PRODUCTS OF NITROBENZENE OXIDATION FROM NON-WOOD LIGNIN ISOLATED BY SULPHURIC ACID

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Lignin is a very attractive and promising raw material to produce vanillin. Lignin was isolated from black liquor by adding sulphuric acid at four levels of concentration (5, 25, 50 and 72 wt%) and subsequently adjusting the pH to 5.The alkaline nitrobenzene procedure for determining lignin structural units has been applied to non-wood lignin and qualitative and quantitative analyses of oxidative-cleavage reaction were evaluated. The total yield of the oxidation products ranged from 34.1 to 36.3 μmolesg^{-1} pure lignin. The dominant phenolic compound was vanillin followed by syringaldehyde. For precipitated lignin samples, the G:S:H ratio varies between 9:3:1 and7:3:1. It was confirmed that the yield of nitrobenzene oxidation and the contentof phenolic products depends on the isolation procedure.

Keywords: precipitated lignin,sulphuric acid, nitrobenzene oxidation, vanillin

INTRODUCTION

A large quantity of black liquor is collected from pulping mills to be used in alkali recovery processes. The majority of lignin available on an industrial scale is the byproduct of pulping processes and it is utilized as a fuel to obtain energy to operate pulping mills. Numerous studies propose the utilization of lignocellulosic biomass as a feedstock for producing lignin.[1-6] The lignin content in plant stems varies between 15 and 40%.[7] Lignin consists of three phenylpropanoid monomers, which are sinapyl alcohol, coniferyl alcohol and *p*-coumaryl alcohol. Each of these monomers gives rise to different aromatic nuclei in the lignin structures and their structural complexity due to the simultaneous presence of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl units (S). The concentration of these released compounds allows establishing the relationship between units S, G and H, and the lignin origin (hardwood, softwood and annual plants). The alkaline nitrobenzene oxidation and its mechanism have been described in several works.[8-10] The degradation of lignin via the oxidation route is applied for conversion of lignin into aromatic aldehyde compounds or phenols.[11] The oxidative-cleavage reaction of lignin to phenolic aldehydes is one of the most useful chemical methods for characterization of these biopolymers. Nitrobenzene oxidation was one of the earliest oxidants used and it produced reasonable quantities of syringylaldehyde and vanillin, depending on the source of lignin. Nitrobenzene oxidation is still probably the most commonly employed chemical degradation for lignin analysis. The acceptance is related to the fact that nitrobenzene oxidation provides a satisfactory yield of *p*-hydroxybenzaldehyde, vanillin and syringaldehyde from lignin H, G and S units, together with smaller amounts of the corresponding benzoic acids.[12] Vanillin represents possibilities of profitableuses.

In this work, lignin was isolated from black liquor by adding sulphuric acid at four levels of concentration (5, 25, 50, and 72 wt%) and subsequently adjusting the pH to 5. Then lignin was oxidized with alkaline nitrobenzene oxidation. The qualitative and quantitative analyses of the oxidative-cleavage reaction were evaluated. The aim of this study was to investigate the behavior of lignin isolated from black liquor by sulphuric acid in the process of nitrobenzene oxidation.

EXPERIMENTAL
Materials
Black liquor (soda-AQ pulping) characterization
The annual plants, hemp and flax, used for obtaining black liquor were kindly supplied by OP Papírna Ltd. (Olšany, Czech Republic). The cooking conditions involved active alkali sodium hydroxide and the presence of anthraquinone (AQ). The obtained black liquor had the following characteristics: dry matter 36.80 ± 0.62 wt%, pH of 12.9 ± 0.3 (determined by a digital Jenway 3510 pH-meter (UK)), and density 1.242 g/mL; C 36.24 ± 0.09 wt%, H 4.93 ± 0.05 wt%, N 1.13 ± 0.01wt%, S 0.24 ± 0.04wt%, ash 45.75 ± 0.32 wt% and Klason lignin 21.23 ± 0.64 wt%.

Lignin recovery from black liquor
The precipitation of lignin from black liquor was initially studied as a single step process in which a dilute solution of sulphuric acid (5 wt% (1.05 N), 25 wt% (6.01 N), 50 wt% (14.22 N), and 72 wt% (23.99 N)) was added to the black liquor with the pH adjusted to the desired value at the temperature of 50 °C. Then, 100 mL of the black liquor was treated with different amounts of diluted acid to obtain a final pH value of 5, while keeping the temperature constant at 50 °C. After precipitation, the content of each flask was filtered through a pre-weighed oven-dried filter paper using a vacuum filtration unit. The precipitated lignin was washed twice with hot water (total volume 400 mL, pH= 6.8) to remove impurities. The lignin was then dried at 25 °C under a pressure of 0.5 mbar, using lyophilisation equipment (LYOVAC (GT2, Germany) until reaching a constant weight.

