. Laccase is the most widely reported enzyme for biomass inhibitor detoxification and is widely distributed in bacteria, fungi, algae, insects, and plants. Laccase possesses four copper ions, which are responsible for the transfer of electrons from the substrate to oxygen and its reduction to water. Laccase is able to oxidize a broad range of substrates including, but not limited to, monophenols, diphenols, polyphenols, and methoxyphenol. Suman et. al. (2018) studied the ability of *Trametes maxima* IIPLC-32 laccase to detoxify phenolics from the sugarcane bagasse and found that it removed 66% of lignin-derived phenolics inhibitors in 55 h (Suman et al., 2018). Giacobbe et al. (2019) reported two laccases in *Pleurotus treatus* and used these for the detoxification of milled brewer's spent grain. They found that it was able to achieve 94% phenol reduction (Giacobbe et al., 2019). Cost of laccase is the main drawback of its utilization in the industrial scale detoxification process. This can be overcome to a certain extent by using laccase of higher activity. Fang et al. (2015) identified a new laccase Glac15 in *Ganoderma lucidum* 77002, which was active at broad pH and temperature ranges and was able to remove 84% of phenolic compounds from prehydrolysate (Fang et al., 2015). The main problem associated with enzymes is their production cost and it can be reduced by binding them with the carrier. Saravanakumar et al. (2016) used bacterial cellulose nanofibers for immobilization of laccase and reported increases in pH and thermal stability of laccase with reusability up to 16 cycles with 85% activity retained. Laccase activity depends on the structural variation of lignocellulosic derivatives as it is able to degrade furfural and coniferyl aldehyde completely and requires redox mediators 1-hydroxybenzotriazole (HOBt) for the detoxification of ketone-based derivatives (Saravanakumar et al., 2016). Use of various mediators (i.e. 2,20-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid) (ABTS), N-hydroxyphthalimide (HPI) violuric acid (VLA), or N-hydroxyacetanilide) have been reported to extend the catalytic activity of laccase against recalcitrant compounds (Fillat et al., 2017).

5.3 Microbe selection and adaptation

Different microbes have different tolerance levels for various inhibitors and their robustness against various inhibitors, which is critical for an efficient fermentation process. Many microbes have been reported for their ability to co-metabolize inhibitors along with other carbon sources for the production of lipids and bioproducts (Bhatia et al., 2017a; Bhatia et al., 2019b). *Rhodococcus* sp. YHY 01 is able to utilize various inhibitors (furfural, HMF, vanillin, 4-HB, and acetate) for biomass production and lipid accumulation. A culture of this strain in hot water treated palm biomass hydrolysate resulted in higher production of biomass and fatty acids without necessitating a pre detoxification step (Bhatia et al., 2017b). *Ralstonia eutropha* 5119 was also able to co-metabolize various inhibitors along with glucose as the main carbon source. Bhatia *et al.* evaluated different plants hydrolysates such as pine, barley, and *Miscanthus* as a carbon source for polyhydroxyalkanoate production by *Ralstonia eutropha* 5119 without necessitating a pre detoxification step (Bhatia et al., 2019b).

Microbial inhibitor tolerance levels can be improved by using approaches like molecular adaptation and metabolic engineering. Wang et al. (2018) used the adaptive evolution approach for improving the inhibitor tolerance of *Corynebacterium glutamicum* S9114. The strain was serially transferred to corn stover hydrolysate containing inhibitors after 24 h for 128 days. The improved strain showed an increased conversion rate for various inhibitors (furfural, HMF, vanillin, syringaldehyde, 4-hydroxybenzaldehyde, and acetic acid) (Wang et al., 2018b). Shen et al. (2-14) used a combined strategy of chemical mutagenesis (ethyl methanesulfonate) and adaptive evolution to improve *S. cerevisiae* vanillin tolerance. The improved strain showed 1.92 fold higher vanillin reduction with 15% higher antioxidant activity as compared to wild strain due to upregulation of oxidoreductase and antioxidant activity (Shen et al., 2014). Moreno et al. (2019) used UV mutagenesis as well as the evolutionary approach to improve the *Candida intermedia* strain. The improved strain was able to ferment glucose and xylose present in lignocellulosic hydrolysate simultaneously and resulted in higher ethanol production (Moreno et al., 2019).

