Review

A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass

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HIGHLIGHTS

- Leading alkaline pretreatments technologies and their features are described.
- Advantages, mechanisms, and effects of alkaline pretreatment are discussed.
- Desirable pretreatment process and alkaline process options are reviewed.
- Various barriers and inhibition mechanism in biomass utilization are discussed.
- Application of alkaline pretreatment in biorefinery is presented.

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ABSTRACT

The native form of lignocellulosic biomass is resistant to enzymatic breakdown. A well-designed pretreatment that can promote enzymatic hydrolysis of biomass with reasonable processing cost is therefore necessary. To this end, a number of different types of pretreatment technologies have been developed with a common goal of making biomass more susceptible to enzymatic saccharification. Among those, a pretreatment method using alkaline reagent has emerged as one of the most viable process options due primarily to its strong pretreatment effect and relatively simple process scheme. The main features of alkaline pretreatment are that it selectively removes lignin without degrading carbohydrates, and increases porosity and surface area, thereby enhancing enzymatic hydrolysis. In this review, the leading alkaline pretreatment technologies are described and their features and comparative performances are discussed from a process viewpoint. Attempts were also made to give insights into the chemical and physical changes of biomass brought about by pretreatment.

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1. Introduction

Lignocellulosic biomass is composed of carbohydrates (cellulose and hemicellulose), lignin, and other extraneous components (proteins, lipids, and inorganic substances) (Kim, 2013). It is a renewable and abundant resource suitable for production of bio-based products such as biofuels and chemicals. Bio-based products produced from renewable sources are desirable alternatives to conventional petroleum-based fuels and chemicals; such bio-based products are also the backbone of the biorefinery concept. According to the International Energy Agency (IEA) report (IEA Bioenergy, 2009), biorefinery is defined as the process for sustainable processing of lignocellulosic biomass into various bio-based products

(food, feed, chemicals, and materials) and bioenergy (biofuels, power and/or heat). The biorefinery concept calls for lignocellulosic biomass to be processed with one of two end goals: (1) to be fractionated into three main components for further conversion to bio-based products with maximal added-value, or (2) to produce primarily biofuels, with residues as co-products. The US Department of Energy (DOE) (Werpy and Petersen, 2004) reported that as many as twelve important building block chemicals can be produced from sugars through either biological or chemical conversions (Holladay et al., 2007). It is anticipated that biorefining technology will play a leading role in creating a new bio-based industrial sector to replace fuels and chemicals currently produced from fossil oil, and to reduce greenhouse gas emissions (Mabee and Saddler, 2010; Park and Kim, 2012).

Commercialization of cellulosic ethanol production, the first step toward biorefinery, still remains challenging because of technical

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and economic barriers (Holladay et al., 2007; Kim and Kim, 2014). Conversion of cellulose and hemicellulose biologically using enzymes and microbes requires five main unit processes: (1) mechanical size reduction (chopping/grinding), (2) pretreatment to make the cellulosic parts more amenable to enzymatic reaction, (3) enzymatic saccharification to hydrolyze cellulose/hemicellulose into monomeric sugars (fermentable sugars), (4) microbial fermentation to convert monomeric sugars into fuels and chemicals, and (5) product recovery and purification. In an integrated biomass conversion process, pretreatment becomes a key element because the design of the subsequent saccharification and fermentation processes rely strongly upon the result of pretreatment. The ultimate goal of pretreatment is to improve the enzymatic hydrolysis of carbohydrates (cellulose and hemicellulose) consequently increasing overall bioconversion efficiency for production of sugars.

In order to achieve this goal, various pretreatment methods, most involving chemical pretreatment, have been proposed, and various reagents and catalysts for different substrates developed. The known methods are: steam/steam explosion (Liu and Chen, 2015), grinding/milling, hot water/autohydrolysis (Timung et al., 2015; Garrote et al., 2002; Vaquez et al., 2001), acid treatment (Jacobsen and Wyman, 2000; Kim et al., 2001), alkali treatment (Ferrer et al., 2000; Choi et al., 2013; Kim et al., 2003; Yoo et al., 2011), and other methods. The outcome of each pretreatment varies widely in terms of its physical and chemical characteristics (Kim, 2013).

