Study of the Ability of Reducing Saccharides to Chemically Transform Lignin

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Article info

Abstract

The efficiency of chemical transformations of lignin obtained from *Picea excelsa* wood under the action of galactose, galacturonic acid and xylose (which can be obtained by enzymatic hydrolysis of hemicelluloses and pectin containing in plant material) was evaluated. The results were compared with use of traditional reducing agent which was borohydride sodium. Using the method of differential UV-spectroscopy was confirmed the increase of a number of phenolic hydroxyl units by Xyl, Gal and GA. The increase of lignin reactivity was controlled to sulfuric acid and to peroxide hydrogen. Similarly to $NaBH_4$, a nucleophilic addition mechanism for the reaction of the reducing saccharides with lignin was revealed. Reduction by $NaBH_4$, Xyl, GA and Gal increased the lignin reactivity to acid solubilisation and to peroxide oxidation.

1. Introduction

Lignocellulosic raw materials can be processed for the isolation of cellulose which has wide range of applications in many sectors, from the pulp and paper production [1, 2], to the textile sector [3] and the food industry [4]. However, independently of the final application, a common problem is the obtaining of a cellulose pulp with high whiteness due to residual presence of lignin. Generally, the lignin is the most difficult to remove component [5] due to its complex structure, being a polymer comprised of variously linked phenylpropane units which are also responsible for the lignin chromaticity [6]. The dominant linkage between the units is the β-O-4 linkage. The lignin macromolecule also contains a variety of functional groups that have an impact on its reactivity: mostly methoxyl groups, phenolic hydroxyl groups, and few terminal aldehyde groups. Only a small proportion of the phenolic hydroxyl groups is free since most of them are occupied in linkages to neighbouring phenylpropane units. Carbonyl and alcoholic hydroxyl groups are also present. Traditionally, the catalytic destruction of the chromophore lignin structures is carried out by oxidizers, such as chlorine-containing agents and hydrogen peroxide. The latter is more eco-friendly, but it has a lower technological efficiency. The use of enzyme-based technologies is an interesting research field since it involves the development of more natural and sustainable processes [7–8]. The search for new effects expanding the possibilities of application of bio-catalysed processes is of great importance also for bleaching of wood cellulose [9, 10].

The present research was carried out in the prospective of developing new methods based on enzymatic catalysis to achieve more sustainable and effective delignification technologies for pulp plant materials. The delignification method proposed by authors in this article is based on the use of reducing sugars as reagents for chemical transformations of lignin. These reducing sugars can be released during enzymatic degradation of polysaccharides in lignocellulosic raw materials. It was previously shown [11] that generated low molecular weight products in the acyclic form have reducing power. In the present work it was assumed that

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the reducing saccharides provide the redox-transformations because unsaturated C=C and C=O bonds present in the lignin structure.

On the bases of these premises, the objectives of this work was to experimentally confirm the ability of reducing sugars, which are products of lignocellulosic raw material hydrolysis, to reduce the lignin.

For this purpose it was necessary to:
• experimentally assess the reduction of auxochrome carbonyl groups in the lignin macromolecule under the action of reducing sugars;
• evaluate the influence of the reduction treatment on the lignin reactivity to sulfuric and hydrogen peroxide.

2. Experimental

All the used chemicals reagents and solvents were of analytical grade («Chemmed» Company, Moscow, Russia).

It is known [12] that lignin with a structure very close to native lignin can be extracted from fir-tree wood. This kind of wood is also characterised by considerable high content of lignin, around 28–30% [13]. Furthermore, lignin from coniferous wood has been the most investigated for its structure and properties [14]. Therefore, coniferous wood, in particular sawdust of fir-tree (*Picea excelsa*) obtained from chipping of a 50-year old fir-tree (*Picea excelsa*) grown up at the gently-continental climate in the Ivanovo region (Russia) was used in the present research.

Xylose (Xyl), galactose (Gal) and galacturonic acid (GA) were selected as potential reducing agents which might be obtained from hemicelluloses and pectin after enzymatic pre-treatment of a lignocellulosic material. Sodium borohydride (NaBH$_4$) was used as traditional reducing agent for reducing C=O bonds, since the lignin ability of reacting with it is well described in literature [13, 15].

