Alkaline Hydrogen Peroxide Delignification of Three Lignocellulosic Biomass under Atmospheric Pressure

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The study's goal was to investigate the delignification characteristics using alkaline hydrogen peroxide (AHP) under atmospheric pressure, intending it as a pre-treatment method for transforming low-value lignocellulosic biomass into high-performance structural materials. The lignocellulosic biomass used in this study were Japanese cedar (*Cryptomeria japonica*), Hyun aspen (*Populus alba* x *glandulosa*), and bamboo (*Phyllostachys pubescens*), which are underutilized and low-value lignocellulosic biomass in Korea. The delignification conditions used were pH 11, liquor ratio 12.5, H_2O_2 (3 to 10%), temperature (25 to 100 °C), and time (1 to 24 h). Japanese cedar exhibited <30% delignification even under the most severe conditions. In contrast, Hyun aspen achieved more than double that level of delignification. However, reaching over 60% delignification was challenging. Bamboo was easily delignified, reaching approximately 80% delignification using similar conditions performed in Japanese cedar and Hyun aspen. These differences observed in AHP delignification among three species were likely to be primarily due to the structural differences and proportion of condensed units in lignin. Consequently, for Hyun aspen and bamboo, the AHP delignification process under atmospheric pressure was considered to be feasible as a pre-treatment method for high-performance structural materials.

INTRODUCTION

There are many types of lignocellulosic biomass that are deemed difficult to use as raw materials for pulping or structural materials, whether in international or local settings. In particular, practical and efficient utilization of lignocellulosic biomass is necessary for a country like Korea, where around 85% of wood resources are imported (KFS 2019).

Japanese cedar (*Cryptomeria japonica*) is a native species of Japan and the most important afforestation wood species in Japan, comprising 43% of their artificial forests (Hayashi and Miyatake 2015). Recently, a considerable amount of Japanese cedar has been imported to Korea for use as interior materials. However, Japanese cedar presents challenges for utilization in pulp production and as structural materials due to its high lignin content and low density (Sakai *et al.* 1983; Hayashi and Miyatake 2015). Hyun aspen is a hybrid variety created through the crossbreeding of *Populus alba* and *Populus glandulosa* (KFRI 2010). While extensively planted in South Korea, the applications of Hyun aspen are primarily limited to specific purposes such as mushroom cultivation or bedding in

livestock farming. Bamboo (*Phyllostachys pubescens*) is abundant in the southern regions of South Korea; however, due to the declining consumption of domestic bamboo products, bamboo forests are becoming desolate (NIFoS 2016). There is an urgent need for the effective utilization of these bamboo resources.

Many studies have been conducted on the utilization of low-valued woody and non-woody biomasses. In recent years, the development of a new type of high-performance structural material through the partial removal of lignin has been gaining attention. Song *et al.* (2018) reported that by treating hardwoods with a mixture of NaOH and Na_2SO_3, followed by hot pressing, a high-strength panel material can be prepared. Specifically, the researchers noted that pressing after approximately 50% delignification enables the production of high-performance panels with bulletproof capabilities. However, with this method there are concerns regarding the economic feasibility due to the substantial amount of chemicals required for delignification. On the other hand, this method has provided new possibilities for the development of new uses for low-grade lignocellulosic biomass through partial delignification.

In this light, H_2O_2 is industrially attractive and currently used as a bleaching reagent in the pulp and paper industry (Gould 1984; Li *et al.* 2014; Ma *et al.* 2015). In addition, H_2O_2 is widely used as an antiseptic and oxidizing agent. The H_2O_2 delignification process can also be applied to the saccharification pre-treatment process of non-woody biomass such as agricultural residues (Huang *et al.* 2020); since the process not only enables effective delignification under atmospheric pressure conditions, but it also minimizes the degradation of carbohydrates. In addition, H_2O_2 is regarded as a relatively environment-friendly and sustainable chemical that can be easily decomposed to water and oxygen as end products (Gould 1984; Kadla and Chang 2001). In alkaline conditions, H_2O_2 can act as a nucleophilic agent by forming hydroperoxide anion (HOO^-), which decompose phenolic and non-phenolic lignin structures *via* nucleophilic attack (More *et al.* 2021; Nge *et al.* 2023).

$$H_2O_2 + HO^- \rightleftharpoons H_2O + HOO^- \tag{1}$$

Although numerous studies have been reported on alkaline hydrogen peroxide (AHP) delignification, actual delignification efforts are predominantly focused on the treatment of non-woody biomass rather than woody biomass.

