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A COMPARISON OF AUTOHYDROLYSIS AND PARTIAL ACID HYDROLYSIS AS A PRETREATMENT OF BEECH SAWDUST FOR THE BIOCONVERSION

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Autohydrolysis and partial acid hydrolysis of beech (*Fagus silvatica*) sawdust were carried out at 185 and 200°C. Rates of formation of pentoses and lignin follow kinetic equation for first order reaction involving all hydrolysis of pentosane, decomposition of monosaccharides and degradation of native lignin. Total sugar yield from the pretreatment and enzymatic hydrolysis beech sawdust was 65.6% after pretreatment with 0.09% hydrochloric acid, and 60.5% after autohydrolysis at 200°C.

Autohydrolysis of wood and agricultural waste as a pretreatment of lignocellulose materials for enzymatic hydrolysis makes it possible to separate these materials into hemicellulose, lignin and cellulose [1, 3, 15, 21]. They may be further processed into useful products such as fuels, chemicals and single-cell protein [22]. The lignin fraction being a residue after autohydrolysis may be utilized for the manufacture of a thermoplastic polymer substitute and as petrochemical phenol replacement in adhesives. Recently, more attention has been paid to the fermentation of pentose sugars originating from hemicellulose [4, 14, 19]. It is essential that not only cellulose, but also lignin and hemicellulose constitute about 50 percent of the plant material and are convertible into useful chemicals (acetone, butanol, 2,3-butanediol, ethanol).

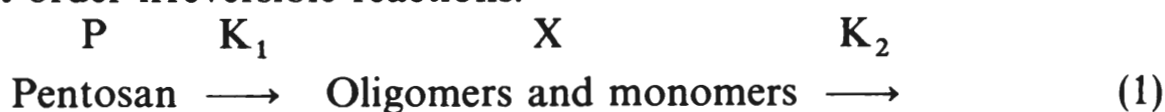
The optimum steaming time for autohydrolysis of typical hardwoods, is not optimal for both lignin and pentose production because the hemicelluloses depolymerize more rapidly, whereas the heating time for lignin degradation should be six times longer [16]. When conditions are optimal for pentose production too much lignin remains on the cellulosic fibers, and the enzymic hydrolysis extent is not too high. At the other extreme, some of the pentose and hexose degrade to 2-fural aldehyde and 5-(hydroxymethyl)-2-fural aldehyde. These by-products react with low molecular weight lignin and they are liable to decrease the susceptibility of cellulose to enzymatic hydrolysis.

Dilute acid hydrolysis has been found by Knappert et al. [7, 8] to be an attractive method of treatment of lignocellulosic materials prior to enzymatic hydrolysis. After the pretreatment of corn stover, oak, poplar followed by enzymatic hydrolysis, glucose yields as high as 100% have been obtained.

The purpose of this study was to compare the autohydrolysis and the partial acid hydrolysis of beech wood as a pretreatment for bioconversion. It was interesting to determine the kinetic rate constants for hydrolysis of pentosan and degradation of lignin. An understanding of the kinetics of wood fractionation is required in order to model reactions of the separation of wood into basic chemical components.

KINETIC MODEL

The kinetic model of xylan removal from various hardwoods during acid and water hydrolysis is a combination of two parallel pseudo-first order reactions (one fast and one slow) [27]. But the beech wood contains only a more reactive hemicellulose fraction [9]. Thus, to make it easier, this kind of wood has been chosen as a model substrate. Pentosan beech wood hydrolysis is characterized by a series of first order irreversible reactions.



The mass balance for this reactions is given in equations

$$\frac{dC_p}{dt} = -K_1 C_p \quad (2)$$

$$\frac{dC_x}{dt} = K_1 C_p - K_2 C_x \quad (3)$$

Integration of equations gives

$$C_p = C_{pi} e^{-K_1 t} \quad (4)$$

$$C_x = \frac{K_1 C_{pi}}{K_2 - K_1} (e^{-K_1 t} - e^{-K_2 t}) + C_{xi} e^{-K_2 t} \quad (5)$$

C_p concentration of pentose in insoluble polymeric form (%)

C_x concentration of oligomers and monomers pentose in solution (%)

C_L concentration of native lignin (%)

t time

K_1, K_2, K_3 the pseudo-first rate constants (min^{-1})

C_{pi} concentration of pentose in insoluble polymeric form at time zero (%)

C_{xi} concentration of oligomers and monomer pentose in solution at time zero (%)

The pseudo-first order rate constants K_1 and K_2 were determined from equation 4 and 5, using the simplex method of Nelder and Mead [17].