Among aforementioned pretreatment options, alkaline pretreatment has emerged as one of the front runners because it has a number of desirable features. Alkaline methods utilize mostly non-polluting and non-corrosive chemicals such as ammonia (aqueous, liquid, and gaseous), sodium hydroxide, sodium carbonate, and calcium hydroxide (lime). Alkaline pretreatment is carried out under milder conditions than those needed for acid pretreatment. Alkaline reagents interact primarily with lignin; therefore, they are more efficient for lignin removal. In this paper, various alkaline pretreatment technologies are reviewed. These include: ammonia recycle percolation (ARP), ammonia fiber explosion/expansion (AFEX), soaking in aqueous ammonia (SAA), low-liquid ammonia (LLA), low-moisture anhydrous ammonia (LMAA), and other alkaline technologies using NaOH, and Ca(OH)$_2$. Reaction mechanisms, major effects, background, processing schemes, and the advantages and disadvantages related to alkaline pretreatment technologies are discussed. Overall, the paper reviews the role of pretreatment technologies using alkaline reagents in biorefinery, which can produce high value products from lignocellulosic biomass.

2. Lignocellulosic biomass

Lignocellulosic biomass is categorized into four major groups based on its source: (1) woody biomass, (2) agricultural residues (rice/wheat/barley straws, corn stover, sugarcane bagasse), (3) energy crops (switchgrass, miscanthus, short rotation hardwood specifically grown for biofuel production), and (4) various types of cellulosic wastes (municipal solid waste, pulp mill and lumber mill wastes). Regarding plant taxonomy, these sources are simply classified as hardwood or softwood tree species, or grass species. However, some agricultural residues and some energy crops such as switchgrass, miscanthus, and elephant grass (*Pennisetum purpureum*), are considered herbaceous plants. The softwoods (gymnosperms) are a group of seed-producing, non-flowering plants, mostly conifers and cycads. The deciduous flowering plants that have broad leaves are called hardwood.

Lignocellulosic biomass is heterogeneous polymeric material comprised of carbohydrates (cellulose and hemicellulose), lignin, and other components. Many factors affect biological conversion of lignocellulosic biomass, and these include chemical and physical barriers that inhibit its enzyme hydrolysis (Kim et al., 2003; Kim, 2013). Carbohydrates, particularly plant cellulose, has a highly crystalline, recalcitrant structure, which makes the enzymatic hydrolysis of cellulose extremely difficult (Kim, 2013). The presence of lignin has also been proven to a major factor hindering enzymatic reaction and microbial fermentation (Brown, 2003; Wyman, 1996; Yang and Wyman, 2006; Kim and Lee, 2006). Lignin is a hydrophobic hetero-polymer of three monomers (coniferyl alcohol, sinapyl alcohol, and *p*-coumaryl alcohol), which is resistant to microbial attack. Lowering the lignin content is desirable during pretreatment because this enhances enzyme saccharification and microbial fermentation.

3. Desirable pretreatment process and process options

The purpose of pretreatment is to make lignocellulosic biomass amenable to enzymatic reactions (saccharification) with reasonable processing cost. Some commonly observed outcomes of pretreatment include decrease of lignin content, increase of surface area, and decrease of crystallinity of the biomass; all of which result in enhanced enzymatic hydrolysis rate and yield (Kim et al., 2003; Kim, 2013). Pretreatment is an essential element in the biorefining of lignocellulosic substrates. Kim (2013) described that pretreatment plays a major role in the economics of the overall bioconversion process because all of the subsequent processes (enzymatic saccharification, fermentation, and the downstream processes) are tailored to the pretreatment results. Ideally, a pretreatment process should (1) improve the enzymatic hydrolysis reaction, (2) produce minimal/no inhibitory compounds, and (3) require reasonable capital and operating costs (with low energy input and minimal waste) (Drapcho et al., 2008; Kim, 2013).