The sawdust of fir-tree was washed with warm water (323–333 K) and oven dried at 378 K until constant weight. The dried sample was milled for 30 min at room temperature in a vibratory ball mill ML-1 (Shanghai Company «Zenith») in the presence of anhydrous toluene. The obtained wood flour (0.43–0.87 mm particle size) was further purified according to the standard method of Bergman for lignin isolation [14]. A mixture of alcohol and benzene (1:1, v/v) was used to purify the wood flour under reflux (in a Soxhlet extractor, solvent to solid ratio 20 mL : 1 g) for 10 h. The solid residue

was dried at room temperature to get the final purified wood flour.

Experimental plan is shown in Fig. 1. The purified wood flour was used for both isolation of pure lignin and its subsequent reduction (process A in Fig. 1) and direct reduction (process B in Fig. 1).

In process A, pure lignin was isolated according to the Bergman's method [14] in order to evaluate the total lignin content (L_{Tot}) and characterize its elemental composition [16] and functional structure (according to the standard methods [15]). For lignin isolation, 5 g of purified wood flour were extracted under reflux (in a Soxhlet extractor) with 100 mL of dioxane:water (9:1, v:v) for 8 h. The extract was evaporated to dryness under vacuum at 50 °C, dissolved in 100 mL of 90% acetic acid and precipitated into 100 mL of hot 1% sodium sulphate. The obtained precipitate was separated by centrifugation (1660 RCF, 8 min, 50 °C), dissolved in 100 mL of dioxane and precipitated in absolute ethyl either (these steps were repeated three times). The final precipitate was oven dried at 50 °C until constant weight. The lignin yield was considered as the content of lignin in flour (L_{Tot}), and analysed as mentioned above.

The obtained pure lignin was reduced by different reducing agents (Xyl, Gal, GA or NaBH$_4$) and the change of content of phenolic hydroxyl groups was monitored over reaction time. The reduced lignin (L_R) was then isolated (according to the same above procedure followed for pure lignin) and characterized for its functional structure to evaluate the modifications occurred during reduction.

