Lignin condensation is assumed to cause these results. In the early stages of the process, lignin is first released from the biomass structure due to the high temperature but is rejoined with hemicelluloses or cellulose as the processes continue (Liu and Wyman, 2004). Ultimately, lignin content in biomass is not changed.

Organosolv pretreatment with 2% sodium hydroxide degraded lignin considerably from 26.17% to 11.72%. Lignin content in sodium hydroxide-control also decreased to 16.29%, but lignin content in ethanol-control did not decrease. Therefore, sodium hydroxide is strong and efficient in dissolving lignin in biomass (David and Shiraishi, 2000).

The glucose amount in organosolv pretreated materials with 1% magnesium chloride was the highest. These results indicate that glucose degradation hardly occurs during organosolv pretreatment with magnesium chloride, while degradation occurs during pretreatment with sulfuric acid or 2% sodium hydroxide.

3.3.3. Surface morphology of pretreated materials

Controls and pretreated materials were observed by FE-SEM for imaging surface changes of lignocellulosic biomass during the pretreatment process. Primarily, pitch pine had a glossy and hard surface. This surface was broken when it was treated by organosolv pretreatment. Surface changes were different from the employed catalysts for organosolv pretreatment, but the organosolv process with magnesium chloride changed the lignocellulosic biomass more similarly to the process with sulfuric acid.

Both images of organosolv pretreated materials with sulfuric acid and with magnesium chloride showed spherical materials, which were called lignin droplets by a previous study (Donohoe et al., 2008). Lignin droplets were also found in ethanol-controls at high temperature.

On the other hand, organosolv-treated material with 2% sodium hydroxide showed features different from the others. The materials decomposed into thin paper-like materials, with the surface looking peeled. Moreover, lignin droplets were hardly found, and it was more similar to that of catalyst-controls than that of ethanol-controls.

3.3.4. ^{13}C NMR for the analysis of lignin droplets

To confirm the spherical shapes on the biomass surface observed from the FE-SEM images of pretreated materials with sulfuric acid and with magnesium chloride, ^{13}C NMR analysis was carried out.

Materials were pretreated by organosolv process with sulfuric acid or with magnesium chloride and were then washed by 1% sodium hydroxide solution (w/v) to extract lignin droplets on the surface. Filtrate was neutralized by 1 N HCl until pH 2. Precipitate was obtained by centrifuge and was analyzed by ^{13}C NMR.

In both NMR spectra, methoxyl signals at 55–60 ppm, aromatic and phenolic signals at 110–150 ppm, C_α signals at 70–80 ppm, C_β signals at 80–90 ppm, and C_γ signals at 60–70 ppm were observed, representing the lignin structure (Lin and Dence, 1992).

Precipitate was also analyzed by solid-state ^{13}C NMR to compare the differences between lignin droplets from sulfuric acid and with magnesium chloride. Both spectra showed the same features, indicating that lignin droplets from sulfuric acid- and magnesium chloride-treated materials have similar structures. However, carbohydrate-signals were not observed from both spectra. Based on the results on the constant lignin contents, lignin droplets from FE-SEM images, and no-carbohydrate-signals from NMR spectra, it was assumed that lignin droplets were formed by condensing only lignin but not by rejoining carbohydrate and lignin as stated previously (Section 3.3.2) (Donohoe et al., 2008).

4. Conclusions

Sulfuric acid showed the best efficiency even at comparatively low temperature. However, low pretreated material yield and high sugar degradation should be overcome. Magnesium chloride was efficient, but required high energy input. However, it obtained high pretreated material yield and high glucose amount. Sodium hydroxide can dissolve lignin effectively, increasing digestibility. However, low pretreated material, considerable glucose degradation and high energy input are drawbacks. A significant problem with a high concentration of sodium hydroxide is that it reacts with the glass, decomposing it. Further, a large amount of salt is produced on the inner surface of the reactor after pretreatment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2010.04.020.

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