Pretreatment can effectively overcome both chemical and physical barriers and enhance the enzymatic digestibility of biomass if proper chemical reagents/catalysts are applied. For selection of optimal treatment reagent/catalyst, a strategy must be set for the removal of lignin and/or hemicellulose. Pretreatment at high temperature with short reaction time (e.g., by dilute sulfuric acid, ARP, or steam explosion), could improve the enzymatic digestibility of the treated solid significantly by removing some of the carbohydrates, mostly hemicellulose. Unfortunately, such a process also degrades sugars into inhibitory compounds. High temperature processes induce high pressure, thus requiring high-pressure materials and vessels and high energy inputs. These lead to high capital and operating costs of the biomass processing facility. Pretreatments designed to operate at low or moderate temperature (e.g., SAA, AFEX, LMAA, LLA, lime treatment) require longer reaction time to compensate for the less severe conditions. During pretreatment, partial degradation of carbohydrates (hexose and pentose), lignin, and other components takes place, producing furfural, 5-HMF, phenols, and various acids. If the reaction conditions (temperature, time, and reagent concentration) are severe enough to remove lignin and hemicellulose, breakdown of the sugars and other soluble components also occurs. Therefore, the selection of proper treatment conditions is necessary, and is the key to effective conversion of lignocellulosic biomass into sugars with minimal formation of inhibitory compounds. For selection of a pretreatment method, not only effective enzymatic hydrolysis, but also undesirable side reactions, especially degradation of carbohydrate and lignin to inhibitory components, must be taken into consideration.

4. Alkaline pretreatment

4.1. Description of alkaline pretreatment technologies

Alkaline pretreatment technologies applying various reagents have been investigated for the common purpose of improving

Table 1
Various alkaline pretreatment reagents and their conditions and effects.

Catalysts		Reaction types and conditions	Major effects	Remarks
Sodium hydroxide		0.5–10.0% NaOH 60–180 °C, 5–60 min Solid loading: 10–30%	50% hemicellulose dissolution, 60–80% delignification, difficulty in recovery of NaOH	High reaction rate
Sodium carbonate		1–30% Na_2CO_3 60–180 °C, 5–60 min Solid loading: 10–30%	20–40% hemicellulose dissolution, 40–60% delignification, Easier recovery than NaOH	Green liquor
Ammonium	Ammonium hydroxide	5–30% ammonia, 30–210 °C, 5–60 min, high pressure (2–17 atm), Solid loading: 10–50%	10–50% hemicellulose solubilization, 0–80% delignification Profound swelling Lignin removal or modification	ARP, SAA, LLA
	Anhydrous gaseous ammonia	Gaseous ammonia 25–80 °C, ~72 h Solid loading: approx. 50% (moisture ~50%)	No need for washing Low liquid loading (only ~50% moisture is enough) Mild reaction condition	LMAA
	Liquid anhydrous ammonia	70–90 °C, ~5 min, high pressure (15–20 atm), anhydrous ammonia, Solid loading: 60–90%	No hemicellulose dissolution. No lignin removal. Rapid evaporation and liquefaction of ammonia	AFEX
Lime		25–130 °C, 1 h–8 weeks 0.05–0.15 g $Ca(OH)_2$ water)/g of biomass Solid loading: 5–20%	20–40% hemicellulose dissolution. 60–80% delignification, de-acetylation. Low energy requirement.	Simple and low-cost process

Source: Tajkarimi et al. (2008); Wyman et al. (2005), Kim and Lee (2007), Li and Kim (2011), Yanga et al. (2012).

enzyme digestibility of lignocellulosic biomass. The ones in this category are: (1) sodium hydroxide (Modenbach, 2013), (2) ammonia (Kim et al., 2003; Kim and Lee, 2005, 2007; Yoo et al., 2011; Li and Kim, 2011), and (3) calcium hydroxide (Karr and Holtzapple, 2000; Kim and Holtzapple, 2005, 2006). Table 1 presents an overview of representative alkaline pretreatment technologies. Alkaline pretreatment reagents are less caustic than acidic reagents such as sulfuric acid and sulfite. Alkaline pretreatments are carried out under milder conditions, some of them even at ambient temperature, as evidenced by soaking in sodium hydroxide, or in ammonium hydroxide. Such methods can eliminate the need for expensive materials and special designs to cope with corrosion and severe reaction conditions. It is also possible to recover and reuse chemical reagents in some of the alkaline pretreatment methods (Table 2).