In process B of Fig. 1, purified wood flour was directly reduced by the same different reducing agents tested on pure lignin in order to assess the influence of such a treatment on reaction ability of lignin to sulphuric acid and peroxide oxidation. The reduced flour (F_R) was washed and oven dried at 50 °C until constant weight and then exposed to the sulphuric acid treatment or to the delignification peroxide oxidation treatment. For the sulphuric acid treatment [17], 1 g of reduced flour was mixed with 15 mL of 72% sulphuric acid. The mixture was stirred for 2.5 h at room temperature and then brought to 200 mL with hot distilled water. The sample was kept in thermostatic bath at 95 °C for 1 h, and the not solubilised solid residue (Klason lignin in reduced flour, KL-F$_R$) was separated by filtration using a qualitative filter paper of medium filtering capacity. The residue was washed by hot water to pH 6, oven dried at 105 °C until constant weight. For the oxidation
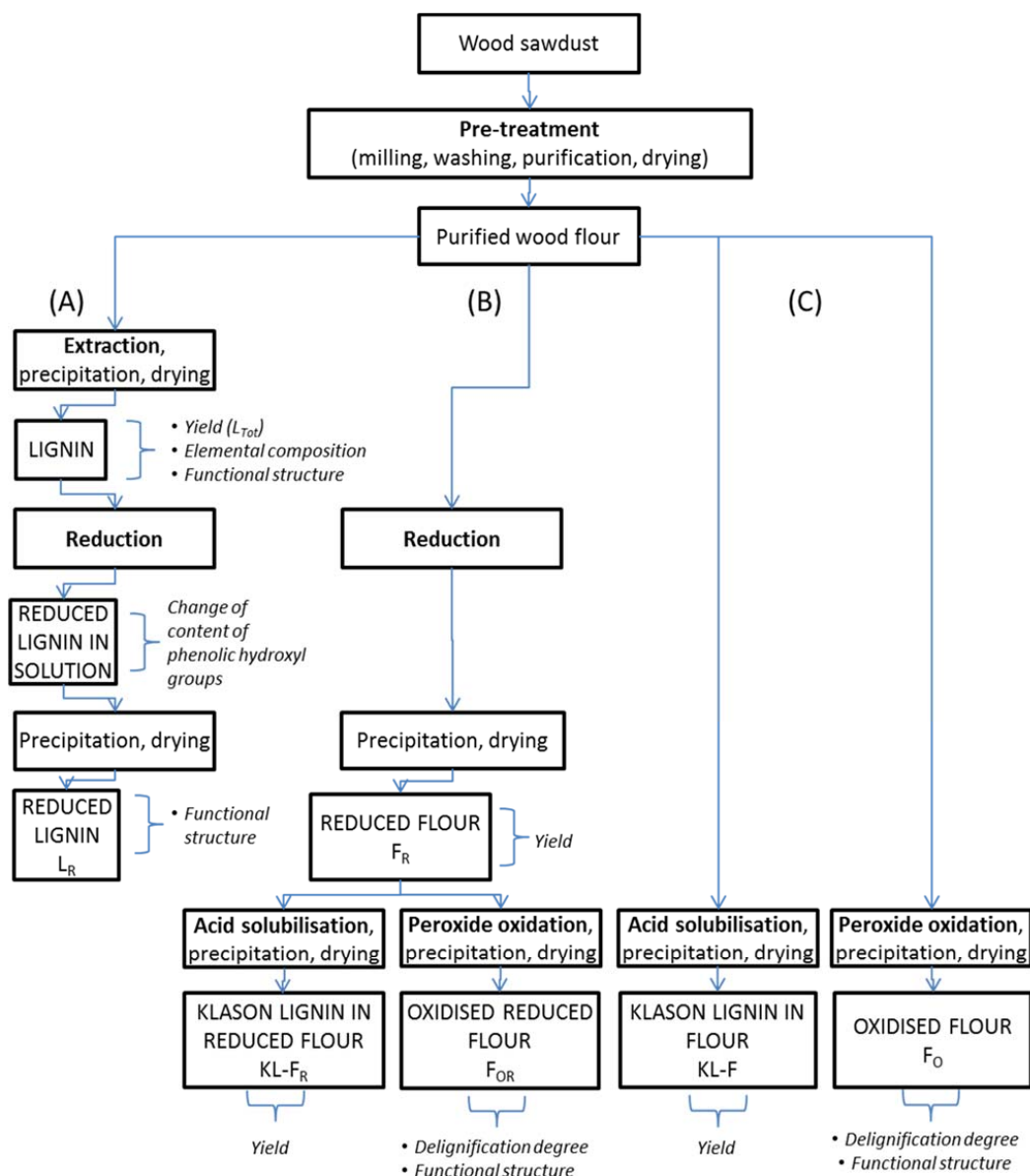
Fig. 1. Experimental plan. (a) Extraction and reduction of pure lignin. (b) Reduction of lignin in flour and next acid solubilisation or oxidation. (c) Direct acid solubilisation or oxidation of wood flour.

treatment, reduced flours were treated [18] at 70 °C for 120 min, with a peroxide solution (final H_2O_2 concentration 1.4 g/L), solvent to solid ratio 6:1 (mL/g). The obtained oxidised reduced flours (F_{OR}) were isolated after cooling by precipitation in absolute ethyl ether, recovered by centrifugation at 1660 RCF, 8 min, 50 °C, oven dried at 50 °C until constant weight and analysed for the total lignin content by spectrophotometric method (in order to evaluate the delignification degree) and for the functional structure (to be compared with the functional structure of reduced lignins).

In process C of Fig. 1, purified wood flour was directly subjected to acid solubilisation or peroxide oxidation as reported for reduced flour in process B.