Methods
Different techniques were used to establish the physicochemical characteristics of the obtained lignins (Table 1).

Ash content
The ash content of all lignins was calculated gravimetrically after in-furnace calcinations at 575 ºC.

C, N, H and S elemental analysis
Total nitrogen (N), carbon (C), hydrogen (H) and sulphur (S) contents of all samples were determined by dry combustion using a Vario Macro Cube C/H/N/S-analyser (Elementar, Hanau, Germany). Measurements were done in two replicates, and the mean standard errors were of 0.35% for C, 0.04% for N, 0.05% H, and 0.01% for S. Oxygen was determined by difference.

Lignin purity determination
Isolated lignin is a raw material containing other substances along with pure lignin. Polysaccharides as accompanying pollutants, were eliminated by a 1 h boiling treatment in 5 wt% H_2SO_4. The lignin (%) was determined based on the mass loss at acidic hydrolysis.

Table 1
Characterization of precipitated lignin with sulphuric acid solutions

Concentration of sulphuric acid (wt%)	Elemental analysis (wt%)					Ash in precipitate % (w/w)	Purity (%)	Total amount of phenolic hydroxyl groups (mmolg^{-1})*
	N	C	H	S	O			
5	1.11	65.75	6.46	0.06	25.59	0.97 ± 0.2	85.1 ± 0.2	1.85 ± 0.4
25	1.12	63.64	6.54	0.07	26.95	0.66 ± 0.3	92.8 ± 0.1	1.91 ± 0.3
50	1.12	64.84	6.33	0.04	27.96	0.72 ± 0.3	90.6 ± 0.4	1.54 ± 0.6
72	1.18	64.90	6.42	0.07	25.66	0.85 ± 0.4	82.4 ± 0.4	1.19 ± 0.4

*Type of phenolic structures determined by the UV method[13]

Nitrobenzene oxidation
Nitrobenzene oxidation of lignin samples (100 mg) was carried out using 2 M NaOH in 10-mL stainless steel vessels at a temperature of 180 °C for 2 h, and the oxidation products were analyzed by isocratic high-performance liquid chromatography under the following conditions: LiChrospher 100 RP-18, 5 μm, 4 × 100 mm ID column (Merck KGaA, Darmstadt, Germany), mobile phase water:methanol:acetic acid (850:150:1), flow rate of 1.0 mL min^{-1}, column temperature of 35 °C, and detection via a diode array detector in the 210-nm to 360-nm region. The quantities of nitrobenzene oxidation products were determined using the method of direct calibration.[12,14]

Measurements were performed in four replicates per sample. An S/G ratio was calculated using the formula:

S/G = (syringyl aldehyde + syringic acid) / (vanillin + vanillic acid)   (1)

H/G = (*p*-hydroxybezaldehyde + *p*-hydroxybenzoic acid) / (vanillin + vanillic acid)   (2)

RESULTS AND DISCUSSION

Many oxidizing agents cause almost complete destruction of the lignin molecule. In contrast, nitrobenzene under alkaline conditions has been shown to yield a considerable quantity of simple compounds in which the benzene nucleus and its substituent groups have survived the degradation. The analysis of lignin by nitrobenzene oxidation[15,16] generally focuses on the H/G/S ratio and the nature of the bonds using the destructive method,[7] which gives rise to patterns in empirically known degradation products.[8-18] It is worth reminding that the oxidation of softwood produces vanillin as the main product.[17] Hardwood forms vanillin and syringaldehyde, while grasses, besides these two aldehydes, produce *p*-hydroxybenzaldehyde. Tables 2-4 present the results of the nitrobenzene oxidation studies, which were used to determine the yields and monomeric composition of the phenolic compounds present in the precipitated lignin with sulphuric acid solutions.

Table 2 shows that all the lignin samples gave rise to vanillin ~18.0 μmolesg-1pure lignin and syringaldehyde~7.1 μmoles g-1pure lignin as the major products. Thus, the dominant phenolic compound was vanillin, followed by syringaldehyde. The formation of phenolic compounds, such as vanillin, is strictly related to the available percentage of its precursor in the lignin structure. The structural units derived from the guaiacyl units are the precursors for vanillin yield. It is known that vanillin is derived from ferulic acid (guaiacyl unit). The total yield of the oxidation products ranged from 34.1 to 36.3 μmoles g^{-1} pure lignin (Table 4).