The pseudo-first order reactions can be assumed to describe the degradation of native lignin to soluble (in water and 1% aq. NaOH) lignin fragments.



Native lignin \rightarrow Soluble lignin (in water and 1% NaOH solution).

For this pseudo-first order reactions

$$\frac{dC_L}{dt} = -K_L C_L \quad (7)$$

The first order rate constant K_L was determined as above (equation 4).

MATERIALS AND METHODS

MATERIAL

Beech sawdust (*Fagus silvatica*) was obtained from a wood yard. The sawdust was air dried and sifted through 0.43-1.5 mm sieves. Chemical analysis of the beech sawdust showed them to consist of $45 \pm 1.5\%$ cellulose, $21.4 \pm 1.1\%$ Klason lignin, $21.8 \pm 1.3\%$ pentosan (see the methods). Cellulase used derived from *Trichoderma reesei* QM 9414 which was obtained by the method described by Mandels et al. [11]. All other chemicals used were of standard grade.

PRETREATMENT

A 250 cm³ stainless steel pressure reactor was used to treat the beech sawdust. The reactor was loaded by adding 20 g of beech sawdust and 75 cm³ of distilled water. A 50 cm³ flask with stopper containing 25 cm³ distilled water or 0.18% or 0.36% hydrochloric acid was introduced into the reactor. The reactor was immersed in an oil bath at a temperature of 220°C. After the desired hydrolysis temperature was achieved in the reactor, it was transferred to a second oil bath maintained at 2°C above the desired temperature. Meanwhile the content of the flask was poured out and mixed with the beech slurry. This eliminated the reaction of the hydrochloric acid with the beech heat up. The time required to bring the reactor's content from room to experimental temperature was 6 min to reach 185°C \pm 1°C and 9 min to reach 200°C \pm 1°C. Reaction times from 0 to 40 min were investigated. After the desired reaction time, the reactor was completely vented. The contents of the reactor were removed and the solids separated from the liquid (I) by filtration and washed with water. The solids were treated with 50 cm³ of 1% aq NaOH for 1 hour and they were separated from the liquid (II) by filtration. The solid residues were neutralized with diluted phosphoric acid, washed with water, weighed and hydrolysed enzymatically.

ENZYMATIC HYDROLYSIS OF THE SOLID RESIDUES

About 0.25 g of the solid residue, calculated as dry mass, was added to 10 cm³ cellulase solution (40 or 80 FPU/g of solids) from *Trichoderma reesei* QM 9414. The hydrolysis was carried out at 45°C in stationary conditions, pH of the

mixture was 4.8. The amount of sugar produced by enzymatic hydrolysis was determined by reaction with 3, 5 dinitrosalicylic acid reagent [13]. The solids were analyzed for their potential sugar content (see the methods) and % sacharification was determined by the following equation:

$$\text{Sacharification (\%)} = \frac{\text{mg reducing sugars} \times 0.9}{\text{mg polisaccharides}} \times 100$$

ANALYTICAL METHODS

The cellulolytic activity was expressed in FPU units by the method described by Mandels et al. [12].

S u g a r a n a l y s i s. After pretreatment, the filtrates were heated in a boiling water bath with 1 N hydrochloric acid during 30 min, neutralized with NaOH to a pH 5.5 to 6.0. The contents of pentose were determined by the orcinol method [6]. The reducing sugars were assayed by reaction with 3.5 dinitrosalicylic acid using the method described by Miller [13]. The cellulose content was determined by the method described by Kürchner and Hanak [10]. The content of pentosan was determined by the orcinol method [6] and that of lignin by the method of Effland [5].