5.4 Metabolic engineering of microbes

Inhibitors affect levels of reactive oxygen species in cells and led to decreased growth and productivity. Ask et al. (2013) engineered *S. cerevisiae* for increased robustness and redox metabolism by over expressing *GSH1, CYS3,* and *GLR1* which are all involved in glutathione metabolism (Ask et al., 2013). Furan aldehyde (furfural) is first oxidized into 2-fucoic acid, then further metabolized into 2-oxoglutaric acid, and finally enter in the TCA cycle to provide energy and building blocks for various biosynthetic pathways. All these steps depend on oxygen thus limiting its application to anaerobic fermentation systems. Under anaerobic conditions, microbes use their native oxidoreductase system to reduce furfural to less toxic furfural alcohol (Nieves et al., 2015). The overexpression of oxidoreductase has improved the reduction rate of furan aldehyde and shortened the lag phase. In *E. coli* under the influence of furfural, a native NADPH dependent oxidoreductase *yqh*D is induced to reduce furfural to furfuryl alcohol. The YqhD deplete the limited NADPH pool that is also required for other biosynthetic pathways and affects the sulphate assimilation pathway, which results in reduced growth. Miller et al. (2009a; 2009b) were able to recover the growth of *E. coli* by addition of cystine, deletion of *yqh*D, and overexpression of *pnt*AB (Miller et al., 2009a; Miller et al., 2009b). Kim et al. (2015) reported that *S. cerevisiae* tolerance towards various inhibitors can also be increased by modulating the intracellular content of spermidine (Kim et al., 2015). There is a need to identify a NADH dependent oxidoreductase to accelerate the furfural reduction and tolerance. Wang et al. (2011) identified an NADH dependent oxidoreductase in *E. coli* and its overexpression resulted in 50% increased furfural tolerance (Wang et al., 2011). To increase furfural tolerance in *E. coli* Song et al. (2017) used a strategy to increase NAD(P)H supply through a nicotine amide salvage pathway by combined expression of *pnc*B and *nad*E genes (Song et al., 2017). Suo et al. (2019) engineered *Clostridium tyrobutyricum* by expressing short-chain dehydrogenase/reductase (SDR) from *Clostridium beijerinckii* NCIMB 8052 with heat shock chaperones GroSEL to improve furfural tolerance and reduction (Suo et al., 2019). Glycerol supplementation to *C. beijerinckii* improved NADH and NADPH levels and able to increase furfural detoxification by 2.3 folds (Ujor et al., 2014). To further improve glycerol utilization in *C. beijerinckii,* Agu et al. (2019) overexpressed two glycerol dehydrogenases (*dhaD1* and *gldA1*) resulting in 43% increased glycerol utilization and 68% furfural detoxification (Agu et al., 2019). By engineering *S. cerevisiae* to increase spermidine content up to 1.1 mg/g cell, Kim et al. (2015) was able to reduce lag phase by 66% and 33% for improved strain in the medium containing furan and acetate (Kim et al., 2015). Xiao et al. (2014) used genome wide RNA-interference (RNAi) screening method to identify genes involved in furfural resistance in *S. cerevisiae* and found the *siz*1 gene encoding E3 SUMO protein ligase involved in furfural tolerance, its deletion results in increased tolerance compared to control strains (Xiao & Zhao, 2014). The regulatory protein IrrE is responsible for resistance to ionizing radiation, DNA damage, and upregulates the proteins involved in stress response, energy metabolism, signal transduction, and transcriptional regulation in *Deinococcus radiodurans* R1. IrrE is thought to function as global regulator and is used to improve tolerance in other microbes also. Luo et al. (2018) demonstrated that IrrE gene promoted resistance against various inhibitors (furfural, 5-HMF, formic acid and acetic acid) in *S. cerevisiae* with a 37% increase in ethanol production (Luo et al., 2018).