The purpose of this study was to investigate the AHP delignification properties of three currently underutilized lignocellulosic biomass sources: Japanese cedar (softwood), Hyun aspen (hardwood), and bamboo (grass) under atmospheric pressure. In particular, there is dearth of literature regarding AHP delignification of softwoods under atmospheric pressure, hence the need for further research. In addition, this work provides preliminary information on the suitability of AHP delignification as a pre-treatment method for the preparation of these underutilized lignocellulosic biomass into high-performance wood materials, that can be plasticized.

EXPERIMENTAL

Materials

Japanese cedar was provided by Happy Home Wood Tech Co., Ltd. (Muan, Korea). Hyun aspen (20 years old) was collected from the mountain side of Yangya-ri (Gosan, Korea), and was cut at Happy Home Wood Tech Co., Ltd. Both Japanese cedar and Hyun

aspen wood were cut and then milled to produce wood powder. The bamboo (2 to 3 years old) powder was provided by Songjuk Industry (Hamyang, Korea). The bamboo powder was air dried at room temperature for a week. In this study, 40 to 80 mesh of wood and bamboo powders were used.

Sodium chlorite ($NaClO_2$, 78%) and sodium hydroxide (NaOH, 93%) were purchased from Duksan Pure Chemical (Ansan, Korea). Hydrogen peroxide (H_2O_2, 34.5%) and sulfuric acid (H_2SO_4, 93%) were purchased from Samchun Pure Chemical (Pyeongtaek, Korea) and Daejung chemicals & Metals (Siheung, Korea), respectively.

Methods

Specific gravity

The specific gravity of the lignocellulosic biomass samples used in this experiment was determined by water immersion method in accordance with ASTM D2395-17 (2022). The biomass samples were dried in a convection oven at 105 °C until practical equilibrium was reached prior to the determination of specific gravity.

Chemical composition

The ash, extracts, and lignin were measured using TAPPI test methods (T 211 om-02, 2002; T 207 cm-08, 2008; T 204 os-76, 1988; T 212 om-02, 2002; T 222 om-02, 2006; UM 250, 1991). The acid-soluble lignin was determined following TAPPI standard method, UM 250 (1991), but the absorption coefficient used was 110 L/g·cm for Hyun aspen and bamboo, and 105 L/g·cm for Japanese cedar, respectively. The holocellulose content was determined by the Wise method (Wise *et al.* 1946), and α-cellulose was determined according to TAPPI test method T203 om-83 (1988). The hemicellulose content was obtained by subtracting the α-cellulose value from the holocellulose value.

AHP delignification

A 2 g (o.d.) sample was placed in a 100 mL of conical beaker and the AHP solution was added. Delignification was performed in a shaking water bath (140 rpm) with the conditions shown in Table 1. For delignification conditions at 100 °C, a 250 mL glass bottle fitted with a 1.5-meter-long glass tube was used as an air-cooling condenser to prevent excessive evaporation of the liquid. After AHP treatment, the sample slurry was filtered through a 1G3 or 1G4 glass filter and washed with 150 mL of hot distilled-deionized water, followed by 30 mL of acetone. The residue was dried overnight in a convection oven at 105 ℃, and the residue yield was determined. All experiments were conducted in duplicate, and the average values were shown.

Table. 1. AHP Delignification Conditions

Dependent		Fixed	
Temperature (°C)	25 to 100	pH*	11.0±0.2
H_2O_2 (%)	3 to 10	Sample size (mesh)	40 to 80
Time (h)	3 to 24	Liquor ratio	12.5

*pH was adjusted with NaOH

Lignin determination of the residue(s) after AHP delignification

The residues obtained from AHP delignification were placed in air to adsorb moisture to attain equilibrium moisture content. Briefly, a 0.5 g was placed in a 30-mL

weighing bottle, to which 7.5 mL of 72% H_2SO_4 was added. The mixture was stirred occasionally and allowed to stand at room temperature for 2 h. Afterwards, this mixture was transferred to a 500-mL Erlenmeyer flask with 273 mL of distilled-deionized (DDI) water, adjusting the H_2SO_4 concentration to approximately 3%. Subsequently, the flask was heated in an autoclave at 120 °C for 1 h. The precipitated lignin was filtered using a 1G4 glass filter and washed with hot DDI water. The filter was then dried at 105 °C for 12 h, followed by the determination of Klason lignin. After the Klason lignin measurement, the obtained filtrate and washing solution were combined, and used for the quantification of acid-soluble lignin. The sum of Klason and acid-soluble lignin was defined as the total lignin.