Reduction trials were carried out with potentiometric equipment including a pH-meter model OP-211/1 with electrodes from platinum and silver chloride and a temperature control system (thermostat (Typ TB25, VEB MLW Prüfgeräte-Werk Medingen). Preliminary trials were carried out to compare the reduction potential of the different tested agents (in terms of oxidation-reduction potential, ORP) and to select the best working conditions for the lignin reduction. Standard 0.56 M solutions of each reducing agent (Xyl, Gal, GA and $NaBH_4$) were prepared at two different pH: 6.5 (using bidistilled water as solvent) and 11 (using NaOH 0.1 N). The solutions were carefully mixed and kept in flasks at a constant temperature of 80 °C

(LTHS 250 device) and under stirring for 60 min. Both aerobic and anaerobic conditions were tested. In the latter case, nitrogen gas was made pass into the mixture through a glass tube. At the end of the experiment, the solutions were left stand for 5 min before measuring ORP. This allowed not distorting experiment results because reaction of the reducing agent with oxygen in the system was avoided [11]. Anaerobic and basic conditions could be selected from these preliminary trials based on ORP, as commented in the results section.

For reduction trials in process A (Fig. 1), 0.002 g of lignin were dissolved in 200 mL of a 9:1 (v:v) mixture of dioxane and aqueous solution of reducing agent (final concentration 0.56 M) at pH 11. The solutions were carefully mixed and kept under nitrogen for 60 min at 80 °C. Reaction rate was monitored at 0, 15, 30, 45 and 60 min, taking out 10 mL of mixture and measuring the ORP.

For reduction trials in process B (Fig. 1), 0.02 g of wood flour was dissolved in 200 mL of aqueous solution of 0.56 M reducing agent at pH 11. The same procedure as for reduction in process A was then followed.

Elemental analysis was carried out according to literature method [16]. Briefly, the carbon and hydrogen content in the lignin preparation obtained after extraction from wood flour was determined by incineration over a catalyst in a quartz tube with the trapping of carbon dioxide and water and gravimetrical determination of their contents. The content of these compounds was subtracted from the initial weight of lignin and the content of oxygen was derived.

Change of content of phenolic hydroxyl groups in lignin structure after reduction was assessed by differential UV-spectroscopy method [15]. This method is based on the wavelength shift between ionized and protonated phenolic hydroxyl groups and, then on the difference in absorption at 250, 300 and 350 nm between phenolic units in slightly acidic (pH 6) and alkaline solutions (pH 12). For each reduced lignin solution, two solutions were prepared. One solution was prepared by mixing 2 mL with 48 mL phosphate buffer at pH 6. The second solution was prepared by mixing 2 mL with 48 mL sodium hydroxide buffer at pH 12. Absorbance measurements were carried out on a Cary 100 Scan spectrophotometer and the difference of absorbance intensity (ΔAbs) at the two pHs was calculated for each wavelength.

Functional structure was evaluated according to the standard TAPPI methods [15]: the total ox-idised groups by the barium and chloride method; total carboxyl groups by the chemisorption calcium-pectate method; total carbonyl groups by the oximation method, while the total phenolic hydroxyl groups were calculated as difference between the contents of the total oxidised and carboxyl groups.

Delignification degree (%) after peroxide treatment was calculated as the percent difference between lignin content before (L_{Tot}) and after the treatment. The lignin content of the oxidised samples was evaluated according to a spectrophotometric method [19]. Briefly, the sample was mixed with dioxane (at a 500:1 mL:g ratio) to solubilise the residual lignin; then the mixture was vigorously stirred for 2 h at 75 °C. After being cooled to room temperature, the dioxane-lignin solution was separated by centrifugation (1660 RCF, 5 min) and diluted twice with distilled water. The final solution was read at 280 nm against a blank sample of dioxane:distilled water, 1:1 (v/v). The optical density (D) at 280 nm was used for determination of lignin concentration (C_l, wt.%) based on a calibration curve made with the pure lignin isolated in process A and treated in the same way. The mass fraction of lignin was calculated taking into account the solution volume, the found lignin concentration and the mass sample.

The results reported in this paper are the average of the replicates ± SD. The statistical software SPSS (version 23, SPSS Inc., Chicago, IL, USA) was used to assess the influence of the reducing agent or of reduction conditions on the functional groups percentage, using univariate analysis of variance (ANOVA) at a confidence level of over 99%. When significance was found, a post-hoc test was carried out for mean discrimination. Variance homogeneity was always verified and then, Tukey's post-hoc test was applied.