The major reactions during alkaline pretreatment include dissolution of lignin and hemicellulose, and de-esterification (saponification) of intermolecular ester bonds (Kim, 2013). These also alter the DP (degree of polymerization) of each component, and bring about changes in the physical properties of treated solids. Such changes might involve surface area, porosity, and crystallinity. The change in the gross crystallinity index is primarily due to removal of amorphous regions (lignin and hemicellulose) of the biomass, rather than to structural change in the cellulose fibers. This is the reason that the gross crystallinity index of treated biomass often rises after pretreatment. Kim and Holtzapple (2006) reported that the activation energy (50.2–54.2 kJ/mol) used for delignification of grass (herbaceous) species (bagasse, corn stover, etc.) by alkaline pretreatment (lime) was much lower than that required for delignification of wood by the Kraft process. It appears that alkaline pretreatment is much more effective for delignification of grass species than of woody species.

4.2. Pretreatment technologies

4.2.1. Sodium hydroxide and sodium carbonate pretreatments

In the Kraft pulping process, a mixture of sodium hydroxide and sodium sulfide, known as white liquor, is used as the pulping reagent. This white liquor then turns into "green liquor", pulping terminology for a solution of sodium carbonate and sodium sulfide (Na_2S). To regenerate the spent white liquor and to recover sodium hydroxide, the green liquor is mixed with calcium hydroxide ($Ca(OH)_2$). Recently, the use of green liquor for pretreatment of lignocellulosic biomass has drawn considerable attention due to its high selectivity for delignification and high retention of carbohydrates (Wu et al., 2011). Pretreatment using sodium hydroxide alone is a technically feasible pretreatment method.

Sodium hydroxide pretreatment, in fact, has a long history (since the 1900s) of study aimed at increasing the digestibility of cellulose by rumen animals. This strong base solubilizes hemicellulose and lignin significantly under certain conditions (Sjostrom, 1993). Sodium hydroxide is one of the strongest base catalysts, so the effectiveness of pretreatment using it is evidenced by a greater degree of enzyme hydrolysis than with other alkaline pretreatments.

Sodium hydroxide effectively attacks the linkage between lignin and hemicellulose in lignin-carbohydrate complexes (LCC); in particular, it cleaves the ether and ester bonds in the LCC structure. Sodium hydroxide is also effective for cleavage of the ester and carbon-to-carbon (C–C) bonds in lignin molecules (ferulic acid).

Table 2
Comparison of various alkaline pretreatments.

Description	NH_3	NaOH	Na_2CO_3	$Ca(OH)_2$
Pretreatment process				
Pressure	High	Low	Low	Low
Temperature	High	Low	High	Low
Concentration or chemical loading	~15–30% aq. or gas	1–5%	5–15%	0.1 g $Ca(OH)_2$/g solid
Recovery process	Simple evaporation, high pressure equipment required	Kraft process, expensive	Partial Kraft process, less expensive	CO_2 carbonating, less expensive
Corrosiveness	Low	High	Medium	Low
Advantages	High selectivity (delignification) & retention of carbohydrates			
	Elimination of liquid stream detoxification			
	High fermentation efficiency because of absence of toxins and inhibitors			

During the NaOH pretreatment reaction, sodium hydroxide is dissociated into hydroxide ion (OH^-) and sodium ion (Na^+) and, as the hydroxide ion concentration increases, the rate of the hydrolysis reaction increases accordingly (Modenbach, 2013).

4.2.2. Ammonia pretreatment

Among alkaline pretreatments, ammonia-based treatments have been investigated most extensively for a number of reasons. It is easily recoverable, non-corrosive, and non-toxic. It is inexpensive to the extent that the majority (~80%) of ammonia produced is currently used for production of fertilizer. Ammonia is a commodity chemical widely used in the chemical, pharmaceutical, and food industries. It is used in many household items such as cleansing and antiseptic solutions (Jensen, 2009). Its industrial use and recovery procedures are well-established. Ammonia can be easily recovered and reused because of its high volatility, and it offers versatile processing options (Kim, 2013; Kim and Lee, 2006).