The selectivity of delignification was calculated according to Eq. 2. In the expression presented, D_L and D_C represent dissolved lignin and dissolved carbohydrate, respectively.

$$\text{Selectivity of delignification} = D_L/D_C \tag{2}$$

where D_L (%) is the total lignin in lignocellulosic biomass (%) minus the total lignin in residue (%, based on each biomass), and D_C (%) is 100 minus [residue yield (%) + D_L (%)].

RESULTS AND DISCUSSION

Wood Analysis

In Korea, Japanese cedar is usually utilized as an interior material. However, due to high lignin content (over 30%) and low specific gravity, Japanese cedar is not suitable for manufacturing pulp and as construction materials (Sakai *et al.*, 1983; Hayashi and Miyatake 2015). As shown in Table 2, the specific gravity of Japanese cedar was 0.28, which was considerably lower compared to Hyun aspen and bamboo.

Table 2. Specific Gravity and Chemical Composition of Japanese Cedar, Hyun Aspen, and Bamboo

Component		Softwood	Hardwood	Grass
		Japanese cedar (*Cryptomeria japonica*)	Hyun aspen (*Populus alba × glandulosa*)	Bamboo (*Phyllostachys pubescens*)
Specific gravity		0.28±0.02	0.41±0.02	0.90±0.01
Ash (%)		0.56±0.01	0.43±0.02	1.24±0.01
Extracts (%)	Cold water	1.28±0.05	2.04±0.25	5.77±0.02
	Hot water	2.49±0.04	3.73±0.01	9.85±0.04
	1% NaOH	10.88±0.13	21.06±0.12	29.69±0.10
	Alcohol-benzene	2.63±0.03	3.11±0.02	4.77±0.07
Carbohydrate (%)	Holocellulose[1]	72.39±0.30	83.63±0.41	69.25±0.44
	α-cellulose[2]	45.62±0.11	49.36±0.21	44.31±0.16
	Hemicellulose (1–2)	26.77	34.27	24.94
Lignin (%)	Klason	31.44±0.18	20.81±0.43	27.20±0.09
	Acid-soluble	0.37±0.03	3.29±0.10	0.51±0.01
	Total	31.81	24.10	27.71

For Japanese cedar, the total lignin content was about 32%, which was higher than that of Hyun aspen and bamboo. This value is typically higher compared to general softwoods from temperate regions. For Hyun aspen, the Klason lignin content was 21%, the lowest among the three lignocellulosic biomass. The acid-soluble lignin content of Hyun aspen was 3.3%, the highest among the biomass samples. The total lignin content of Hyun aspen was 24%, which was within the range of lignin content from general hardwoods from temperate regions. For bamboo, the total lignin content was about 28%, similar to that of softwoods from temperate regions.

AHP Delignification of Lignocellulosic Biomass

H_2O_2 decomposes into water and oxygen, and this property makes H_2O_2 environmentally friendly and widely used in various fields. By adjusting the pH of H_2O_2 solution to alkaline conditions, selective delignification is possible while stabilizing carbohydrates. In pulp industry, AHP is used not only in the production of non-wood pulp, but also in the conservation bleaching of mechanical pulp (Smith 2012). When H_2O_2 is used for the delignification of lignocellulosic biomass, pH 11.5 showed the best delignification performance (Gould 1985). Indeed, for best delignification results, a high concentration of H_2O_2 must be used, and a large amount of alkali is required to adjust to pH 11.5. For this study, the characteristics of AHP delignification using three different lignocellulosic biomass *i.e.* Japanese cedar, Hyun aspen, and bamboo were investigated at a slightly lower pH of 11 for practical and economical purposes.