2. Results and discussion

Arabinose, glucose, galactose, mannose, xylose, galacturonic acid and other saccharides are formed when the polysaccharides in cellulosic material is subjected to enzyme hydrolysis. In this work, xylose and galactose (typical hemicelluloses monosaccharides) and galacturonic acid (a sugar acid, pectin component) were investigated. Reducing properties of monosaccharide solutions are linked to the presence, in the acyclic form of their molecule, of aldehyde functional groups and to their ability to oxidise to the corresponding aldonic acid

and aldaric acid, as shown in scheme (in the latter not only the aldehyde group but both ends of the aldose linear forms are oxidized).

Reducing power of monosaccharide and acid monosaccharide solutions depends on features of the chemical and steric-isomeric structure of the molecule in the cyclic form. These features influence the nature of intermolecular interactions in the pyranosic cycles and the ability of their transition to the free aldehydic form [11].

Table 1 shows the results of ORP measurements for the different analysed reducing agents.

For all the tested compounds, the reducing power is significantly enhanced by both alkaline conditions and anaerobic environment, because alkalinity promotes the course of retroaldonic decay reactions of the monosaccharides, with a break of C-C bond and formation of a mixture of reducing compounds with shorter carbon chains.
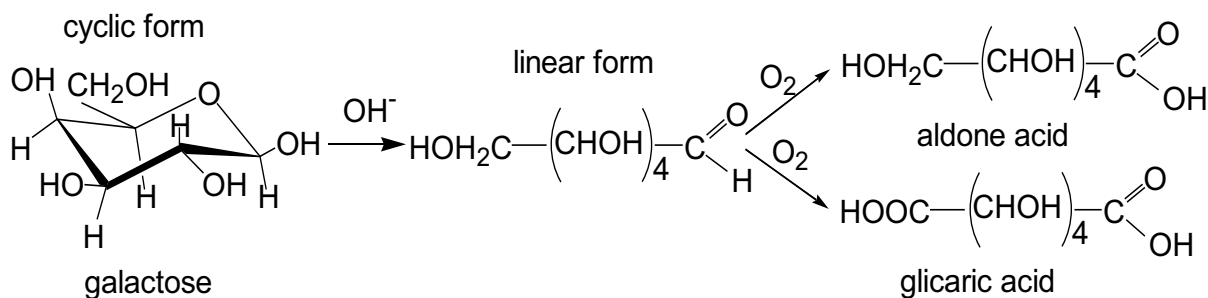
Table 1
Redox potential of different reducing agents, at different conditions, after 60 min at 80 °C

Reducing agents	ORP, mV			
	aerobic condition		anaerobic condition	
	pH 6	pH 11	pH 6	pH 11
Galactose	-102.17	-580.83	-132.00	-825.33
Galacturonic Acid	-201.83	-586.17	-245.33	-846.17
Xylose	-255.33	-619.50	-318.83	-905.67
NaBH$_4$	-	-761.50	-	-1080.67

The presence of oxygen reduces the reducing power since part of the reagent reacts with oxygen [20]. Interestingly, under anaerobic conditions and at pH 11, the reducing properties of Gal, GA and Xyl solutions were comparable (even though statistically lower) to the reference reducing agent sodium borohydride. The most powerful monosaccharide was Xyl, followed by GA and Gal. Alkaline and anaerobic conditions were then selected for the lignin reduction trials.

(R=H; OCH$_3$)

I   II   III

Table 2

Functional structure of pure lignin isolated from purified wood flour of *Picea excelsea*; pure lignin reduced by different agents (Xylose; Galactose; Galacturonic acid); oxidised wood flour and oxidized reduced flour

Lignin type	Functional groups*, wt.%		
	–C=O	–OH$_{ph}$	–COOH
Pure isolated lignin	4.15^a ± 0.03	3.17^e ± 0.02	0.41^f ± 0.00
Pure isolated lignin reduced by			
NaBH$_4$	0.46^e ± 0.02	5.40^a ± 0.04	0.42^f ± 0.02
Xylose	1.27^d ± 0.03	4.91^b ± 0.05	0.42^f ± 0.03
Galacturonic acid	1.71^c ± 0.01	4.65^c ± 0.04	0.42^f ± 0.01
Galactose	2.03^b ± 0.02	4.45^d ± 0.02	0.42^f ± 0.02
Oxidised wood flour	4.13^a ± 0.03	0.44^e ± 0.03	14.76^e ± 0.12
Oxidised wood flour from flour reduced by			
NaBH$_4$	0.45^e ± 0.02	0.44^e ± 0.03	26.70^a ± 0.21
Xylose	1.25^d ± 0.01	0.45^e ± 0.01	24.04^b ± 0.19
Galacturonic acid	1.70^c ± 0.01	0.47^e ± 0.02	22.52^c ± 0.18
Galactose	2.01^b ± 0.02	0.46^e ± 0.01	21.55^d ± 0.17
* Values are reported as mean ± s.d., according to ANOVA and Tukey's post-hoc test.			

