Lignocellulosic structural changes after physico-chemical pretreatment monitored by near infrared spectroscopy

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Introduction

Agro-residues composed mainly of cellulose, hemicelluloses and lignin are abundant renewable resources for second generation biofuel production. Delignifying pretreatment is an important step to improve enzyme susceptibility for biomass-to-bioenergy conversion. In the work to be described, wheat straw was subjected to physico-chemical pretreatments at different degrees of severity in order to get rid of lignin, and increase the amount of hydrolysable sugars regarded as precursors for biofuel production. Fourier transform near-infrared (FT-NIR) spectroscopy was applied to characterise the structural changes of wheat straw i.e. lignin removal, degree of acetylation of polysaccharides, mainly xylan, as well as amorphous, semi-crystalline and crystalline structural changes of cellulose moieties after pretreatment.

Materials and methods

Samples of 20 g wheat straw (1 cm length) were treated with alkaline or alkaline/hydrogen peroxide in 200 ml solution for 4 h. Effects of temperature (50 – 121°C) and H_2O_2 concentration (3 – 10 %w/w) were investigated. After pretreatment, treated straw was washed with 1 L deionised water and dried at 50°C for extractives, Klason lignin, acid-soluble lignin and weight loss analyses.[1] Ash content was determined after ignition at 550°C for 1 h. Hemicellulose and amorphous cellulose were analysed by acid methanolysis and GC/MS (Aligent 7890A GC/MS system, USA).[2] Crystalline cellulose content was calculated by subtraction of total carbohydrate from acid methanolysis results. For enzyme hydrolysis, 100 mg pretreated straw samples were hydrolysed by 1 ml Viscozym L (www.novozymes.com) in 9 ml Na-acetate buffer pH 4.0 at 40°C for 48 h and the

amount of reducing sugars was determined by DNS assay. Dry samples were milled to 80 µm, for FT-NIR spectroscopy by an Equinox 55 instrument (Bruker, Germany) using a fiber optic probe measuring from 10000 to 4000 cm^{-1} with 100 scans and 8 cm^{-1} resolution. The apparent absorbance, Log (1/*R*), was measured. Second derivative spectra of averaged spectra (Savitzky–Golay algorithm) were used for straw characterisation (OPUS software 6.0).

Results and discussion

Effect of temperature on alkaline pretreatment

Increase of temperature in alkaline pretreatment of wheat straw led to more lignin removal (Table 1).

However, loss of xylan, the major constituent of wheat straw hemicelluloses was also found. The change of lignin and xylan composition was clearly observed by FT-NIR spectra [Figure 1(a)].

The lignin minima near 5980 cm^{-1} assigned to CH stretch 1st overtone of aromatics[3] decreased when temperature was increased from 50°C to 121°C. Acetyl groups of xylan side chain, absorbing near 5960 cm^{-1} and 5990 cm^{-1}, attributed to CH stretch 1st overtone of CH_3, were strongly degraded or extracted from straw at higher temperatures. In addition, minima of lignin and xylan overlapping bands near 5800 cm^{-1} decreased due to either xylan degradation or deacetylation and delignification.

Table 1. Alkaline and alkaline/H_2O_2 pretreatment of wheat straw.

Sample	Reducing sugar (mg g^{-1} straw)	Weight loss (%)	Extractive (%)	Total lignin (%)	Ash (%)	Hemicelluloses (%)	Crystalline cellulose (%)
Untreated wheat straw	128.0	–	11.4	21.5	4.2	33.9	29.0
Alkaline pretreatment[1]							
50°C, 4 h	220.4	14.5	7.4	13.5	2.9	22.3	39.4
90°C, 4 h	247.8	15.0	8.6	12.9	2.3	18.1	43.1
121°C, 20 min	274.9	17.0	10.0	11.7	1.2	18.5	41.6
Alkaline/H_2O_2 pretreatment[2]							
3.4%H_2O_2	215.8	18.5	8.0	13.4	3.9	13.4	42.8
6.8%H_2O_2	296.5	22.0	7.2	10.2	3.6	13.9	43.1
10.2%H_2O_2	294.8	23.5	6.3	6.1	3.5	16.4	44.2

[1]Alkaline pretreatment at pH 12 adjusted by 1 M NaOH.

[2]Alkaline/H_2O_2 pretreatment at pH 12, 90°C for 4 h.