Japanese cedar

Table 3 shows the results of AHP delignification on Japanese cedar under atmospheric pressure. Delignification using 3% H_2O_2 at temperatures below 50 °C resulted in a negative delignification. This result suggests that under these AHP delignification conditions, the dominant reaction is likely due to the condensation of lignin, even under mild AHP delignification conditions. When the temperature was between 50 and 100 °C, obtaining >5% delignification was not possible. To improve delignification, H_2O_2 was used at its maximum concentration of 10%. As a result, delignification increased with the increasing temperature; however, obtaining >26% delignification proved to be challenging. Thus, the results indicate that even under the most severe AHP conditions (10% H_2O_2, 100 °C, and 7 h), achieving 30% delignification is unattainable. These results were thought to be similar to other softwood species, which may be the reason why there are limited reports on AHP delignification under atmospheric pressure regarding softwoods. AHP is known to degrade the conjugated double bonds as well as the phenolic and non-phenolic units of lignin by hydroperoxide anion (HOO^-) as active species (Fig. 1, More *et al.* 2021). In Fig. 1, since the number of double bonds in the lignin side chain is very limited, in order to achieve a high degree of delignification, the phenolic and non-phenolic units of lignin must be extensively degraded by AHP. However, even under harsh AHP conditions, the delignification of Japanese cedar occurred to a limited extent. Therefore, in terms of AHP delignification reactions shown in Fig. 1, it is quite difficult to comprehend why the delignification of Japanese cedar cannot achieve >30%. Although not fully understood, it is thought to be an interplay of factors such as the amount of condensed structures and β-O-4 units, and molecular weight of Japanese cedar lignin.

Table 3. Results of AHP Delignification on Japanese Cedar

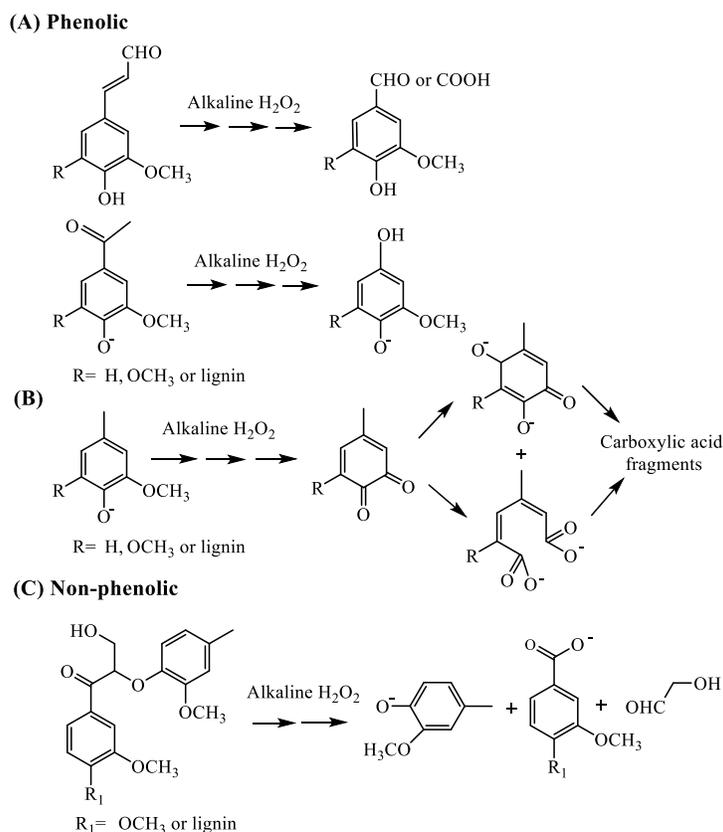| H$_2$O$_2$ (%)	Temp. (°C)	Time (h)	Residue Yield (%)	Residual Lignin (%)	Delignification (%)
3	25	3.5	96.0	32.9	-4.6
		7	95.5	32.1	-2.2
	50	3.5	95.1	31.5	-0.2
		7.0	94.7	34.8	-10.7
	70	3.5	93.0	30.7	2.3
		7	92.8	30.4	3.3
	84	3.5	91.6	30.2	4.0
		7	90.9	30.0	4.5
	100	3.5	90.5	30.0	4.7
		7	89.8	29.8	5.1
10	25	24	95.0	31.4	0.0
	70	3.5	91.1	27.5	12.4
		7	88.7	26.4	16.0
	100	3.5	80.3	23.7	24.5
		7	77.8	23.2	26.2

Fig. 1. AHP oxidation of phenolic and non-phenolic lignin structure. (A) phenolic lignin with conjugated side chain, (B) phenolic lignin oxidation to benzoquinone or aromatic ring opening, (C) non-phenolic lignin with aryl-α-carbonyl conjugated moieties. The lignin decomposition reactions under AHP conditions were based on Ma *et al*. (2015) and Kadla and Chang (2001), with some modifications.