Fig. 2. Difference of absorbance intensity at pH 6 and pH 12 (ΔAbs) of lignin reduced with NaBH$_4$ (a), Galactose (b), Galacturonic acid (c) and Xylose (d) at different processing times.

In process A (Fig. 1), pure lignin was isolated with a 15.32 ± 0.5 wt.% yield which was, then, considered as the total lignin content of purified floor. Elemental analysis showed a content of 62.72, 6.24 and 31.04 wt.% for C, H and O, respectively. These values are in agreement with other lignin compositions found in the literature [16], confirming also the high purification level of the obtained fraction in terms of removal of cellulose, hemicellulose and pectin. Functional structure characterization is reported in Table 2. Changes in the structure of lignin macromolecule under the action of reducers are caused by transformations of non saturated C=C and C=O bonds in the propane chains. The content of C=O bonds in the pure untreated lignin is rather big (4.15%) because more than 20% of the phenylpropane units in the lignin present the carbonyl group [15]. Furthermore, in one third of them, the keto-group is in α-position giving the auxochrome effect.

Change in the auxochrome functions, such as the content of α-o-4 linkages and of free phenolic hydroxyl groups, can be experimentally identified by differential UV-spectroscopy method, as reported in section 2. The results of difference in absorbance for pure lignin reduction trials (process A of Fig. 1) are reported in Fig. 2. The maximum absorbance at 350 corresponds to absorption by structural phenolic fragments in the form (I) containing the auxochrome carbonyl group. Peaks at 300 and 250 nm reflect the presence of links in the form of coniferyl (II) and n-coumaryl (III) alcohols.

The transformation of the lignin absorption spectrum after reduction by sodium borohydride is presented in Fig. 2a. It can be seen that at increasing reduction time, intensity of the absorption by carbonyl-containing phenylpropanic units (at 350 nm) is reduced up to 5.1 times after 60 min. This is in agreement with functional structure analysis which revealed a 9 times reduction in the content of carbonyl groups (Table 2).

On the opposite, increase in intensity of absorption at 300 and 250 nm is observed during reduction, which indicates an increase in the content of free phenolic hydroxyl groups. All the registered curves intersect at a point between 320 and 340 nm, confirming the reduction of the auxochrome carbonyl groups and the increase in the total number of the ionized guaiacylpropanic lignin fragments without destruction of the chromophore molecule nucleus.

According to literary data [15] the reaction at the base of lignin reduction by sodium borohydride is a typical nucleophilic addition:

Results of the reduction treatment in the presence of Gal, GA and Xyl, are presented in Fig. 2b-d. The spectral curves reveal lignin modifications were the same as those observed with NaBH$_4$ according to the nucleophilic addition mechanism, since they presented the intersection point at 330 nm, increase in absorption at 250 nm and at 300 nm, and a decrease in absorption at 350 nm.

The degree of substratum transformation in a reduced form will obviously depend on the reducing power of the system that is its ORP. In fact, the results showed that the decrease of the maximum absorption at 350 nm increased with increasing reducing properties of the used solutions in the following order Gal < GA < Xyl: the highest decrease (2.85 times) was observed for Xyl.