5.5 Feedstock selection and engineering

Lignin is an important component of plant cell wall that acts as a scaffolding material to surround the cells and provide strength to plants to grow against gravity. High lignin content is considered to be problematic by researchers interested in converting plant biomass into fuel and other bioproducts (Bhatia et al., 2017c). Lignin restricts the accessibility of enzymes to cellulose and hemicellulose and hinders their hydrolysis into fermentable sugars. Delignification of plants requires various pretreatments (physical and chemical) which results in the production of various side products and affects microbial fermentation. Engineering plants with low lignin content may be a strategy to improve plant biomass hydrolysis with the maximum sugar release. Almost ten genes are involved in lignin biosynthesis and up- or downregulation of these genes using sense, antisense, or RNAi approaches may alter lignin content (Lu et al., 2010). Using RNAi mediated suppression of *p*-coumaroyl-CoA 3-hydroxylase in hybrid poplar Coleman et al. (2008) were able to decrease the lignin content by 56-59% (Coleman et al., 2008). Shafrin et al. (2017) used hpRNA-based vectors for downregulation of monolignoid biosynthetic genes-caffeic acid *O*-methyltransferase (*COMT*) and cinnamate 4-hydroxylase (*C4H*) and able to reduce 16–25% acid soluble lignin content in the jute stem (Shafrin et al., 2017). Hu et al. (2018) performed high-throughput assays to study the effect of lignin composition and content on enzymatic digestibility of rice biomass, and reported that lignin monomer has a negative effect rather than lignin content, and ratio of *p*-hydroxyphenyl unit to guaiacyl unit has a positive role (Hu et al., 2018). Shi et al. (2016) studied the effect of ionic liquid pretreatment on different *Arabidopsis* mutants with variable lignin monomer composition *fah1-2* (G-lignin dominant), *C4H-F5H* (S-lignin dominant), and *med5a med5b ref8* (H-lignin dominant) and reported that H- and S- lignin mutants showed increased cleavage of *β*-O-4 linkages compared to G-lignin and have low recalcitrance. Incorporation of monolignol like molecules instead of conventional monolignol units in lignin polymers may also generate easily digestible lignin (Shi et al., 2016). Lee et al. (2017) engineered *Arabidopsis* to shift metabolic flux towards disinapoyl esters, which resemble monolignol sinapyl alcohol by overexpressing sinapoylglucose:sinapoylglucosesinapoyl transferase and showed its integration in the cell wall (Lee et al., 2017). Cai et al. (2016) engineered *Populus* wood by expressing monolignol 4-O-methyltransferase that modifies the chemical precursors of lignin and prevents their incorporation into the lignin polymer. This approach results in a 62% increase in sugar release and 49% increase in ethanol yield (Cai et al., 2016).

6. Conclusions

Lignocellulosic biomass based biorefinery is a renewable and ecofriendly approach which may lead to economic gain. In spite of advancements in various pretreatment methods (ILs, supercritical fluid based, low temperature steep delignification, and cosolvent-enhanced lignocellulosic fractionation) which results in maximum sugar yield with minimum inhibitors production, still there is a huge scope for improved biomass pretreatment and detoxification-based innovations. Apart from advancements in pretreatment and detoxification technologies, combined pretreatment with simultaneous saccharification and fermentation, there is still a need for cost-effective methods that can offer maximum sugar yield with minimum inhibitory compounds, energy, and chemical consumption.

Acknowledgements

The authors would like to acknowledge the KU Research Professor Program of Konkuk University, Seoul, South Korea. This study was supported by Research Program to solve social issues of the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (2017M3A9E4077234), National Research Foundation of Korea (NRF) (NRF-2015M1A5A1037196, NRF-2019M3E6A1103979, 2017R1D1A1B03030766). In addition, this work was also supported by polar academic program (PAP, PE18900). The consulting service of the Microbial Carbohydrate Resource Bank (MCRB, Seoul, South Korea) is greatly appreciated. This work also supported by the US Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number(s) DE-SC0018420.

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Figure captions

Figure 1. Lignocellulosic biomass composition and formation of inhibitory by-products after various pretreatments.

Table 1. Effect of lignocellulosic biomass composition and physical structure on recalcitrance

Pretreatment method	Component	Role in recalcitrance	Reference
Chemical composition	Lignin	Lignin acts as a physical barrier and restrict accessibility to cellulose. Lignin derived compounds have inhibitory effect on hydrolases.	Wang et al., 2019
	Hemicellulose	Hemicellulose acts as a physical barrier and restricts the accessibility of cellulase to cellulose.	Kumar et al., 2018
	Acetyl group	Interfere with enzyme recognition by inhibiting hydrogen bonding between cellulose and catalytic domain of cellulase.	Wang et al., 2019
	Proteins	Cell wall proteins have positive as well as negative effect on hydrolysis.	De Bhowmick et al., 2018
Physical structure	Crystallinity	Hydrolysis rate of amorphous cellulose is almost 30 times higher than crystalline cellulose.	Liu et al., 2019b
	Degree of polymerization	Hydrolysis of biomass is a depolymerization process. Lower degree of polymerization provides more accessibility to enzymes and increase hydrolysis.	Liu et al., 2019b
	Particle size	Reduction of particle size increase reactive surface area and decrease crystallinity and degree of polymerization.	Zhai et al., 2019
	Pore size	Pore size is an important factor in hydrolysis as cellulase can only enter pores bigger than 5.1 nm.	Ponnusamy et al., 2019
	Surface area	Higher accessible surface area provides more access to enzyme and increases hydrolysis rate.	Cho et al., 2019