The main effects of ammonia pretreatment methods include removal or modification of lignin, increase of surface area and/or pore size, and modification of cellulose and hemicellulose structures. In aqueous form, it causes swelling of the biomass, which brings significant morphological change. It also affects the degree of crystallinity of the integral biomass, if not the core of cellulose fibers, as compared to other alkaline pretreatment methods. Ammonia pretreatment opens up the structure of the biomass, making it amenable to enzymatic hydrolysis (Kim et al., 2009).

4.2.2.1. Aqueous ammonia pretreatment.
As the first-of-its-kind ammonia-based process, ammonia recycle percolation (ARP) was investigated for pretreatment of herbaceous and hardwood biomass (Kim and Lee, 1996; Kim et al., 2003). It employs a fixed-bed reactor operated in flow-through mode (also called a percolation reactor). A unique feature of this reactor is that it can minimize re-polymerization and re-precipitation of soluble lignin onto the biomass surface because the soluble lignin is discharged from the reactor as soon as it is formed. It was built and tested as a proof-of-concept laboratory reactor. Although a continuous reactor is desirable in a commercial scale operation, the continuous version of it (co-current auger-driven reactor where the flow of liquid is faster than the solid flow) is yet to be developed. The main advantage of the ARP is its efficient lignin removal. More than 80% lignin removal is not uncommon in ARPs, and satisfactory improvement of digestibility has been observed in a number of studies using herbaceous biomass and hardwood feedstock (Kim and Holtzapple, 2005; Kim and Lee, 1996, 2005; Kim et al., 2003, 2006). The reaction conditions in ARPs are typically 10–15% ammonia, 150–210 °C, and ~2.3 MPa. From a process viewpoint, ARP has its shortcomings. It is a high temperature process that requires high energy input and high liquid throughput. The recovery of ammonia is also an energy intensive process. In addition, approximately 50% of the hemicellulose is lost along with the soluble lignin during operation of the ARP. Although hemicellulose sugars are not decomposed during ARP, they are considered lost because they are recovered at low concentrations in oligomer form, already contaminated with lignin degradation products and extraneous components, which makes them unusable for bioconversion.

To alleviate the problem of hemicellulose loss during ARP, a simple batch process was developed using aqueous ammonia (i.e., soaking in aqueous ammonia or SAA). In SAA tests performed on corn stover, the biomass was soaked in 15–30% ammonia at low temperature (30–80 °C) for extended periods (4–24 h), which resulted in retention of more than 80% of the hemicellulose and near 100% of the cellulose (Kim et al., 2009). However, it appears that SAA is effective only for herbaceous biomass. It should also be noted that commercial "cellulase" invariably contains enough xylanase activity to concurrently hydrolyze both xylan and glucan in alkaline-treated solids. This is evidenced by successful conversion of corn stover to ethanol by simultaneous saccharification and cofermentation (SSCF) (Kim and Lee, 2005). The advantage of retaining hemicellulose as a solid is that this makes it possible to avoid the formidable task of biologically converting the contaminated lean sugar oligomers into desired product, a costly, multistage process. The SAA pretreatment is effective in removing lignin (60–70% removal). Other issues with SAA are its long reaction time and high liquid throughput.

In order to reduce the chemical input and water use, the low liquid ammonia (LLA) pretreatment based on aqueous ammonia was developed (Li and Kim, 2011). The LLA process requires less liquid and ammonia loading than those of SAA and ARP. In LLA, minimal ammonia and liquid (1:0.2–5.0 w/w of solid-to-liquid ratio) are applied for an extended period (4–12 weeks). This enhances the enzymatic hydrolysis yield significantly. It is also conducted under mild conditions (~30 °C, ~ambient pressure), and yielded ethanol at 73% of the theoretical maximum based on the amount of total carbohydrates (glucan + xylan) present in the untreated material.

Because LLA uses aqueous ammonia, it is anticipated that LLA could more easily be applied to the industry than could pretreatments using gaseous ammonia because ammonia gas is more difficult to handle than ammonia liquid, without increasing pressure.

