



Pretreatment of Lignocellulosic Biomass Based on Improving Enzymatic Hydrolysis

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Abstract

Lignocellulosic materials among the alternative energy resources are the most desirable resources that can be employed to produce cellulosic ethanol, but this materials due to physical and chemical structure arranges strong native recalcitrance and results in low yield of ethanol. Then, a proper pre-treatment method is required to overcome this challenge. Until now, different pre-treatment technologies have been established to enhance lignocellulosic digestibility. This paper widely describes the structure of lignocellulosic biomass and effective parameters in pre-treatment of lignocelluloses, such as cellulose crystallinity, accessible surface area, and protection by lignin and hemicellulose. In addition, an overview about the most important pre-treatment processes include physical, chemical, and biological are provided. Finally, we described about the inhibitors enzymes which produced from sugar degradation during pre-treatment process and the ways to control this inhibitors.

Keywords: Lignocellulosic Biomass, Crystallinity, Pre-treatment Technologies

Introduction

Bioethanol derived from biomass is regarded as a most promising alternatives to fossil fuels due to its higher octane, clean burning, higher flame speeds and higher heats of vaporizations than gasoline, which can be blended with gasoline or used sprightly as clean alcohol in dedicated vehicles (Choi *et al.*, 2015). Anyway, in the first generation bioethanol production, expensive starch and sugar derived from sugar cane and maize are involved as feed stocks but in the second generation process, lignocellulosic materials are used, because it's cheap, abundant and renewable (Dias *et al.*, 2013). Lignocellulosic biomass is mainly composed of cellulose (38-50%), hemicelluloses (23-32%) and lignin (15-25%), in a complex structure, which the recalcitrancy of these compositions is very high. One of the best methods to convert such biomass into soluble sugars is enzymatic hydrolysis because of its low energy demand; despite, the main problem is the low cellulose accessibility due to strong linkage of cellulose with lignin (Gupta *et al.*, 2011). This leads to difficulties within the conversion process; therefore an appropriate pre-treatment technology (Fig. 1) is needed to overcome this recalcitrance and makes polysaccharides comfortably feasible for enzymatic hydrolysis (Gupta *et al.*,

2011; Singh *et al.*, 2015; Wang *et al.*, 2016). In other words, pre-treatment is the crucial and expensive unit process in converting lignocellulosic biomass into ethanol (Kim *et al.*, 2016).

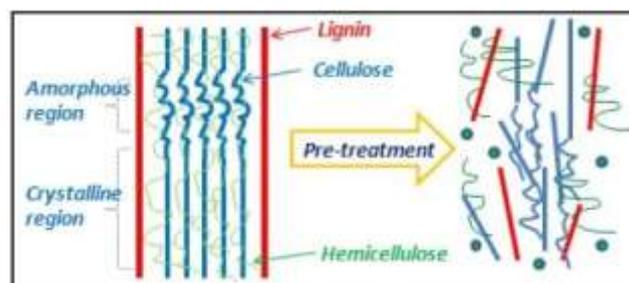


Fig. 1: Schematic pre-treatment of lignocellulosic biomass

A suitable pre-treatment method involves (1) breaking hydrogen bonds in crystalline cellulose (CrI), (2) breaking down cross-linked matrix of hemicelluloses and lignin, and (3) improving cellulose accessible surface for following enzymatic hydrolysis (Huang *et al.*, 2015; Si *et al.*, 2015). Until now, various pre-treatment methods have been developed, which can be extensively categorized into physical (chipping, grinding, irradiation, steam explosion and hot water), chemical (alkaline, acid and ionic liquid),

physico-chemical and biological process (Si *et al.*, 2015; Wang *et al.*, 2016). On the other side, regardless of the pre-treatment method used, during the process many inhibitory compounds are produced, which have negative impact on microbial activity in the hydrolysis step. Generally, inhibitors are classified into three main group: (1) weak acids like levulinic, acetic and formic acids, (2) furan derivatives like 5-hydroxy-2-methyl furfural (HMF), and furfural, and (3) phenolic compounds as well (Behera *et al.*, 2014; Kim *et al.*, 2016). This review first present the structure of lignocellulose and effective factors in pre-treatment lignocelluloses, then describes the most commonly used pre-treatment methods in improving the digestibility of lignocellulosic biomass and finally briefly review inhibitors enzymes and efficient techniques to remove components that inhibit microbial growth and enzymatic hydrolysis of lignocellulose.