It is possible to assume that the carbonyl groups in the phenylpropane units with free phenolic

hydroxyl groups (whose presence is registered by the differential UV-spectroscopy method) are not the only ones exposed to such transformations. Also the C=O groups in median structural fragments of lignin macromolecules with the phenolic hydroxyl involved in covalent bonds, and also the carbonyl groups present in *β*-position of propane links will likely be reduced. The increase in the content of free phenolic hydroxyl groups may be connected with destabilisation and destruction of simple ether bond near a reducing carbonyl between structural lignin units.
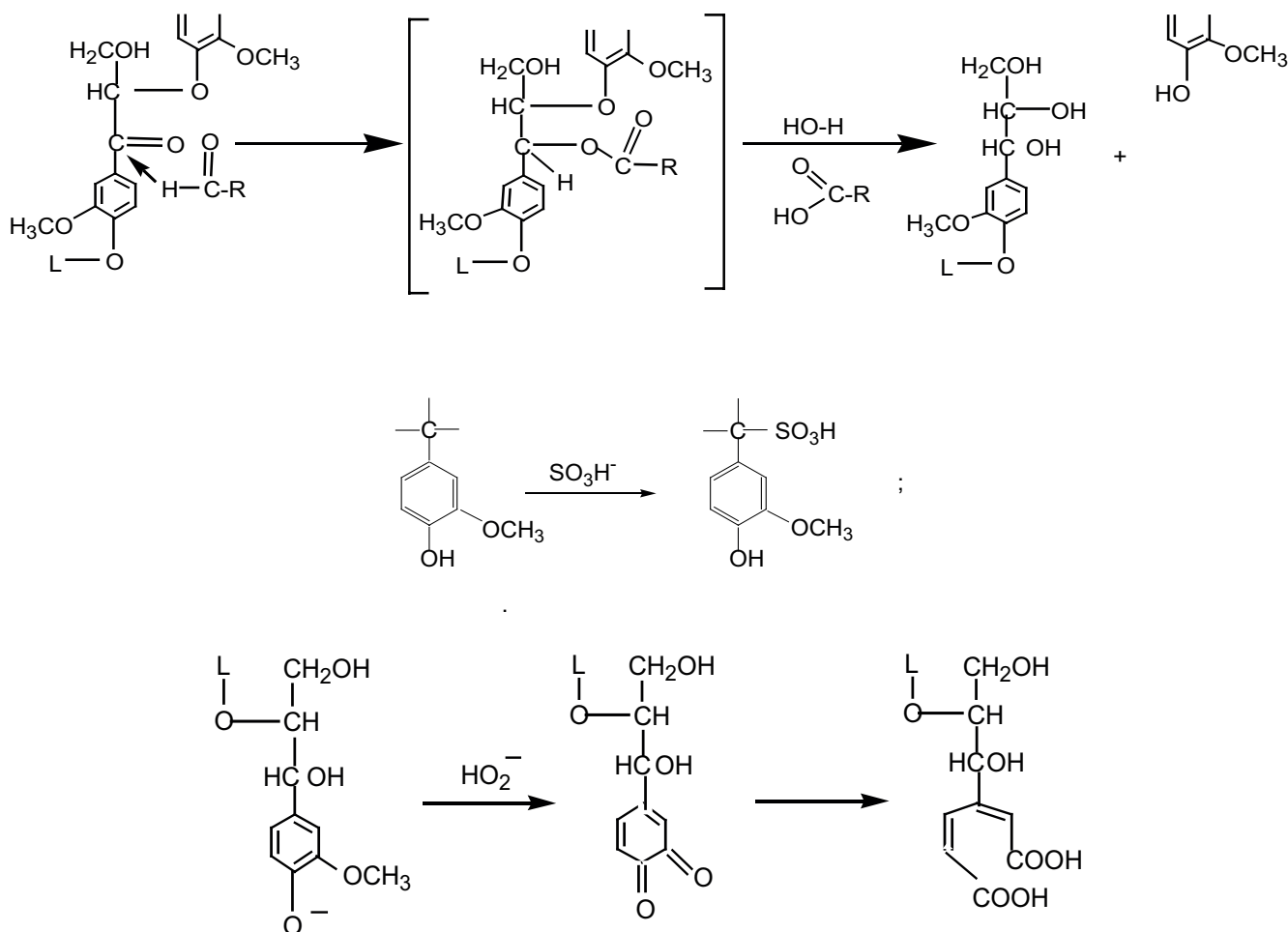
It is known that sulphating and peroxide oxidation reactions for lignin proceed only in the units which are in a free phenolic form.