4.2.2.2. Anhydrous ammonia pretreatment.
The LMAA process uses anhydrous ammonia to overcome problems inherent with conventional pretreatment processes using aqueous ammonia. Previous methods such as ARP and SAA require high chemical loading and excessive water use for both pretreatment reaction and washing steps because they are liquid-phase reactions (Yoo et al., 2011). Use of LMAA can significantly reduce the water and energy input compared to those involving aqueous ammonia pretreatment.

The LMAA process includes three steps: ammoniation under ambient conditions in the first step, pretreatment (40–150 °C for 72–96 h) in the second step, and evaporation/recovery of excess ammonia in the third step. The biggest advantage of LMAA is that it does not require a washing step to remove and recover residual ammonia because it contains little water (moisture) and ammonia loadings. The residual ammonia in the biomass can provide antimicrobial effects during long- and mid-term storage in the biorefinery facilities. The adsorbed ammonia can also serve as a nitrogen source for the microorganisms used in the subsequent fermentation process (Yoo et al., 2011, 2014). It was reported that 89% of the theoretical ethanol yield (based on glucan + xylan in corn stover) was attained during simultaneous saccharification and co-fermentation (SSCF) of LMAA-treated corn stover (Yoo et al., 2011). The optimal conditions reported for LMAA were: 0.1 g-NH_3/g-solid, 1.0 solid/liquid ratio, 80 °C, and 84 h of pretreatment time.

The AFEX (ammonia fiber explosion/expansion) process was introduced as a pretreatment method for lignocellulosic biomass (Dale et al., 1985). Its effect is similar to that of LMMA except that it utilizes anhydrous liquid ammonia. Because it was introduced in the early 1980s, several different and improved versions of AFEX have been developed. In the AFEX process, biomass is contacted with liquefied anhydrous ammonia at elevated temperature (60–120 °C). After treatment, the pressure is rapid released. Due to expansion of the liquid ammonia trapped in the biomass, its structure is disrupted, which is a beneficial pretreatment effect. The ammonia used in the pretreatment is recovered at this stage in the form of a low-pressure gas, and reused. The pretreatment conditions of AFEX are moderate for temperature and high for pressure. Because most of it is recovered, net consumption of ammonia is low. During AFEX, neither component dissolution nor weight loss of solids occurs. According to a recent report, AFEX

de-crystallizes the cellulose, hydrolyses hemicellulose, depolymerizes lignin, and increases the micropores in the cell wall structure (Mosier et al., 2005). Therefore, the structure of the biomass is changed, resulting in increased water holding capacity and higher digestibility (Stuart and El-Halwagi, 2012).

AFEX treatment also alters the structure of the cellulose and lignin, which results in modification and redistribution of lignins (Martinez et al., 1991). Like other ammonia pretreatments, the residual ammonia left in the pretreated biomass can be used as a nutrient for microorganisms, thus facilitating fermentation (Dale et al., 1985). AFEX does not degrade biomass carbohydrates significantly.

The AFEX process does have drawbacks. The use of high pressure treatment raises the cost of equipment. The energy requirement is high for ammonia recovery, which involves recompression of anhydrous ammonia gas to liquid. Because pressure swings occur in the reactor operation and ammonia recovery, scale up of the process may encounter technical difficulties (Drapcho et al., 2008).

4.2.2.3. Ammonolysis. Ammonolysis is a chemical reaction analogous to hydrolysis, only in this case an ammonia ion (NH_2^-) plays the role of water molecules in hydrolysis. The ammonia molecule is dissociated producing H^+ and NH_2^- species, which then induces breakage of chemical bonds in LCCs and lignins. The presence of LCCs (lignin-carbohydrate complex) in lignocellulosic biomass has been confirmed, and various chemical linkages have been identified between lignin and the carbohydrates present in LCC structures (Du et al., 2013). The major linkages in biomass include ether (R-O-R') and ester (R-CO-OR') bonds in LCCs, and ether (C–O–C) bonds in lignin polymers. Ammonia molecules promote very effective selective cleavage of these linkages, leading to lignin dissolution and eventually to lignin removal (Kim, 2013).