Structure of Lignocellulose and Its Major Components

Cellulose

In nature, the major abundant polysaccharide is cellulose and mostly serves as the main scaffolding constituent for plant cell wall. Cellulose is composed of linear chain of D-glucose connected by β -(1,4)- glycosidic bonds together. The Cellulose strain are associated to each other to make cellulose fibril. Cellulose fibers are connected by a number of intra- and intermolecular hydrogen bonds (Li *et al.*, 2010). Then, cellulose is insoluble in water and most organic solvents (Swatloski *et al.*, 2002).

Hemicellulose

Hemicelluloses, mainly located in secondary cell walls, are heterogeneous branched biopolymers containing pentoses, hexoses, and urgonic acids (Girio *et al.*, 2010). They are relatively easy to hydrolyse due to their amorphous, branched structure, and their lower molecular weight as well (M. F. Li *et al.*, 2010). In order to increase the cellulose digestibility, large amounts of hemicelluloses need to be removed as they cover cellulose fibrils limiting their accessibility for the enzymatic hydrolysis (Agbor *et al.*, 2011). Hemicelluloses are somewhat sensitive to operation condition, hence, factors like retention time and temperature need to be regulated to avoid the formation of by-products like furfurals and hydroxymethyl furfurals that later inhibit the yeast fermentation process (Palmqvist and Hahn-Hägerdal, 2000).

Lignin

Lignin is a heterogeneous three-dimensional network macromolecule generally formulated from the oxidative combination of monolignols, in particular *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) (Ralph *et al.*, 2004). Besides the three main monolignols other products such as traces of hydroxyl cinnamyl aldehydes, acetates, *p*-coumarates, *p*-hydroxybenzoates, and tyramineferulate

were also observed (Boerjan *et al.*, 2003). In various plant species, plant tissues and cell wall layers these monolignols present different abundance. In addition, there are many possible linkages among these monolignols, like b-O-4, b-5, b-1, b-b, 5-5, and 4-O-5 (Sette *et al.*, 2011). The actual structure of the lignin macromolecule is completely complicated because of the variety of monolignols and the “randomness” of linkages among these monolignols (Ralph *et al.*, 2004).

Lignin content is one of the most prominent limiting parameters for making lignocellulose impressionable to microbial attack. Studies have proved that lignin reduce facilitates hydrolysis of cellulose. The mechanism by which lignin hinder cellulose hydrolysis is still not entirely known. Although, two explanations have been recommended that could be the lead to this phenomenon. Firstly, lignin is a structural polysaccharide that by covalent linking to hemicellulose imparts strength to the plant cell wall (Yuan *et al.*, 2013). The covalent bonding between lignin and cellulose prevents the carbohydrate to be exposed for enzymatic hydrolysis. Secondly, woody plants (woody biomass) as compare with herbaceous crops (grass biomass) is harder to treat. A comparison of lignin structures in both substrates present that the extent of cross-linking and the phenyl content lignin found in wood is far more complex than that of grassy substrates (Khalil *et al.*, 2014; Ververis *et al.*, 2004).

Effective parameters in pre-treatment lignocelluloses

The intrinsic features of native lignocellulosic biomass make them resistant to enzymatic attack. The purpose of pre-treatment is to change these features in order to make the materials ready for enzymatic degradation. Since lignocellulosic materials are extremely complex, their pre-treatment is very complicated either. The best procedure and conditions of pre-treatment rely entirely on the type of lignocelluloses (Huang *et al.*, 2015; Wang *et al.*, 2016). The cellulose crystallinity (Crl), degree of cellulose polymerization (DP), accessible surface area, and lignin and hemicellulose protection are the major parameters regarded as influencing the rate of lignocelluloses degradation by the enzymes. These parameters will be discussed briefly below.