Hyun aspen

The AHP delignification results for Hyun aspen are shown in Table 4. Although various AHP delignification conditions were tested, the relationship between delignification and residue yield had a high correlation. The highest delignification attained was 54%. Springer (1990) reported that treatment of aspen (*Populus tremuloides*) wood with AHP (pH 11, 5% H_2O_2) removed at most 36% of the original lignin. This indicates that AHP delignification under atmospheric pressure of hardwoods is also challenging, similar to softwoods. However, the delignification of hardwoods is relatively more effective than for softwoods. The reason is primarily due to the structural differences between softwood and hardwood lignin. Most likely, the difference in amount of β-O-4 bonds, the most abundant linkage in lignin, and the condensed structures greatly affected their delignification. Hyun aspen might have more β-O-4 bonds and less condensed structures. In addition, while the aromatic moieties in softwood lignin is mostly composed of guaiacyl (G) units, hardwood lignin also contains syringyl (S) units. These differences in the aromatic moieties notably affect AHP delignification on hardwoods.

Table 4. Results of AHP Delignification on Hyun Aspen

H_2O_2 (%)	Temp. (°C)	Time (h)	Residue Yield (%)	Residual Lignin (%)	Delignification (%)
3	25	24	88.0	14.5	30.5
	70	7	85.2	13.9	33.3
5	25	24	83.8	12.6	39.3
	70	3.5	81.5	11.6	44.1
7	25	24	83.0	12.3	41.0
	70	3.5	78.8	10.8	48.1
		7	77.4	9.6	53.7
	100	3.5	76.2	10.9	47.7
10	25	24	82.5	12.0	42.2
	70	3.5	76.5	10.1	51.6
		7	74.8	9.9	52.4
	100	3.5	73.9	9.8	52.9

Bamboo

When bamboo was treated with AHP, delignification occurred more readily, despite the higher lignin content than that of Hyun aspen (Table 5). Delignification was able to reach up to almost 80% at similar AHP conditions performed on Japanese cedar and Hyun aspen. Thus, the feasibility of utilizing bamboo for chemical pulp production under AHP delignification conditions became evident, as AHP treatment already had been established as a suitable treatment for non-woody biomass. From the result, the high delignification of bamboo was attributed to the differences in aromatic nuclei and the amount of condensed structures in lignin. In case of grass lignin, not only G and S units but also *p*-hydroxyphenyl (H) units were present. The H units do not have methoxy groups. Therefore, the delignification of bamboo easily proceeded compared to other lignocellulosic biomass used in this experiment.

Table 5. Results of AHP Delignification on Bamboo

H_2O_2 (%)	Temp. (°C)	Time (h)	Residue Yield (%)	Residual Lignin (%)	Delignification (%)
3	25	3	86.0	23.2	14.8
		12	85.3	22.1	18.7
	100	3	77.1	17.6	35.4
		12	75.4	16.3	40.1
6.5	63	7.5	65.5	9.9	63.5
10	25	3	78.8	17.4	36.0
		12	69.1	12.9	52.8
	100	3	56.8	6.8	74.9
		12	54.7	5.8	78.9

Comparison of AHP delignification for the three lignocellulosic biomass

Figure 2 illustrates the relationship between delignification and residue yield after AHP delignification for three types of lignocellulosic biomass. In the case of bamboo, there was a linear correlation between delignification and residue yield. The delignification in bamboo proceeded smoothly with AHP treatment under atmospheric pressure, allowing for almost 80% delignification, despite the high lignin content and high specific gravity. As expected, the delignification results obtained from bamboo agreed with the previous findings, stating that AHP delignification under atmospheric pressure was suitable for herbaceous plants (Huang *et al.* 2020). As mentioned earlier, this is attributed to the amount of non-condensed units and structural differences in bamboo lignin, facilitating easy decomposition and dissolution during the AHP delignification process. For Hyun aspen, up to about 60% delignification was attainable, but no further delignification occurred when the residue yield reached 75 to 80%. Achieving a high level of delignification under these experimental conditions was challenging for Hyun aspen. However, this process could be potentially used for wood pre-treatment that does not require a high level of delignification, as it is possible to achieve up to 60% delignification. The delignification of Japanese cedar was challenging, and no further delignification occurred near 80% residue yield. Therefore, AHP delignification process employed may not be suitable for softwoods such as Japanese cedar. This is due to the fact that the condensation reaction took precedence over lignin degradation under AHP conditions, or that the lignin degradation reaction was very limited. Table 6 shows the delignification selectivity of three lignocellulosic biomass at maximum AHP conditions. Although Hyun aspen exhibited high delignification selectivity, attaining >60% delignification was improbable due to the degradation of effective H_2O_2 during delignification. Kadla *et al.* (1999) reported that although the reaction mechanism of AHP and lignin is highly dependent on H_2O_2 stability, H_2O_2 is very unstable at elevated temperatures and it mostly degrades in a short period of time in the absence of stabilizers. Thus, in the future, achieving higher delignification was thought to be possible by using stabilizers for H_2O_2.