4.2.3. Lime pretreatment

Pretreatment using lime ($Ca(OH)_2$) has also been developed (Karr and Holtzapple, 2000; Kim and Holtzapple, 2005). Although the lime pretreatment was introduced as one that could operate under mild reaction conditions, it can be operated in a variety of modes and conditions: (1) high temperature for short reaction time (100–160 °C, <6 h, ~200 psig) with or without oxygen; (2) low temperature for long-term pretreatment (55–65 °C, 2–8 weeks, atmospheric pressure) with or without air; or as (3) simple pretreatment requiring 1 h in boiling water, without air or oxygen (Sierra et al., 2009). Normal lime loading is in the range of 0.05–0.15 g-$Ca(OH)_2$/g-biomass (at concentration of 5–20% solids). The key points of lime pretreatment are that it is simple and effective, especially for herbaceous (grass) biomass (Chang and Holtzapple, 2000; Karr and Holtzapple, 2000). The role of lime in the process is that it removes most of the acetyl groups and about 30% of the lignin from the biomass, low lignin concentration being the main factor promoting enhanced enzymatic saccharification. Although the pretreatment effect with lime is not as strong as that with ammonia or sodium hydroxide, it is a simple and low-cost process, which makes it an attractive pretreatment option (Kim, 2013).

5. Internal barriers to enzymatic hydrolysis

5.1. Lignins

The effectiveness of alkaline pretreatment relies primarily on lowering the lignin content in lignocellulosic biomass, which in turn increases the rate and yield of enzymatic hydrolysis of carbohydrates. In addition, alkaline reagents are more effective for delignification of herbaceous biomass than woody biomass. This is because woody material contains more lignin and is quite different from herbaceous biomass in that weight of molecules is greater and the molecules more tightly structured.

Lignin is an amorphous and highly complex polymer of three aromatic alcohol units (*p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) (Freudenberg and Nash, 1968). Lignols are incorporated into the lignin polymeric structure in the form of phenylpropanoid units (*p*-hydroxyphenyl, guaiacyl, and syringyl lignins).

The various groups of biomass-source species have different types of lignins, which contain different amounts of methoxy groups within them (Xu et al., 2014). Softwoods contain mostly guaiacyl (a polymer of coniferyl alcohol) with small quantities of *p*-hydroxyphenyl. On the other hand, hardwoods contain more syringyl units than softwood lignin, either a mixture of guaiacyl and syringyl (a copolymer of coniferyl and sinapyl alcohol; found in most dicots) or a mixture of all three types of lignins (in most monocots) (Boerjan et al., 2003). Dominant lignins in grasses and palms are *p*-hydroxyphenyl (in addition to syringyl + guaiacyl) and syringyl, respectively (Kuroda et al., 2001). In terms of lignin size and structure, hardwood has lignin of lower DP and more methoxyl groups than softwood. Softwood lignins are much more difficult to delignify (Brown, 2003; Sjostrom, 1993). For this reason, only a few pretreatment methods have been developed for softwood: sulfite pretreatment (SPORL) and ethanol organo-solv pretreatment (Zhu et al., 2009; Pan et al., 2008).

According to the experimental work of Shimizu et al. (2012) performed on various model lignin dimers; sodium hydroxide under certain conditions (1 N NaOH, 40–70 °C for 3–7 h) cleaves the dimer containing syringyl units more rapidly than guaiacyl-guaiacyl dimers. In other words, the lignocellulosic biomass with higher content of syringyl units tends to be more easily delignified at higher pH; thus, hardwoods (syringyl + guaiacyl) is more suitable biomass for alkaline pretreatment than softwoods are. It was also reported that grass species tend to have a 'syringyl: guaiacyl' ratio similar to that of hardwood (Modenbach, 2013).