Cellulose Crystallinity (Crystallinity Index, Crl)

Cellulose Crl exists in various polymorphs and is seen in microfibrils in plant cell wall. They are formed by hydrogen bonds by long chains of (1,4) β -D glucan bonded to each other. The existence of hydrogen bonds between chains with stand microbial and enzymatic attack while breaking them increase depolymerisation (Chundawat *et al.*, 2010). Researches have exhibited that during hydrolysis digestion of amorphous cellulose takes place before Cellulose Crl (Zhao and Chen, 2013). Cellulose Crl is a key feature of cell walls that not only accounts for the amorphous and

crystalline regions of native cellulose but also reflects cellulose interactions with other wall polymers. Using X-ray diffraction, the crystalline index (CrI) has been applied to detect cellulose CrI in various biomass materials (Bansal *et al.*, 2010). It has been presented that lignocellulosics pre-treatment enhance its hydrolysability although in many cases improves the cellulose CrI fraction. This fact has been recommended to be because of the elimination or decreasing of more simply accessible amorphous cellulose after pre-treatments (Hu *et al.*, 2014; Meng and Ragauskas, 2014). In contrast, in many cases high pH pre-treatments have been displayed to have less influence and even decrease biomass crystallinity (Kumar and Wyman, 2009)

Degree of Polymerisation (DP)

The degree of polymerization (DP) is mainly related to other substrate features, such as crystallinity and it is highly variable among different plant species. Although the function of glucan chain length is not absolutely known, it is believed to influence cellulose hydrolysis (Puri, 1984). Cellulose DP is a noticeable factor in determining biomass digestibility. Pre-treatment of lignocellulose as well as enzymatic hydrolysis is a depolymerisation process of cellulose (Meng and Ragauskas, 2014). The influence of various pre-treatments on cellulose chain length has been studied presenting decreased DP in solids prepared by different pre-treatments recommended that xylan reduction had a more rigorous effect on cellulose chain length than lignin removal. (Kumar and Wyman, 2009).

Accessible Specific Surface Area

The accessible specific surface area is a critical parameter that influences the enzymatic hydrolysis of lignocellulosic materials. The particle size and porosity or pore volume present an important effect on the accessible specific surface area of lignocellulosic materials. A high enzymatic hydrolysis rate and glucose yield can be obtained with the decreasing of the lignocellulose particle size (Chen *et al.*, 2011; Yeh *et al.*, 2010). As lignocellulosic biomass are dispersed into nanoscale, the accessible specific surface area of lignocellulose will improve remarkably. Almost the cellulose digestibility is complete in a short time because more cellulose is exposed to the enzymes (Martin-Sampedro *et al.*, 2012). The lignocellulose digestion can be noticeably improved when the pores of the substrate are large enough to assist both large and small enzyme constituents to maintain the synergistic action of the cellulase enzyme system.

Hemicelluloses and Lignin

Hemicellulose and lignin are closely connected to cellulose through non-covalent and covalent linkages, the content and distribution of hemicellulose and lignin also show obvious influence on the lignocellulose enzymatic hydrolysis (Alvira *et al.*, 2010; Wang *et al.*, 2016). The presence of hemicelluloses and lignin greatly obstructs the contact of enzymes with cellulose, leading to a very low enzymatic hydrolysis rate of raw materials. Moreover, the non-productive binding of enzymes to lignin obstructs cellulose hydrolysis as well (Jönsson and Martín, 2016; Pan *et al.*, 2005). Lignin and hemicelluloses reduction may improve the pore size and accessible surface of the lignocellulosic materials and reduce the non-productive adsorption of enzymes to lignin. Therefore, the removal of hemicelluloses and lignin can significantly improve the digestibility of lignocellulosic materials (Mussatto *et al.*, 2008; Puro *et al.*, 2016; Wang *et al.*, 2016). Many pre-treatment processes are purposed to improve the digestibility of lignocellulose materials by hemicelluloses and/or lignin reduction.