RESULTS AND DISCUSSION

Table 1 presents the kinetic rate constants for the water and partial acid hydrolysis of pentosan (K_1 and K_2) and that lignin (K_L) of beech sawdust at 185°C and 200°C. An increase of pentosan hydrolysis temperature from 185°C to 200°C made it possible to obtain a more favourable ratio of rate constant K_1 for sugar production to the rate constant K_2 for sugar degradation. For the hydrolysis of pentosan in 0.09% hydrochloric acid at 185°C the ratio of K_1 to K_2 was 2.9 higher than in the case of autohydrolysis at the same temperature. Thus

Table 1. Reaction rate constants for pentosan and lignin removal from beech wood

Pretreatment	Temp. °C	Acid % w/v	K_1 min ⁻¹	K_2 min ⁻¹	K_{La} min ⁻¹	K_1/K_2	K_1/K_L	K_L/K_2
Autohydrolysis	185	H ₂ O	0.051	0.027	0.022	1.9	2.3	0.81
Acid	185	0.045	0.127	0.032	0.048	4.0	2.6	1.5
Acid	185	0.09	0.244	0.045	0.078	5.5	3.1	1.7
Autohydrolysis	200	H ₂ O	0.108	0.047	0.044	2.3	2.55	0.94
Acid	200	0.045	0.32	0.060	0.13	5.4	2.5	2.2

an increase in temperature and a decrease in pH hydrolysis favoured a lower degradation of sugars and an increase in the yield of pentoses (Fig. 1-2). The same factors caused an increase in the rate constant K_1 for degradation of native lignin, but a decrease in pH hydrolysis limited applicability of the equation 6. This

