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PRETREATMENT OF BEECH SAWDUST WITH AUTOHYDROLYSIS AND ALKALINE PROCESSING IN PREPARATION FOR ENZYMATIC HYDROLYSIS

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A pretreatment with sodium hydroxide and calcium hydroxide was studied with the view of increasing saccharification yields from enzymatic hydrolysis of autohydrolyzed-extracted beech sawdust. The beech sawdust was heated with water at 200°C for 20 min, and extracted with hot water and 1% solution of sodium hydroxide. Secondary treatments were carried out at temperatures ranging from 20 to 170°C, and with sodium hydroxide content ranging from 1 to 4%. The pretreated materials were hydrolyzed with "Novo" cellulase (SP 122) and with *Fusarium sp.* 27 cellulase. After secondary treatment the susceptibility of cellulosic substrates to enzymatic hydrolysis was more than twice higher than that of autohydrolysed-extracted beech sawdust.

The effectivity of enzymatic hydrolysis of lignocellulosic substrates depends to a large extent on the accessibility of cellulose to cellulolytic enzymes. Size reduction of the lignocellulosic substrate or the elimination of a part of its hemicelluloses and lignin increases the accessibility of cellulose for the enzymes and thereby enhances the rate of enzymatic hydrolysis. Kerr and Goring [5] showed that the removal of hemicelluloses from birch sawdust with 3% NaOH solution resulted in an increase of the diameter of cell wall capillaries from 9.2 Å to 16.9 Å, which made possible the penetration of these capillaries by cellulolytic enzymes.

In recent years, particular attention was devoted to autohydrolysis of lignocellulosic substrates as a method of their pretreatment for enzymatic hydrolysis [10, 18]. The result of such pretreatment is a material deprived of a considerable amount of hemicelluloses and of a part of the lignins following extraction with organic solvents or alkalies. However, the enzymatic conversion of cellulose to glucose requires the use of cel-

lulolytic preparations of high cellulolytic activities of the order of several dozen FPU units per g substrate; this considerably increases the cost of obtaining the hydrolysates [10, 12].

The present study concerned the effect of dilute sodium hydroxide and calcium hydroxide solutions on the increased susceptibility of autohydrolyzed-extracted beech sawdust to enzymatic hydrolysis.

METHODS

PRETREATMENT OF BEECH SAWDUST

The experiments were performed with beech sawdust of particle size ranging from 0.43 to 1.5 mm. The sawdust was autohydrolyzed in a 250 cm^3 autoclave containing 20 g of substrate and 100 cm^3 of distilled water. The autoclave was immersed in an oil bath until the temperature inside it attained 200°C. This temperature was maintained for 20 min and then the autoclave was cooled in cold water. The obtained material was washed with distilled water on a Buchner funnel to obtain about 200 cm^3 of filtrate. Next, 100 cm^3 of 1% NaOH solution was added and the mixture was left to stand for 1 h. The lignocellulosic material was separated from the solution on a Buchner funnel, and then either washed with water, neutralized with dilute phosphoric acid, again washed with water to get pH 5 and incubated with the cellulases, or subjected to another extraction with 1-4% sodium hydroxide solution. The repeated processing of lignocellulosic material was carried out in a 250 cm^3 autoclave at 135 and 170°C for 1 h, with 100 cm^3 of sodium hydroxide solution or 100 cm^3 of water and 0.5 g of calcium hydroxide added to the lignocellulosic material obtained from the first extraction. The lignocellulosic material processed with sodium hydroxide was filtered, washed with water, dilute phosphoric acid and then again with water, and incubated with cellulolytic enzymes. The lignocellulosic material treated with calcium hydroxide was separated from the precipitated lignin fraction by sedimentation (the lignocellulosic substrate particles sediment in a water solution much quicker than the lignin fraction). The material thus obtained was subjected to enzymatic hydrolysis.

The processing of the substrate with alkalies at 100°C was done in Erlenmayer flasks heated for 1 h in a boiling water bath.

ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSIC MATERIAL

To about 0.25 g (dry mass) of lignocellulosic substrate there was added 10 cm^3 of cellulase preparation containing 50 mg of *Fusarium sp.* 27 mycelium biomass with the cell-bound cellobiase [17]. The cellulolytic prepara

tion was a mixture of equally active "Novo" cellulolytic preparations (SP 122) and a preparation from a *Fusarium sp.* 27 culture [16]. The activity of the preparation was 1.6 FPU units cm^3 or, in conversion to protein, 0.52 FPU units/mg protein. The activity of cell-bound cellobiase of *Fusarium sp.* 27 mycelium was 0.164 mmol/cm^3×min×g biomass. The hydrolysis of lignocellulosic substrates was carried out in stationary conditions at 45°C and pH 4.8, with samples periodically taken for analysis.

ANALYTICAL METHODS

Sugar content in reaction mixtures was determined in reaction with 3,5 dinitrosalicylic acid [8]. Cellulase activity was expressed in FPU units according to Mandels et al. [6a]. Cellobiase activity was determined by the method of Sternberg [14] adapted to determinations of the activity of cell-bound cellobiase [17]. Cellulose content was assayed by the method of Kürchner-Hanak [6], and lignin content by the method of Jayme-Knolle [9]. The specific surface of the lignocellulosic material was determined on the basis of the isotherm of methylene blue adsorption [11]. Dry mass content in the substrate was determined by drying at 105°C.

RESULTS AND DISCUSSION

Autohydrolysis of lignocellulosic substrates led to a degradation of part of the lignins which in turn made possible their extraction with dilute sodium hydroxide solutions. The effect of the temperature of lignin extraction with 1% NaOH solution is described in Table 1. The increase of extraction temperature from 20 to 135°C caused a drop in the mixture's pH, thereby worsening the conditions of lignin extraction and failing to increase the effectivity of enzymatic hydrolysis of carbohydrates contained in beech sawdust. A twice repeated alkaline processing was applied to improve the susceptibility of the lignocellulosic material obtained by auto-

Table 1. Effect of extraction temperature on the degree of enzymatic saccharification of autohydrolyzed and extracted with aqueous NaOH solution

Extraction temperature °C	pH of extract	Amount of extracted lignin %	Degree of saccharification after*):		
			1 hr	4 hrs	24 hrs
20	12.2	9.6	8.4	24.0	41.8
100	10.5	9.2	8.3	23.9	40.3
135	9.2	2.1	8.5	25.1	42.6

Cellulase activity — 20 FPU/g substrate

*) Based on the amount polisaccharides present in autohydrolyzed-extracted beech sawdust.

hydrolysis of beech sawdust to enzymatic hydrolysis. In the first stage the lignins were extracted with 1% NaOH solution at 20°C, and then the processing with alkalies was repeated at a higher temperature. Table 2 presents the effect of temperature during the action of 1% NaOH solu-

Table 2. Effect of lignin secondary extraction with 1% aqueous NaOH solution on chemical compositionand the degree of saccharification of autohydrolyzed-extracted beech sawdust

Extraction temperature °C	Amount in mass		Cellulase activity*) FPU/g	Degree of saccharification after: %		
	cellulose %	lignin %		1 hr	4 hrs	24 hrs
20	72.1	21.4	64	28.3	45.5	88.3
			32	16.8	31.9	61.3
			64	34.7	54.3	91.3
100	73.7	20.2	32	23.3	44.9	72.0
			16	14.0	29.4	52.8
			64	54.7	74.3	95.9
135	74.7	18.9	32	30.4	52.4	92.1
			16	17.1	37.8	72.8
170	63.2	28.5	64	42.4	59.0	88.5

*) Cellulase preparation was combined with cell-bound cellobiase *Fusarium sp.* 27.

tion during the secondary processing on the dynamics of saccharification of carbohydrates in the obtained lignocellulosic material and on cellulose and lignin contents. An increase of processing temperature to 135°C favoured the increase of cellulose susceptibility to enzymatic hydrolysis, while at 170°C the conditions of lignin extraction worsened in view of the drop of the mixture's pH, which also adversely affected the composition of the obtained lignocellulosic material.

An increase of the concentration of the NaOH solution from 1% to 2% and then to 4% increased the susceptibility of beech sawdust cellulose to enzymatic hydrolysis (Table 3). The specific surfaces and cellulose content in the lignocellulosic material obtained in this way were also greater. An increased NaOH concentration in the secondary process, however, led to lower lignocellulosic material yield and increased the cost of the neutralization of the NaOH solution. The lignin fraction dissolved in the NaOH solution can be separated by acidifying the solution or by adding calcium chloride, but only when the extraction was performed with 1% or 2% NaOH solution (Table 4). This is important given the possibility of regenerating the NaOH and using it once again in the same process after removing the lignin fractions with calcium chloride.