Pre-Treatment Technologies for Lignocellulose Biomass

During the last decades, different pre-treatment technologies to enhance various lignocellulosic biomass enzymatic digestion have been reported and investigated. Table 1 shows the percentage of biomass digestibility by different pre-treatments in various plant species. All these pre-treatment methods are presented to decrease lignocellulose recalcitrance to enzymes mostly by decreasing the level of hemicelluloses and lignin, improving accessible surface area, cellulose CrI reduction, improving pore volume, or changing lignin structure (Wang *et al.*, 2016). The past two decades have observed an enormous amount of study being done in the area of pre-treatments. The main features of different pre-treatment methods on the compositions of lignocellulosic biomass and their limitation summarized in Table 2. Various methods have been occupied to examine the productivity as a pre-treatment estimate. The synergistic influences on efficient lignin reduction have also been investigated on a large scale. Pre-treatment methods possibly categorized in different ways. For example it can be classified into two groups: acidic and alkaline according to the pH that is maintained during the pre-treatments process (Singh *et al.*, 2015; Wu *et al.*, 2014). Therefore, pre-treatment technologies can be broadly categorized into physical, chemical, physico-chemical and biological. In this section, we described the most used pre-treatment in the last decades.

Table 1. The percentage of lignocellulosic biomass digestibility by different pre-treatments in various plant species

Pre-treatment method	Plant species	Wall polymer alteration	Pre-treatment	Lignocellulosic biomass digestion (%)	References
Chemical and Physico-chemical pre-treatments	Miscanthus stem	Cellulose, Hemicellulose level	NaOH, H ₂ SO ₄	100	Vancov <i>et al.</i> , 2012
	Miscanthus stem	lignin level	NaOH	93-100	Ming Li <i>et al.</i> , 2014
	Miscanthus stem	Hemicellulose, lignin level	NaOH, H ₂ SO ₄	99	Xu <i>et al.</i> , 2012
	Wheat and rice straw	Cellulose, lignin, Hemicellulose level	NaOH	60-93	Wu <i>et al.</i> , 2013
	Maize straw	Cellulose, lignin level	NaOH, H ₂ SO ₄	98	Jia <i>et al.</i> , 2014
	Sweet sorghum bagasse	Cellulose, lignin level	NaOH, H ₂ SO ₄	40-100	Meng Li <i>et al.</i> , 2014
	Rice straw	Cellulose level	(NH ₄)CO ₃	72	Kim <i>et al.</i> , 2014
	Miscanthus stem	Cellulose level	NaOH, H ₂ SO ₄	26-86	Zhang <i>et al.</i> , 2013
	Miscanthus stem	lignin level	NaOH, H ₂ SO ₄	100	Si <i>et al.</i> , 2015
	Switchgrass stem	Cellulose level	Ionic liquid	80	Sathitsuksanoh <i>et al.</i> , 2015
	Sugarcane bagasse	Cellulose level	Ionic liquid	98	Gao <i>et al.</i> , 2013
	Poplar wood	Cellulose level	Ionic liquid	97	Wu <i>et al.</i> , 2014
	Rice straw	lignin level	Microwave, alkali	70	Ma <i>et al.</i> , 2009
	Sugarcane bagasse	Hemicellulose level	Hot water, NaOH, HCl	72-77	Vanholme, 2014; Yu <i>et al.</i> , 2013
	Cotton stalk	Cellulose level	Steam explosion, NaOH	78	Huang <i>et al.</i> , 2015
Mustard stalk	Cellulose level	Steam explosion, alkali, dilute acid	81	Kapoor <i>et al.</i> , 2015	
Physical Pre-treatments	Poplar wood	lignin level	Hot water	91	Studer <i>et al.</i> , 2016
	Oilseed rape straw	Pectin level	Steam explosion	86	Wood <i>et al.</i> , 2014
	Wheat bran	Hemicellulose level	Irradiation microwave	91	Aguedo <i>et al.</i> , 2015
Biological Pre-treatments	Rice straw	lignin level	Trichoderma viride	56	Ghorbani <i>et al.</i> , 2015
	Poplar wood	lignin level	White rot fungi	85	Wang <i>et al.</i> , 2013
	Wheat straw	lignin level	Pleurotus ostreatus	35	Hatakka, 1983

Table 2. Effect of different pre-treatment methods on the compositions lignocellulosic biomass and their limitation