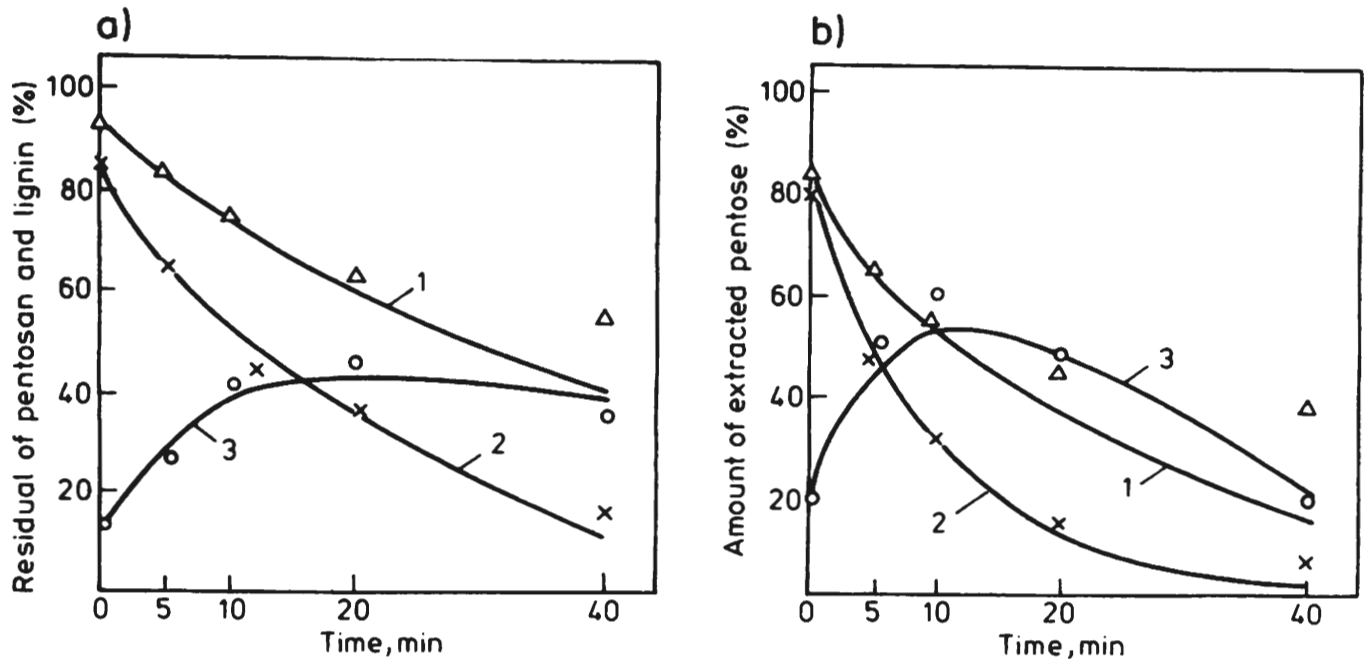


Fig. 1. Residual amounts of pentosan (2), lignin (1) and extracted pentose (3) as a function of autohydrolysis time of beech sawdust at 185°C (a) and 200°C (b). Symbols represent actual data points; solid lines represent predictions of kinetic model described in equation (1) and (6)

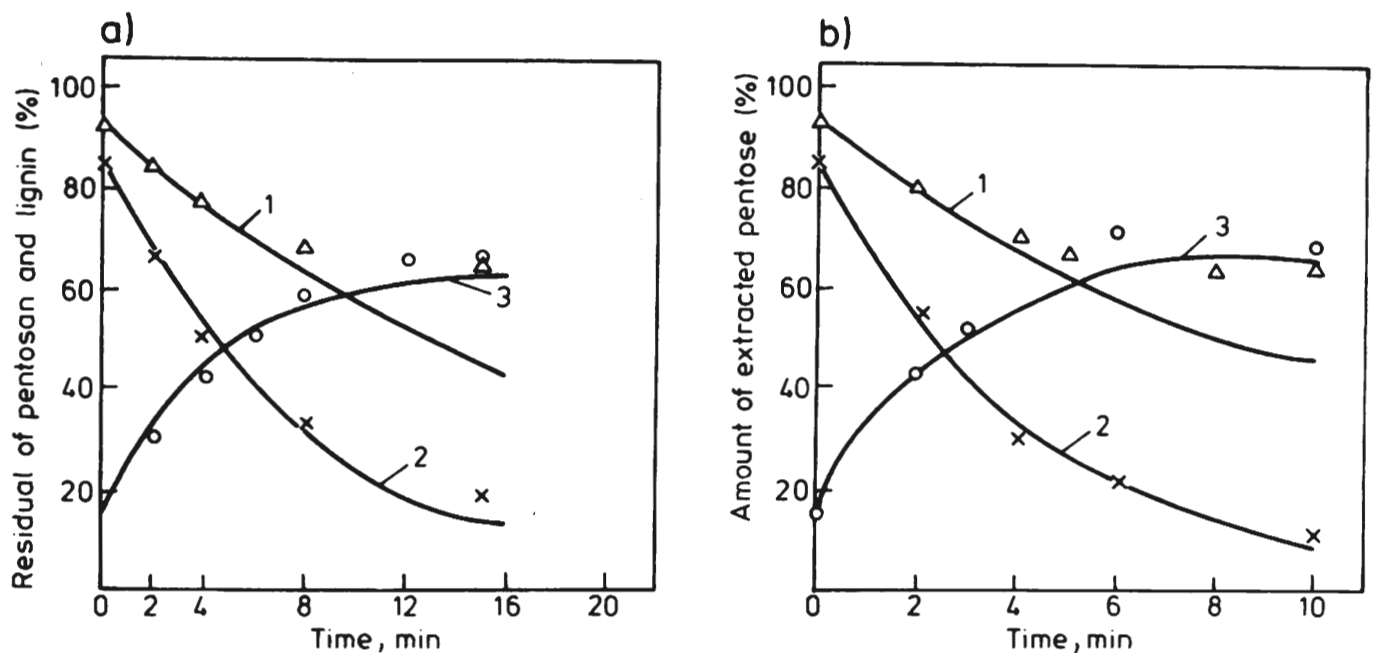


Fig. 2. Residual amounts of pentosan (2), lignin (1) and extracted pentose (3) as a function of time of partial acid hydrolysis of beech sawdust at 185°C using 0.045% (a), and 0.09% (b) hydrochloric acid. Symbols represent actual data points; solid lines represent predictions of kinetic model described in equation (1) and (6)

equation described correctly the degradation of native lignin to the fractions soluble in water and dilute aq. NaOH within 0-55% loss of lignin (Fig. 1) for autohydrolysis at 200°C and only 0-30% lignin loss for treatment in 0.09% hydrochloric acid at 185°C. This limitation resulted from an increase in the intensity of condensation of lignin fragments in relation to the degradation rate of native lignin and formation of non reactive lignin, insoluble in dilute sodium hydroxide solutions.