Table 2. Lignocellulosic biomass pretreatment methods and related pros and cons

Pretreatment method	Advantages	Disadvantages	Reference
Ionic liquid	Effectively solubilizes plant cell wall at mild temperature. Properties can be adjusted. Reusable and solubilizes lignocellulose.	Strong tendency to denature enzyme. Expensive.	Singh, 2018
Supercritical CO_2	Transportation in solid, liquid, or gas form. Effectively improves cellulose hydrolysis. Does not form inhibitory compounds. Easy recovery.	High pressure requirements. Does not modify lignin or hemicellulose.	Liang et al., 2017
Low temperature steep delignification (LTSD)	Low inputs of non-toxic chemicals. High conversion rate and yield. Mild operating conditions. Recovery of chemicals.	Toxic products can be generated. Expensive.	Kumar & Sharma, 2017

Co-solvent enhanced lignocellulosic fractionation (CELF)	Effective reduction in biomass recalcitrance. Low boiling, renewable solvents. Enhances hydrocarbon fuel precursor yields. Increases digestibility of biomass.	Low boiling, renewable solvents. Enhances hydrocarbon fuel precursor yields from lignocellulosic biomass. Increases digestibility of biomass.	Nguyen et al., 2017

Table 3. List of ionic liquids used for delignification of lignocellulosic biomass

Name	**Biomass**	**Conditions**	**Lignin removed (%)**	**Reference**
1,3-Dimethylimidazolium methyl sulfate $[C_4C_1im][MeSO_4]$	Miscanthus	$[C_4C_1im][MeSO_4]$ 80%, 120 ºC, 2 h	27.2	Gschwend et al., 2018
1-Butyl-3-methylimidazolium hydrogen sulfate $[C_4C_1im][HSO_4]$	Miscanthus	$[C_4C_1im][HSO_4]$ 80%, 120 ºC, 2 h	43.78	Gschwend et al., 2018
	Miscanthus	$[C_4C_1im][HSO_4]$ 80%, 120 ºC, 22 h	92.84	Gschwend et al., 2018
	Willow	$[C_4C_1im][HSO_4]$ 80%, 120 ºC, 22 h	85.07	Gschwend et al., 2018
	Pine	$[C_4C_1im][HSO_4]$ 80%, 120 ºC, 22 h	65.5	Gschwend et al., 2019
1-Butylimidazolium hydrogen sulfate $[C_4Him][HSO_4]$	Miscanthus	$[C_4Him][HSO_4]$ 80%, 120 ºC, 4 h	81.14	Gschwend et al., 2018
	Miscanthus	$[C_4Him][HSO_4]$ 80%, 120 ºC, 20 h	79.63	Gschwend et al., 2018
	Miscanthus	$[C_4Him][HSO_4]$ 95%, 120 ºC, 20 h	92.83	Gschwend et al., 2018
1-Butyl-3-methylimidazolium chloride $[C_4C_1im]Cl$	Miscanthus	$[C_4C_1im]Cl$ 80%, 120 ºC, 22 h	15.1	Gschwend et al., 2018
1-Butyl-3-methylimidazolium acetate $[C_4C_1im]MeCO_2]$	Miscanthus	$[C_4C_1im]MeCO_2$] 80%, 120 ºC, 22 h	26.23	Gschwend et al., 2018
	Willow	$[C_4C_1im]MeCO_2$] 80%, 120 ºC, 22 h	17.43	Weigand et al., 2017
	Pine	$[C_4C_1im]MeCO_2$] 80%, 120 ºC, 22 h	17.26	Gschwend et al., 2019

Switchgrass	$[C_4C_1im]MeCO_2$] 100%, 160 ºC, 3 h	65	Williams et al., 2018
Maple wood	$[C_4C_1im]MeCO_2$] 100%, 130 ºC, 1.5 h	27	Williams et al., 2018
Oak	$[C_4C_1im]MeCO_2$] 100%, 110 ºC, 16 h	34.9	Williams et al., 2018

Table 4. Examples of supercritical CO_2 pretreatment methods on glucan conversion yields after enzymatic hydrolysis (Liu et al., 2019b).