Pre-treatment method	Lignin removal	Hemicellulose removal	Cellulose de-crystallization	Increase accessible area	Disadvantages	References
Alkaline	H ^a	H	ND	H	High pollution and high chemical recovery cost	Ming Li <i>et al.</i> , 2014
Acid	M ^a	H	ND	H	Chemical recovery problem	Kim <i>et al.</i> , 2014
Ionic liquid	M	L ^a	H	H	High cost of ionic liquid	Gao <i>et al.</i> , 2013
Steam explosion	L	H	L	H	High equipment cost and generation of inhibitors	Huang <i>et al.</i> , 2015
Microwave irradiation	L	M	H	H	High equipment cost	Aguedo <i>et al.</i> , 2015
Hot water	H	H	ND	M	High equipment cost and generation of inhibitors	Studer <i>et al.</i> , 2016
Biological	H	M	ND	H	Low hydrolysis rate, large space requirement, watchful control condition of microorganism growth	Ghorbani <i>et al.</i> , 2015

^a H: high effect; M: medium effect; L: low effect ; ND: not determined.

Physical Pre-Treatments

The main purpose of physical pre-treatments are to improve the accessible specific surface area of lignocellulosic materials to enzymes by decreasing their particle size or disturbing their structural regularity. Different types of physical processes, such as mechanical comminution (chipping and grinding), irradiation (gamma rays and microwave), hot water have been used to enhance the lignocellulosic materials digestion (Puro *et al.*, 2016; Wang *et al.*, 2016). Steam explosion, hot water and irradiation microwave have been applied as powerful physical pre-treatments in different biomass materials (Table 1) (Aguedo *et al.*, 2015; Studer *et al.*, 2016; Wood *et al.*, 2014).

Chipping and grinding among the mechanical comminution processes, is preferred to disperse immense lignocellulosic materials into small pieces and promote the following pre-treatment procedures. Milling can remarkably decrease the particle size and cellulose CrI, and as a result by extending the milling time enhance their enzymatic hydrolysis. Nevertheless, the energy utilization of mechanical comminution is closely related to the final particle size of lignocellulosic materials. Then, to obtain a high enzymatic hydrolysis value and fermentable sugar yield a high energy input is demanded. This makes milling technology not economically valuable (Martin-Sampedro *et al.*, 2012; Yeh *et al.*, 2010). Other physical pre-treatment technologies have been applied to enhance enzymatic hydrolysis of lignocellulosic biomass such as irradiation and steam explosion. In recent years generally physical pre-treatment used as assistance to chemical pre-treatment method because, physical cannot remove hemicelluloses or lignin from the lignocellulosic biomass or reduce their particle size. It was reported that by many researchers that physical pre-treatment assisted to chemical pre-treatments are more effective in lignocellulose biomass digestion than the conventional heating chemical pre-treatments (Huang *et al.*, 2015; Kapoor *et al.*, 2015; Ma *et al.*, 2009; Vanholme, 2014; Wei *et al.*, 2016)

Chemical Pretreatments

Alkaline pretreatment

The most commonly used method for hemicelluloses and lignin removal in the lignocellulosic biomass is alkali pre-treatment and it disperses bulk lignocellulosic materials into lignocellulosic fibers. The ester linkages in hemicelluloses and lignin comfortably broken down under alkaline conditions. At the relatively high temperature, the ether linkages in hemicelluloses and lignin can also be destroyed. The cleavages of these linkages considerably encourage the solubilisation of hemicelluloses and lignin, hence, enhancing the porosity of biomass. Different alkaline reagents, such as sodium hydroxide (NaOH), calcium hydroxide (CaOH₂), potassium hydroxide (KOH), and aqueous ammonia, have been used to pretreat many lignocellulosic materials. Among these alkaline reagents,

NaOH and CaOH₂ are the most commonly used (Hendriks and Zeeman, 2009; Wang *et al.*, 2016). Alkali pre-treatment at relatively low temperatures could lead to much higher biomass enzymatic digestibility and does not demand complicated reactors, which are appealing to be employed on-farm. Anyway, the main drawbacks are long residence time (from hours to days) and the need for neutralization of the pretreated slurry (Jia *et al.*, 2014; Wang *et al.*, 2015; Wu *et al.*, 2013; Xu *et al.*, 2012). Combination of NaOH and CaOH₂ were used to increase the cost productivity of alkaline pre-treatment in switch grass. The raw material was first pretreated by NaOH, and then biomass was pretreated by Ca(OH)₂ which led to glucose and xylose yields of 59.4% and 57.3%, respectively (Xu and Cheng, 2011). Moreover, Li *et al.* (2014) reported that mild alkali pre-treatment effectively extracts G-rich lignin for high biomass digestibility in Miscanthus.