Pretreatment of beech sawdust with hydrochloric acid solutions made it possible to obtain a more favourable ratio K_L to K_2 (Tab. 1), as compared with

Table 2. Effect of autohydrolysis and acid hydrolysis of beech sawdust on yields, chemical composition and enzymatic conversion of the residual solids

Pretreatment	Temp. °C	Acid % w/v	Time min.	Yields %	Chemical composition (%)			Degree of saccharification (%)			Amount of extracted lignin with 1% NaOH %
					Klason lignin	cellulose	pentosan	1 hr	24 hr	48 hr	
Unpretreated	—	—	—	100	22.8	45.0	22.3	—	—	—	0
Autohydrolysis	200	H ₂ O	10	59.2	27.1	68.2	3.9	9.4	41.2	57.8	7.6
			20	55.7	22.3	72.4	2.0	21.2	83.2	95.8	10.8
Acid	200	0.09	2.5	56.0	27.0	69.0	2.1	9.0	44.8	62.3	7.8
			5	52.6	27.3	70.4	trace	18.6	78.1	95.4	7.9

autohydrolysis, and consequently produced a decrease in the amount of pentose degradation products at the same degradation degree of native lignin.

Yield, chemical composition and susceptibility to enzymatic hydrolysis of lignocellulosic residues obtained after 10 and 20 min. autohydrolysis and after 2.5 and 5.0 min. hydrolysis in 0.09% hydrochloric acid at 200°C are presented in Table 2. Table 3 presents the output of sugars obtained as a result of prehydrolysis of beech sawdust and of the enzymatic hydrolysis of lignocellulosic residues. The high degree of beech sawdust pentosan hydrolyzation after 10 min. autohydrolysis and 2.5 min. acid hydrolysis indicated that there is no need to extend treatment from the point of view of pentose production. However, under such conditions, delignification of beech sawdust was insufficient to obtain a complete saccharification of cellulose (Tab. 2). A high degree of cellulose saccharification was obtained extending the time of treatment twice, which in turn caused a considerable degradation of pentose, especially in the process of autohydrolysis. After treating the beech sawdust with 0.09% hydrochloric acid for 5 min. at 200°C, 7.9% lignin fractions were extracted with 1% aq. NaOH (in relation to beech sawdust treated), whereas after 20 min. autohydrolysis 10.8% of lignins were extracted. In spite of this, the susceptibility of cellulose obtained in this way, to enzymatic hydrolysis was similar. A smaller amount of lignin extracted from beech sawdust after treatment with hydrochloric acid points to an increased reaction intensity of lignin fragments condensation.

Table 3. Sugar yields from the pretreated beech wood and enzymatic hydrolysis of the residual

Hydrolyzate	Sugar released (% w/w of beech)			
	autohydrolysis, 10 min.	200°C 20 min.	acid, 200°C, 2.5 min.	0.09% 5 min.
Liquid (I)	14.6	11.8	21.1	21.4
Liquid (II)	2.9	3.1	2.7	2.8
Liquid after hydrolysis of residual solids	27.5	45.6	28.0	41.4
Total	45.0	60.5	51.8	65.6

Knappert et al. [7] compared the results of the enzymatic hydrolysis of poplar, which had previously been autohydrolysed and had undergone partial acid hydrolysis. An application of the second method made it possible to saccharify cellulose with doses of cellulolytic preparations which were several times smaller than after the process of autohydrolysis. Although the acid treatment did not remove lignin from the substrate, its solubility in ethanol was increased. According to Scott et al. [20] hydrolysis of red oak chips, after long acid impregnation, helped to obtain more xylose and less furfural as compared with acid hydrolysis without impregnation. Puri and Mamers [18] have shown a favourable effect of carbonic acid, during autohydrolysis of bagasse, wheat straw and *Eucalyptus regnan* on their digestibility.