5.2. Inhibition mechanism of lignin

Cellulase enzymes (glucanase + xylanase) are known to be inhibited by various degradation products, such as organic acids, phenolic compounds, furfural, and hydroxymethyl furfural (HMF) (Hodge et al., 2008; Kim et al., 2011). The fact that lignin and phenolic compounds inhibit enzymatic reaction indicates that enzymatic saccharification can be improved by lowering the lignin content in the biomass prior to enzymatic hydrolysis. Although the alkaline pretreatment is effective for delignification, a high percentage of lignin removal is not necessary to effectively convert lignocellulosic biomass into fermentable sugars. Among alkaline methods, AFEX and LMAA leave the lignin content intact (zero delignification), while SAA removes 50–60% of the lignin, which is significantly less lignin removal than that of ARP (Alizadeh et al., 2005; Yoo et al., 2011; Kim and Lee, 2006). It is not just the gross amount of lignin in biomass that controls enzymatic hydrolysis. The location of lignin in biomass, the lignin surface area, and the properties and surface conditions of the lignin also play significant roles. One example is non-productive binding of the enzyme on lignin that makes the enzyme irreversibly inactive. Such binding is related to the lignin surface area (not the gross amount) and the location of the lignin, as well as to the properties of the lignin surface that affects its affinity with protein. In this regard, blocking active lignin sites has emerged as a supplementary tool for pretreatment because this can improve enzyme efficiency. It has been proven that non-productive adsorption of enzyme on lignin can be prevented using surfactants and proteins, and these have significantly improved the yield from enzymatic

saccharification (Eriksson et al., 2002; Yang and Wyman, 2006). The mechanism of lignin inhibition of the enzymatic hydrolysis reaction has been verified.

6. Role of alkaline treatment in biomass refining

Alkaline pretreatment can play an important role in the biorefinery of lignocellulosic biomass because alkaline treatments can be applied not only for pretreatment but also as a method for fractionation of lignocellulosic biomass. Fractionation of lignocellulosic biomass into lignin, carbohydrates, and other components can be the first step toward realization of biorefinery. Alkaline reagents used in pretreatment are highly selective for the separation of lignin. They can be used to recover a large fraction of lignin with relatively high purity. Alkaline pretreatment retains more hemicellulose than acidic or neutral pretreatments (dilute-acid or steam treatment). Pretreatment using dilute acid or hot water is effective for hemicellulose recovery. With proper selection of pretreatments and their operation sequence, effective fractionation can be achieved. This is an important attribute of the biorefinery concept as it offers flexibility in utilizing biomass. If alkaline treatment is applied in the first stage, low-lignin biomass and lignin are obtained as the products. The lignin produced in alkaline pretreatment is sulfur-free, unlike that produced by pulping processes (Kim et al., 2009). Such lignin will then be used in further processing. Fuel additives and bio-based polymers (adhesives and asphalt extenders) are potential products (Holladay et al., 2007).

If the fractionation of hemicellulose is achieved by acidic treatment, its sugars (primarily xylose and its oligomers) are obtained as second-stage products, which can be further converted into value-added products that include fuels and platform chemicals (Werpy and Petersen, 2004). The solids remaining after hemicellulose removal is glucan-rich biomass, which is a versatile feedstock with numerous biorefinery conversion applications.

Alkaline treatment of biomass gives another option for biomass fractionation. Li and Kim (2011) reported that sequential two-step enzymatic hydrolysis/fermentation (SSF), using xylanase and glucanase, can effectively produce glucan-rich and xylan-rich products. With this method, xylanase is used to produce xylose, and then cellulase is used to produce glucose in the second stage. In addition to the pretreatment methods by acids and bases, the use of combined chemical reagents for alkaline pretreatment has been attempted. For example, hydrogen peroxide and carbon dioxide have been tested as additional reagents (Kim and Hong, 2000; Kim and Lee, 1996; Kim et al., 2000; Tyson, 1991).

7. Conclusions

A variety of alkaline reagents have been used to improve enzymatic saccharification of lignocellulose. Alkaline pretreatment has been considered one of the most effective methods because it has many advantages. These include effective delignification, minimal interaction with hemicellulose, and mild reaction conditions. In addition, it is possible to recover and reuse many of the reagents used in alkaline pretreatment. They are highly selective for separation of lignin. It is then anticipated that alkaline pretreatment could play a critical role in the biorefinery industry because it can be used not only as a pretreatment but also as a method for fractionation.

Acknowledgements

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