Table 3 also presents the relative total moles of *p*-hydroxybezaldehyde, represented by H, hydroxyphenyl lignin units; the relative total moles of syringaldehyde and syringic acid represented by S, syringyl lignin units (Table 2); and the relative total moles of vanillic acid, vanillin, represented by G, guaiacyl lignin units (Table 2), occurring in the precipitated lignins determined by the nitrobenzene oxidation.

Based on the nitrobenzene oxidation results, the relatively low S/G ratio could mean that lignin fractions with more guaiacyl units were easier to extract than the lignin fractions with more syringyl units or that the guaiacyl units were less condensed or crosslinked than the syringyl units.[19] The presence of a lesser amount of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid is considered most likely to be indicative of non-condensed *p*-hydroxyphenyl units (Table 3). G:S:H ratios were also calculated for precipitated lignins (Table 4). The values of the G-units for lignin samples were in the range of 21.7 to 24.5 μmolesg^{-1} pure lignin. The values for S-units and H-units of precipitated lignin samples were in the range of 8.7 to 9.7 μmoles g^{-1} pure lignin and 2.6 to 3.1 μmoles g^{-1} pure lignin, respectively. For precipitated lignin samples, the ratio of G:S:H varies between 9:3:1 and 7:3:1, which falls in the same category as straw or grass type lignin.[20,21] The yield of nitrobenzene oxidation and the composition of phenolic products depends on the chemical treatment.[22] The difference in G:S:H content depends on the mode of precipitation and the formation of lignin aggregates, which are influenced by the acid ionic strength, hydrodynamic conditions and other factors. The concentration of H_2SO_4 thus influenced both the content of individual groups and their total content (Table 1). It was confirmed that the yield of nitrobenzene oxidation and the content of phenolic products depends on the isolation procedure.

Table 2
Yield of degradation products of the precipitated lignin samples by nitrobenzene oxidation

Concentration of sulphuric acid (wt%)	Vanillin		Vanillicacid		Syringaldehyde		Syringicacid	
	AVG	SD	AVG	SD	AVG	SD	AVG	SD
5	17.99	0.03	5.43	0.02	6.92	0.01	1.96	0.01
25	17.61	0.02	5.03	0.03	6.84	0.03	1.88	0.01
50	19.05	0.07	5.42	0.03	7.15	0.08	2.00	0.02
72	17.35	0.38	4.32	0.13	7.60	0.44	2.07	0.17

Table 3
Yield of degradation products of the precipitated lignin samples by nitrobenzene oxidation

Concentration of sulphuric acid (wt%)	*p*-hydroxybezaldehyde (μmoles g^{-1}pure lignin)		*p*-hydroxybenzoic acid	
	AVG	SD	AVG	SD
5	0.43	0.01	2.17	0.02
25	0.55	0.01	2.24	0.04
50	0.54	0.02	2.18	0.01
72	0.53	0.05	2.53	0.04

Table 4
Total yield and molar ratio of degradation products of the precipitated lignin samples by nitrobenzene oxidation

Concentration of sulphuric acid (wt%)	Total (μmoles g^{-1}pure lignin)	Molar ratio		G:S:H
		S/G	H/G	
5	34.90	0.38	0.11	9:3:1
25	34.13	0.38	0.12	8:3:1
50	36.34	0.37	0.11	9:3:1
72	34.40	0.45	0.14	7:3:1

CONCLUSION

Lignin is a very attractive and promising raw material to produce vanillin, biopolymers and biofuels. In this study, we isolated lignin from black liquor – a waste product of annual plants, hemp and flax – and precipitated it. According to the distinct acid precipitation behaviors, the fractionation of lignin with various concentrations of sulphuric acid (5, 20, 50 and 72 wt%) at pH = 5 was established in this study. Then the oxidation of lignin to produce vanillin with nitrobenzene was studied. The alkaline nitrobenzene oxidation of precipitated lignin from black liquor produced vanillin, vanillic acid, syringaldehyde, syringic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid in average yields of approximately 18, 5, 7.1, 2, 0.5 μmoles g^{-1} and 2.3 μmolesg^{-1} pure lignin, respectively. Thus, the dominant phenolic compound was vanillin, followed by syringaldehyde. It was confirmed that the yield of nitrobenzene oxidation and the content of phenolic products depends on the isolation procedure. For precipitated lignin samples, the ratio of G:S:H varies between 9:3:1 and 7:3:1.

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