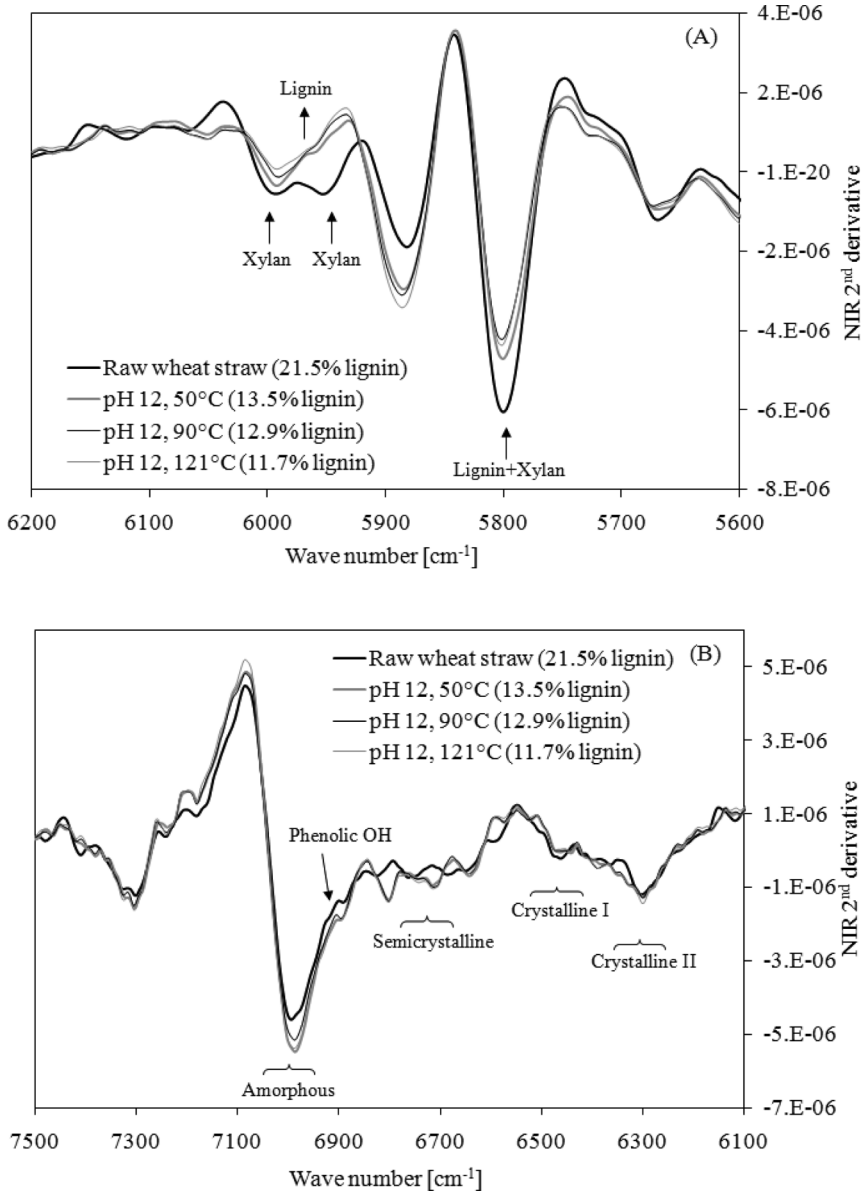

Figure 1. Lignin and polysaccharide change (A) and cellulose modification (B) of alkaline treated wheat straw at different pretreatment temperatures.

Total hemicellulose content was found to decrease at higher temperatures, mainly due to solubilisation (Table 1) and therefore crystalline cellulose content relatively increased. FT-NIR spectral changes of crystalline cellulose II absorbing near 6280 cm^{-1} were not significant at these degrees of delignification [Figure 1(b)], in contrast crystalline cellulose I absorbing near

6460 cm^{-1} (attributed to OH stretch 1st overtone[4,5]) decreased, mainly caused by the fiber swelling effect. Semi-crystalline cellulose with intermediate strength of H-bonded OH near 6722 cm^{-1} and the amorphous part with weakly H-bonded OH groups near 7000 cm^{-1} increased.[4–6] This finding suggests that alkaline pretreatment at higher temperature alters the strength of hydrogen bonds of cellulose from crystalline to either semi-crystalline or amorphous forms that are