In order to limit the degradation of sugars originating from hemicellulose of hardwoods Bierman et al [1] removed them during rapid steam hydrolysis together with steam condensate. This pretreatment made it possible to fractionate wood leaving a fraction rich in cellulose and partly in reactive lignin. The drawback of this method consisted in a considerable dilution of sugars. Regulation of pH during the pretreatment of lignocellulosic materials makes it possible to control the process of separation functioning of individual components, but there is an antagonism between gaining highly reactive lignin and production of sugars from hemicellulose. In the future, the conditions of treatment process will to a large extent depend on the cost of intermediate products including pentose and reactive lignin.

CONCLUSIONS

1. Although the actual reactions mechanism is certainly more complex, a simple series model of the form of equation (1) and (6) is adequate to describe the net production of pentose oligomers and pentose monomers, and fragments of lignin (in a limited range), when beech sawdust is subjected to heating in aqueous media of 0.45% and 0.09% hydrochloric acid at the temperature of 185°C and 200°C.

2. The addition of hydrochloric acid to the autohydrolysis medium enhances the rate of all reactions in beech sawdust, but it has a greater effect on the hydrolysis of pentosan than on the degradation of pentose. At given temperature, the addition of hydrochloric acid increased the maximum achievable recovery of pentose and decreased the maximum yield of lignin solubilized in 1% dilute sodium hydroxide solution.

3. Autohydrolysis and partial acid hydrolysis are an effective pretreatment for enhancing the enzymatic conversion of beech wood cellulose to glucose. Under optimal pretreatment conditions more than 90% saccharification was achieved, but relatively high enzyme (substrate ratios/80 FPU/g) are required in order to achieve a high degree of cellulose conversion.

4. The pretreatment and enzymatic saccharification of beech sawdust pretreated with 0.09% hydrochloric acid at 200°C and with water at 200°C gave 65.6% and 60.5% reducing sugars, respectively.

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PORÓWNANIE AUTOHYDROLIZY I CZĘŚCIOWEJ KWASOWEJ HYDROLIZY JAKO METOD WSTĘPNEGO PRZYGOTOWANIA TROCIN BUKOWYCH DO BIAKONWERSJI

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Streszczenie

Przebadano kinetykę hydrolizy pentozanów i ligniny podczas autohydrolizy i częściowej kwasowej hydrolizy kwasem solnym trocin bukowych w temp. 185 i 200°C. Otrzymane dane posłużyły do opracowania kinetycznych modeli opisujących hydrolizę pentozanów, degradację powstałych cukrów prostych oraz degradację naturalnej ligniny do ligniny rozpuszczalnej w 1% roztworze Na OH w 20°C. Wzrostowi stężenia kwasu solnego od 0 do 0,09% podczas obróbki trocin bukowych towarzyszyła większa wydajność pentoz, co potwierdził stosunek stałych kinetycznych reakcji hydrolizy pentozanów (K_1) i degradacji cukrów prostych (K_2) od 1,9 dla autohydrolizy do 5,5 dla 0,09% roztworu kwasu solnego podczas obróbki w temp. 185°C. Obecność kwasu solnego w mieszaninie reakcyjnej zwiększyła szybkość hydrolizy zarówno pentozanów, jak i degradacji ligniny. Wzrostowi stężenia kwasu solnego towarzyszył intensywniejszy proces kondensacji lignin co objawiło się zmniejszoną wydajnością ekstrakcji ligniny po procesie obróbki.

W wyniku obróbki wstępnej trocin bukowych, a następnie enzymatycznej hydrolizy celulozy uzyskano łącznie 65,6% cukrów redukujących gdy trociny traktowano 0,09% kwasem solnym w temp. 200°C, a 60,5% cukrów redukcyjnych w stosunku do użytej masy trocin stosując autohydrolizę również w temp. 200°C.