Acid and dilute-acid pre-treatments

Acid pre-treatment especially by using sulfuric acid (H₂SO₄) are the most used chemical pre-treatment for lignocellulosic materials where polysaccharides are hydrolysed to monosaccharides leading to high cellulose approachability to enzymatic hydrolysis. Acid pre-treatment can be presented either under low acid concentration and high temperature or under higher acid concentration and lower temperature (Chen *et al.*, 2017). Anyway, the concentrated acid pre-treatments are lead to cause severe cellulose and monosaccharides reduction i.e. glucose, production of inhibitors (like HMF and 2-furfuralaldehyde) and oxidation of equipment, then the concentrated acids are less attractive. On the other hand, diluted acid because of lower generation of inhibitors is more desirable (C. Li *et al.*, 2010; Xu *et al.*, 2012; Zheng *et al.*, 2009). There are many different diluted reagents acids, like H₂SO₄, HCl, H₃PO₄, and HNO₃ which have been applied to pre-treat different lignocellulosic biomass (Wang *et al.*, 2016). The influence of dilute sulphuric acid pre-treatment conditions on the recovery of xylose and glucose during the pre-treatment and following enzymatic hydrolysis procedures was examined by many researches (Chen *et al.*, 2017; Hsu *et al.*, 2010). In different investigations reported that for enhancing biomass digestibility and enzymatic hydrolysis two-step acid and alkaline pre-treatments are more effective (Table 1) (Jia *et al.*, 2014; Si *et al.*, 2015; Xu *et al.*, 2012). For instance, lately, Si *et al.* (2015) have reported pre-treatments with 2% NaOH and 1% H₂SO₄ increasing biomass digestibility (100%) in hemicelluloses-rich samples via the efficient co-extraction of hemicelluloses and lignin. Additionally, Jia *et al.* (2014) have shown that two-step 2% NaOH and 1% H₂SO₄ improving biomass digestibility (98%) in maize straw with reduction of cellulose and lignin.

Commonly, lignin reduction by mineral acid pre-treatment is not highly advantageous. It was reported by many studies

that after pre-treated with mineral acids, lignocellulosic residues were mainly contain high contents of lignin. A subsequent delignification procedure is always demanded to further enhance lignocellulosic residues digestibility (Xu et al., 2012; Zhang et al., 2012).

Ionic liquids (ILs) pre-treatment

Ionic liquids or salts are usually constituted of large organic cations and small inorganic anions. By modifying the compounds of anions and cations the influence of ILs can be different (Gao et al., 2013). This pre-treatment has high effects on lignin degradation, enhancing cellulose CrI, increasing accessible surface area. Some of the ILs recognised such as 1-allyl-3-methylimidazolium-chloride, 1-ethyl-3-methylimidazolium-acetate, 1-butyl-3-methylimidazolium chloride and 1-ethyl-3-methylimidazolium diethyl phosphate have recently received much attention because of their capability in cellulose decrystallinity (Wu et al., 2014). The main drawbacks of ILs method in the industrial scale are very costly, recycling of pure ILs is energy-intensive and, during pre-treatment, solution becomes viscous that makes it difficult to handle. Then, for enhancement of ionic liquids solubility for lignocellulosic biomass more research is required to increase the economics of ILs pre-treatments and construct effective IL recovery proses (Gao et al., 2013; Sathitsuksanoh et al., 2015). It has reported in sugarcane bagasse and poplar wood cellulose degradation by ionic liquids occurred around 98% and 97%, relatively (Gao et al., 2013; L. Wu et al., 2014).