Treatment method	**Biomass**	**Conditions**	**Reducing sugar yield (%)**	**Reducing sugar yield from untreated biomass (%)**
Supercritical CO_2	Rice straw	160ºC, 15 bar, 50 min	79.4	71
	Rice straw	110ºC, 300 bar, 30 min	32.4	27.7
	Wheat straw	180ºC, 50 bar, 10 min	79.6	70.8
	Eucalyptus chips	160ºC, 50 bar, 80 min	92.2	73.28
	Switchgrass	170ºC, 200 bar, 60 min	66	14
	Mixed perennial grasses	170ºC, 50 bar, 60 min	68	15.7
	Mixed hardwood	170ºC, 50 bar, 60 min	77	9
	Big bluestem	170ºC, 50 bar, 60 min	66	22.1
	Yellow pine	165ºC, 200 bar, 30 min	84.7	14.5
	Aspen	165ºC, 200 bar, 30 min	27.3	12.8

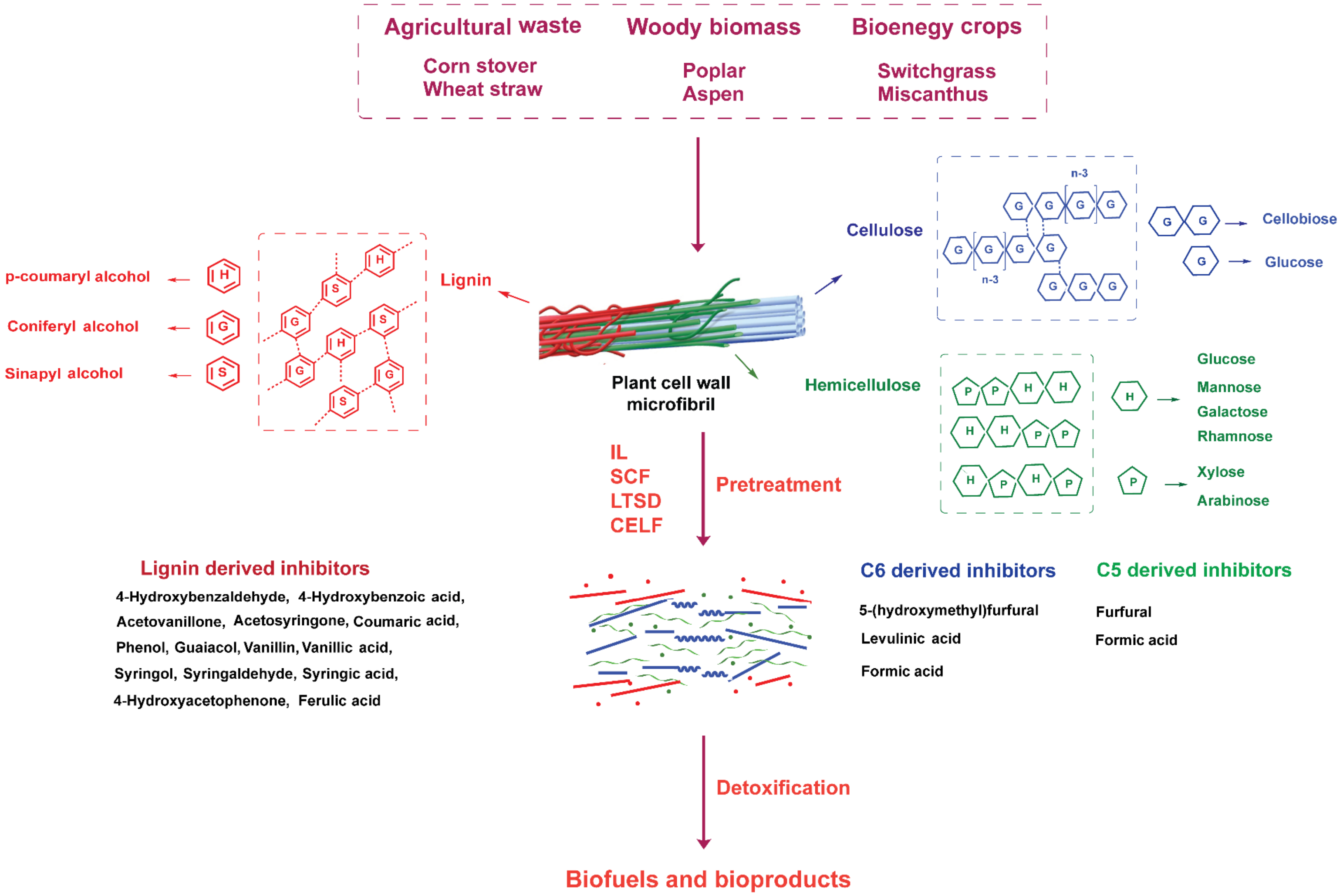
Table 5. Effect of various lignocellulose derived inhibitors on microbes