The use of calcium hydroxide instead of sodium hydroxide in the separation of lignins from previously autohydrolyzed beech sawdust may considerably lower the cost of the entire process of initial preparation of

Table 3. Effect of lignin secondary extraction with different concentration of aqueous NaOH solutions on chemical composition and properties of autohydrolyzed-extracted beech sawdust

NaOH concentration in solution %	Mass yield*)	Specific surface area of mass m^2/g	Amount in mass		Degree of saccharification after 1 hr %
			cellulose %	lignin %	
0	55.8	55.2	72.1	21.4	8.4
1	44.3	110.8	74.8	18.9	16.3
2	38.2	139.0	77.8	15.7	17.7
4	34.0	164.0	80.0	14.6	23.4

Cellulase activity 20 FPU/g substrate
*) Calculated in relation to untreated substrate

Table 4. Amount of precipitated lignin fraction from extracts obtained after lignin secondary extraction of autohydrolyzed-extracted beech sawdust with different concentration of aqueous NaOH solutions

NaOH concentration in solution %	Amount of lignin fraction after precipitation*)	
	acid %	calcium ions %
1	5.5	6.4
2	8.5	7.7
4	11.0	not precipitate

*) Calculated in relation to untreated substrate

the substrate for enzymatic hydrolysis. Calcium ions combine with lignins to form compounds insoluble in water, and the calcium-lignin complex was removed from the sawdust by sedimentation thanks to the much quicker precipitation of beech sawdust particles than of lignin calcium salts molecules. The use of calcium hydroxide in the secondary alkaline processing of previously autohydrolyzed beech sawdust increased the susceptibility of the obtained lignocellulosic material to enzymatic hydrolysis, and made it possible to obtain the same degree of saccharification as in the material subjected to autohydrolysis and lignin extraction at 20°C but with the use of enzymatic preparations of activity reduced by more than half (Table 5).

DISCUSSION OF RESULTS

There are numerous methods of pretreating lignocellulosic substrates for enzymatic hydrolysis [13]. The choice of the method frequently depends on the kind of hydrolysed lignocellulosic substrate. For example, Matsumura et al. [7] attained 70-80% of saccharification of soft wood sawdust after first vibratory grinding the material to particles measuring 10-30 μm

Table 5. Enzymatic hydrolysis of autohydrolyzed-extracted beech sawdust secondary pretreated with calcium hydroxide

Extraction temperature °C	Specific surface area substrate m^2/g	Cellulase activity FPU/g substrate	Degree of saccharification after %		
			1 hr	4 hrs	24 hrs
20	55.2	64	26.5	47.7	87.5
		32	16.4	30.8	58.4
135	148.3	32	29.3	51.1	93.4
		16	18.3	39.6	74.8

Cellulase preparation was combined with cell-bound cellobiase *Fusarium sp.* 27

in diameter; similarly grinded birch wood sawdust, on the other hand, saccharified only in 37.1%. In turn, autohydrolysis gives good results as a method of pretreating lignocellulosic substrates only in the case of hardwood [13].

The effect of alkalies on the various components of lignocellulosic substrates depends on numerous parameters such as alkalie concentration, reaction temperature, reaction duration, etc. The treatment of hard wood sawdust with dilute alkalie solutions leads to the extraction of, first of all, xylan and a certain amount of lignins [7], to the reaction of alkalies with ester bonds and acetyl groups of wood, as well as to the swelling of cellulose and reduction of its crystallinity [3].

The autohydrolysis of beech sawdust followed by extraction with water and sodium hydroxide removed considerable amounts of hemicelluloses and lignins without affecting the cellulose content. According to Puls et al. [12], however, during hydrolysis the degree of cellulose polimerization decreases, and the changes increase with the increase of hydrolysis time.

The treatment of previously autohydrolysed beech sawdust cellulose with dilute alkalies increased the susceptibility of cellulose to enzymatic hydrolysis but at the same time led to considerable losses of cellulose from the substrate; these losses were particularly apparent when sodium hydrogen solutions of higher concentration were used. The lignin fraction dissolved in the alkaline solution may be precipitated by acidifying this fraction. The obtained mass of the lignin fraction greatly exceeded the amount of extracted lignin, and this indicates that in addition to lignin the fraction also contains carbohydrates. Cinite et al. [1] acted with a water solution of sodium hydroxide on wheat straw at 170°C and discovered in the solution a lignin-carbohydrate complex with the carbohydrate-to-lignin ratio ranging from 3:1 to 1.5:1. Gierer [4] suggests that during pretreatment of wood with sodium hydroxide at elevated temperatures there occurs condensation and polimerization of lignins which may lead to the formation of unwanted chemical compounds.