These differences observed in AHP delignification between three species were thought to be primarily due to the structural differences and proportion of condensed units in lignin. Consequently, for Hyun aspen and bamboo, the atmospheric pressure AHP delignification process was thought to be feasible as a pre-treatment method for high-performance structural materials.
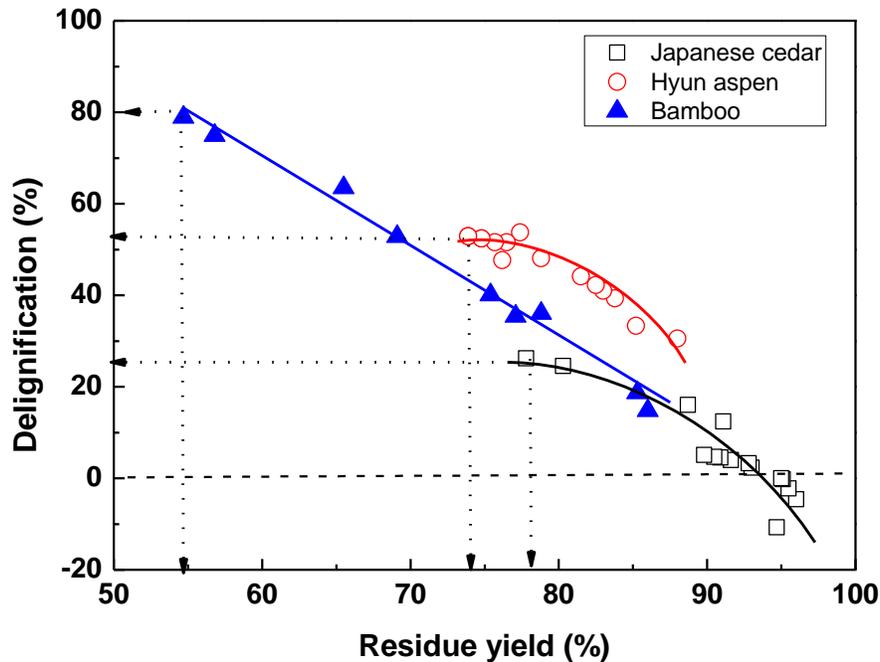
Fig. 2. Relationship between delignification and residue yield of Japanese cedar, Hyun aspen, and bamboo.

Table 6. Comparison of the Selectivity of Delignification at Maximum AHP Delignification on Each Lignocellulosic Biomass

Species	H_2O_2 (%)	Temp. (°C)	Time (h)	Residue Yield (%)	Residual lignin (%*)	Delignification (%)	D_L/D_C
Japanese cedar	10	100		77.8	23.2	26.2	0.6
Hyun aspen	7	70	7	77.4	9.6	53.7	1.8
Bamboo	10	100		54.7	5.8	78.9	0.9
D_L: dissolved lignin, D_C: dissolved carbohydrate, *: Percent on original biomass.							

Future Work

In this study, the AHP delignification under atmospheric pressure of three types of low-value and underutilized lignocellulosic biomass in Korea was examined. The results showed that more than 50% delignification on Hyun aspen (hardwood) and bamboo (grass) was attainable. Thus, it was confirmed that AHP treatment delignification under atmospheric pressure can be used as a pre-treatment for the conversion of these lignocellulosic biomass to high-density and high-performance materials. Future investigations will include applications of the AHP delignification conditions obtained in this study to wood panels of varying thicknesses, intending to manufacture wood panels with various degrees of delignification. The feasibility of producing high-density and high-performance wood panels through hot pressing will be assessed.

CONCLUSIONS

Three different types of lignocellulosic biomass were treated by alkaline hydrogen peroxide (AHP) under atmospheric pressure, and the delignification characteristics were investigated.

1. Even under severe AHP conditions, Japanese cedar was difficult to delignify beyond a level of 30%.

2. Hyun aspen and bamboo reached maximum delignification at 60% and 80%, respectively, under similar AHP conditions performed with Japanese cedar. Thus, AHP delignification under atmospheric pressure can be considered as a suitable pre-treatment method for transforming low-value lignocellulosic biomass into high-performance structural materials, particularly for Hyun aspen and bamboo.

3. The difficulty in the AHP delignification under atmospheric pressure of Japanese cedar was probably due to the structural differences in softwood lignin compared to hardwood and grass lignin.

ACKNOWLEDGMENTS

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