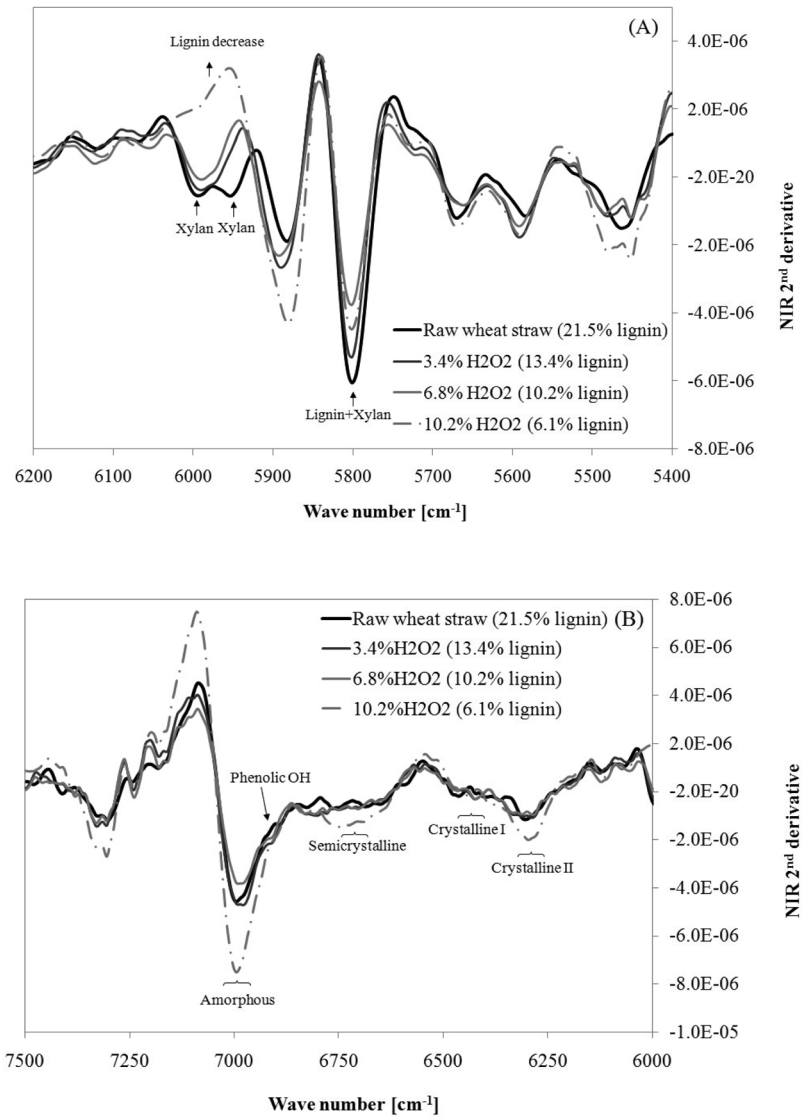

Figure 2. Lignin and polysaccharide change (A) and cellulose modification (B) of alkaline/H_2O_2 treated wheat straw at different H_2O_2 concentrations.

more digestible. Consequently, a higher yield of hydrolysable reducing sugar was obtained (Table 1).

Effect of hydrogen peroxide concentration on alkaline/H_2O_2 pretreatment

Lignin was more degraded from wheat straw when the H_2O_2 concentration was increased from 3.4% to 10.2% during alkaline/H_2O_2 pretreatment at pH 12 (Table 1). The higher weight loss was substantially obtained mainly due to the loss of hemicelluloses. Crystalline cellulose structure seemingly increased after alkaline/H_2O_2 pretreatment because of the high severity of pretreatment, that led to the higher H–OH binding strength. Minima of lignin and acetylated xylan absorbing near 5980 cm^{-1}, 5960 cm^{-1} and 5990 cm^{-1}, together with the overlapping band of lignin+xylan minima near 5800 cm^{-1}, obviously decreased with the increase of H_2O_2 concentration [Figure 2(a)].

Changes of amorphous, semi-crystalline and crystalline structures of pretreated wheat straw were clearly monitored [Figure 2(b)]. Relative to untreated straw, alkaline/H_2O_2 pretreatment at moderate H_2O_2 concentration (3.4 and 6.8 %H_2O_2) at pH 12, 90°C yielded higher amorphous and semi-crystalline moieties near 7000 and 6460 cm^{-1} respectively. Therefore, hydrolysable reducing sugar yield was substantially enhanced. Though less reactive than crystalline I, crystalline cellulose structure II was also decreased at this condition. Nevertheless, the most severe condition (10.2 %H_2O_2, pH 12, 90°C) apparently yielded a higher degree of crystalline structure. This very high H_2O_2 concentration showed a significant improvement of reducing sugar yield (Table 1) due to the very high strength of H bonded OH within crystalline cellulose moieties.

Though overshadowed by the amorphous polysaccharides absorbance near 7000 cm^{-1}, a shoulder of phenolic hydroxyl groups near 6913 cm^{-1} was visible. This shoulder disappeared after the high degree of delignification, when residual lignin content was less than 10%. Compared with alkaline pretreatment, phenolic hydroxyl groups after alkaline/H_2O_2 pretreatment appeared with less intensity, due to the oxidative degradation by hydroperoxy anion (OOH^-) and hydroxyl radicals (·OH) generated from H_2O_2.[7]

Conclusion

FT-NIR spectroscopy is an efficient tool to monitor structural changes of lignocellulose after physico-chemical pretreatment. Degrees of delignification, deacetylation and modification of crystalline structure of cellulose were clearly monitored by FT-NIR spectroscopy.

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