Biological Pretreatment

Microorganisms or enzymes have also been applied to pre-treat various lignocellulosic biomass before enzymatic hydrolysis. For the degradation of lignin and hemicelluloses many different microorganisms like brown-, white- and soft-rot fungi have been selected and used (Sánchez, 2009). Moreover, the most widely used fungi among these microorganisms is white-rot fungi. (Alvira et al., 2010). White-rot fungi has many various species like *Phanerochaete chrysosporium*, *Ceriporia lacerata*, *Cyathus stercoletus*, *Ceriporiopsis subvermispora*, *Pycnoporus cinnabarinus* and *Pleurotus ostreatus* which have been applied to pre-treat different lignocellulosic biomass (Hatakka, 1983). Furthermore, white-rot fungi produce lignin-modifying enzymes, such as laccase, ligninperoxidases, and manganese peroxidases, which present prominent influence on lignin reduction (Kumar and Wyman, 2009). It was reported by Ghorbani et al. (2015) in rice straw white rot fungi (*Trichoderma viride*) can remove lignin about 56% and also in poplar wood white rot fungi can effectively deconstruct lignin construction (85%), resulting in enhancement of biomass enzymatic saccharification (Wang et al., 2013). Hatakka (1983) has presented pre-treatment of wheat straw for five weeks by white rot fungi (*Pleurotostreatus*) reduced lignin in the

original wheat straw about 34%, but in the un-treated samples only 12% lignin reduction occurred.

Inhibitors Enzymes and Overcome of the Inhibitors

During the pre-treatment procedure of lignocellulose many compounds formed which can confirm prohibitory to microbial growth and metabolism. Capability Inhibitory chemicals that can be released from cellulose, hemicellulose and lignin when employing thermochemical treatments are aliphatic acids like acetic, formic and levulinic acid, furfuraldehydes like furfural and 5-hydroxymethylfurfural (HMF) and, uronic acid, 4-hydroxybenzoic acid, vanillic acid, vanillin, phenol, cinnamaldehyde, and formaldehyde (Jönsson and Martín, 2016). By thermal breakdown of pentose and hexose sugars furan by-products are formed. The two furan derived illustrative, furfural and HMF arise from the dissolution of pentose and hexose sugars on treating lignocellulose with diluted H₂SO₄. By-products of furan are harmful to the activity of particular enzymes that are engaged in microbial metabolism like hexokinase, aldolase, phosphofructokinase, triosephosphate dehydrogenase, and alcohol dehydrogenase (Behera et al., 2014; Kim et al., 2016).

On the fermentation procedure by several methods, we can control the influence of inhibitors (Jönsson and Martín, 2016; Rabemanolntsoa and Saka, 2016). Generally, to remove the negative impact of inhibitors, there can be four methods: (1) prevent the formation of inhibitors during pre-treatment and hydrolysis (2) detoxify the hydrolyzate prior to fermentation; (3) develop genetically modified microorganisms that can resist attack of inhibitors (4) conversion of toxic compounds into neutral products (Rabemanolntsoa and Saka, 2016). To prevent the formation of toxic compounds, a simple strategy would be the careful choice of lignocellulosic material and the application of mild pre-treatments. This may not agree with the industrial demand as a high cellulose and subsequent sugar yield is desired which can only be obtained by implementing harsh pre-treatment methods (Kim et al., 2016; Rabemanolntsoa and Saka, 2016). Addition of chemicals to the fermentation process is one way for detoxification (Behera et al., 2014; Duque et al., 2016).

Conclusion

In order to improve fermentable carbohydrate recovery, produce effective by-product, reduce inhibitors, low-cost bioethanol procedure, and increase ethanol production various effective pre-treatment technology have been identified for lignocellulosic materials. In general, pre-treatment methods are divided into four major groups i.e. physical, chemical, physico-chemical and biological. The main parameters to obtain an efficient hydrolysis are crystallinity of cellulose, its accessible surface area and

protection by lignin and hemicellulose. Despite most of the pre-treatment have many advantages, but one method could not be the best selection for all types of biomass. Therefore, basic understanding of various pre-treatment technologies, different composition of biomass feedstock and the relationship between composition of biomass feedstock and pre-treatment methods would considerably help in matching the best pre-treatment method for a specific biomass feedstock.

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