Inhibitor	Effect on microbial growth and fermentation	Reference
Weak acids	Weak acid in undissociated form permeates through and inside the cells to release the anion and proton which disrupts intracellular pH.	Wang et al., 2018a
	Formic acid has higher permeability and toxicity than acetic acid.	
	Weak acid anions affect cell turgor pressure and inhibit growth.	
	Formic and propionic acids slow down the synthesis of macromolecules (DNA, RNA and protein).	
	Intracellular pool of various amino acids reduced (glutamate and aspartate)	
Phenolics	Phenolics are more toxic than aliphatic or furans with the same functional groups.	Pattrick et al., 2019
	Vanillin causes partial disruption of K^+ gradient in microbes.	
	Able to inhibit activity of various lignocellulosic hydrolases.	
	Shows antibiofouling effect on biofilm formation in Gram negative bacteria.	
Furan	Furfural is a key inhibitor of lignocellulosic hydrolysate.	Liu et al., 2019a
	2-furoic acid and furfuryl alcohol cause membrane leakage.	
	These have negative effects on glycolytic and fermentative enzymes.	
	Furfural acts as a mutagen and causes double stranded DNA breaks.	

Table 6. Various detoxification methods for the removal of inhibitors from lignocellulosic plant biomass hydrolysate, and their advantages and disadvantages

Method	Advantages	Disadvantages	Reference
Evaporation	Able to remove volatile compounds (acetic acid, furfural and vanillin).	Increases the concentration of nonvolatile compounds.	Coz et al., 2016
Adsorptive membrane	Scaling up of the process is easy and pressure drop is significantly low. Sugar loss is low.	Process is costly and allows selective removal of inhibitors.	Fayet et al., 2018
Ionic resins	Resins can be regenerated and reused. Successfully applied for removal of lignin-derived inhibitors (acetic acid and furfural).	Pore diffusion is slow and requires high processing time and pressure. Scaling up is difficult.	Wang & Yang, 2017
Biochar	Low cost and able to remove hydrophobic compounds (phenolics and furan). Sugar loss is also low.	Higher energy demand for the preparation of biochar.	Lee & Park, 2016
Liquid-liquid extraction	Solvents can be recycled and reused. Process is reported to remove acetic acid, vanillin, furfural, 4-HB, and low molecular weight phenolics	Organic solvents are costly and require complete removal before using hydrolysate for further purpose.	Kim, 2018
Biological	Utility cost is low as detoxification is performed under mild conditions. Eco-friendly approach, and less waste is generated.	Requires prolonged detoxification time. Enzyme production cost is high.	Shobana et al., 2017

Figure. 1


Agricultural waste
Corn stover
Wheat straw
Woody biomass
Poplar
Aspen
Bioenegy crops
Switchgrass
Miscanthus
p-coumaryl alcohol
Coniferyl alcohol
Sinapyl alcohol
H
G
S
Lignin
Cellulose
n-3
Cellobiose
Glucose
Plant cell wall
microfibril
Hemicellulose
P
Glucose
Mannose
Galactose
Rhamnose
Xylose
Arabinose
IL
SCF
LTSD
CELF
Pretreatment
Lignin derived inhibitors
4-Hydroxybenzaldehyde, 4-Hydroxybenzoic acid,
Acetovanillone, Acetosyringone, Coumaric acid,
Phenol, Guaiacol, Vanillin, Vanillic acid,
Syringol, Syringaldehyde, Syringic acid,
4-Hydroxyacetophenone, Ferulic acid
C6 derived inhibitors
5-(hydroxymethyl)furfural
Levulinic acid
Formic acid
C5 derived inhibitors
Furfural
Formic acid
Detoxification
Biofuels and bioproducts