Based on this, the change in the content of free phenolic forms in the phenylpropanic units of lignin in wood flour after reduction should modify the substratum solubilisation in sulphuric acid solution or the substratum delignification in alkaline peroxide solution.

Acid insoluble lignin content (Klason lignin) in untreated wood flour (process C in Fig. 1) was 28.7 ± 0.5 wt.%, while in flour reduced by NaBH$_4$, Gal, GA and Xyl was 14.6, 18.4, 17.9 and 16.7 wt.%, respectively. This indicates that the reduction treatment increased the lignin reactivity towards acid solubilisation (Fig. 3). Functional structure analysis (Table 2) showed that the action of sodi-

um borohydride led to almost complete reduction (89%) of the carbonyl groups, while Xyl, GA and Gal led to 69.4, 58.8 and 51% (Fig. 3). The illustrated dates have variances about the mean. The error bars is not exceed 5%.

Analysis of lignin content in the oxidised samples revealed a 88% delignification degree for the flour previously reduced by NaBH$_4$ compared to the 58% for the untreated wood flour, which again reveals the increase in lignin reactivity due the reduction pre-treatment (Fig. 3). Delignification degree in case of reduction pre-treatment with Gal, GA and Xyl was 60.9, 65.2 and 73.1%, respectively. The obtained results incontestably prove the increase of phenylpropanic bonds in free phenolic forms after the reduction treatment, leading to a higher reactivity of the polymer.
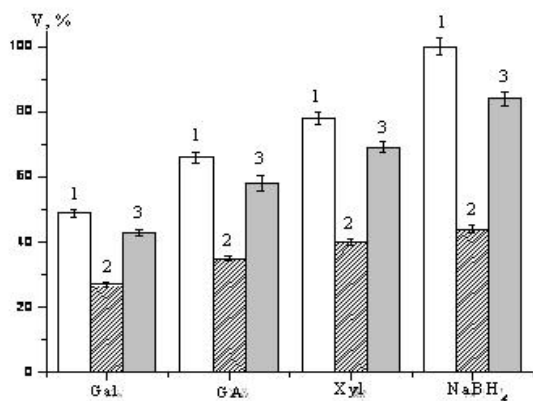
Fig. 3. Completeness (V) reduction (1) of lignin under action of reducing systems and influence of reduction treatment to increase solubility of lignin in sulfuric acid (2) and extraction its from wood flour by hydrogen peroxide (3).

Table 2 reports the change in the content of functional groups in the pure lignin and after treatment by different reducing agents and after oxidation. Content of the carboxyl groups was not influenced by the reduction treatment, while it was obviously greatly influenced by the peroxide treatment. However, if a reduction pre-treatment is carried out, the increase in the wt.% of carboxyl groups is enhanced after oxidize wood and reached 26.70% for the NaBH$_4$ agent, compared to the 14.76% of oxidised wood flour without reduction pre-treatment. The increase in the content of oxidized groups after the reduction treatment is caused by the increase in the content of free phenolic hydroxyl functions (which contribute the 88% of the total oxidised groups in the original lignin, and the 91–93% in the reduced lignins). As observed for the variation in carbonyl groups and in UV-spectra, the percent increase in the oxidised groups content was related with reducing power of the solution, being a 81, 63, 53 and 46% for NaBH$_4$, Xyl, GA and Gal, respectively (Fig. 3).

The received results incontestably proved the appearance of the additional quantity of the phenylpropanic units in the free phenolic form. At that the enhance reaction ability of the modified polymer are symbate to increase of the speed redox-transformations on the preliminary stage of the reducing treatment.

3. Conclusions

This research suggests that an enzymatic pre-treatment of a lignocellulosic material could enhance the reactivity of lignin to acid solubilisation or to peroxide oxidation. It was, in fact, demonstrated that the reducing sugars increase the number of phenylpropane units of lignin in the free phenolic form and that the reaction occur according to the same nucleophilic addition mechanism found for lignin reduction by sodium borohydride. The reduction of lignin by reducing sugars lead to a higher delignification degree. Further research is needed to evaluate the effect of mixed sugars which can be generated in real systems; to optimise the process and to assess its real environmental and economic feasibility.

Acknowledgments

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