The increased susceptibility of beech sawdust cellulose to enzymatic hydrolysis due to the action of alkalies is a result of the increased specific surface of the hydrolysed substrate. This is indicated by the mutually proportional increases of the degree of lignocellulosic material saccharification, and of the specific surface. According to Targoński [15], other features characterizing cellulose, such as the degree of polimerization or the degree of its crystallinity, have a much smaller bearing on the effectivity of enzymatic hydrolysis of cellulose than the specific surface.

The treatment of lignocellulosic substrates with concentrated alkalies causes swelling of cellulose, the change of cellulose structure from form I to structural form II, and a reduction of cellulose crystallinity. According to David and Thiry [2], the susceptibility of cellulose II to enzymatic degradation is much greater than that of cellulose I: after 48 h of hydrolysis of cellulose II it was saccharified in over 90%, while cellulose I, after 72 h, was saccharified in only 35%. On the other hand, Matsumura et al. [7] did not observe any increase of helocellulose reactivity after treatment with alkalies. Indeed, after treatment with a concentrated NaOH solution at 60°C the degree of helocellulose saccharification was reduced, and the authors explain this as being due to the formation of unwellcome compounds containing lignin.

CONCLUSIONS

1. Autohydrolysis of beech sawdust at 200°C for 20 min and extraction of hemicelluloses and a part of the lignins gave a cellulose material containing 72.2% cellulose and 21.4% lignin. The polisaccharides contained in the cellulose material were saccharified in 88.3% during 24 h by a cellulolytic preparation with an activity of 64 FPU/g substrate augmented with cellobiase. In analogous conditions the polisaccharides of untreated beech sawdust were saccharified in a mere 9.3%.

2. The treatment of autohydrolyzed and extracted beech sawdust with 1-4% NaOH solution at 135°C increased the specific surface of the substrate and its susceptibility to enzymatic hydrolysis by about two to three times.

3. A repeated treatment of autohydrolyzed and extracted beech sawdust with NaOH solutions caused a reduction of solid lignocellulosic material by about 20 to 39%, depending on the NaOH concentration.

4. The heating of autohydrolyzed beech sawdust at 135°C for 1 h with calcium hydroxide made it possible to reduce by more than half the dose of enzymatic preparation needed to attain the same degree of saccharification of the polisaccharides contained in the substrate as in the case of the substrate before processing.

5. The autohydrolysis and alkaline processing of lignocellulosic sub-

strates is among the cheapest and most effective methods of preparing the cellulose in lignocellulosic substrates for enzymatic hydrolysis, despite the considerable energy consumption involved.

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WSTĘPNE PRZYGOTOWANIE TROCIN BUKOWYCH DO ENZYMATYCZNEJ HYDROLIZY PRZEZ AUTOHYDROLIZĘ I ALKALICZNĄ OBRÓBKĘ

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Streszczenie

Autohydroliza jest efektywną metodą wstępnego przygotowania substratów lignocelulozowych do enzymatycznej hydrolizy, polegającą na ogrzewaniu w wodzie lub

parze wodnej substratu w temp. 180-200°C przez 5-20 min. Trociny bukowe ogrzewano z wodą w temp. 200°C przez 20 min, po czym ekstrahowano wodą i 1% roztworem NaOH uzyskując masę o powierzchni właściwej 55,2 m^2/g zawierającą 72,1% celulozy i 21,4% ligniny. Tak otrzymaną masę celulozową poddawano enzymatycznej hydrolizie celulazami pochodzącymi z firmy „Novo" (SP 122) oraz z hodowli *Fusarium sp.* 27; 88,3% polisacharydów zawartych w masie celulozowej ulegało scukrzeniu w ciągu 24 h w obecności preparatu celulaz o aktywności 64 FPU/g substratu uzupełnionego dodatkiem celobiazy. W celu zmniejszenia dawek preparatu celulolitycznego użytego do hydrolizy stosowano powtórne działanie na autohydrolizowane trociny bukowe wodorotlenku sodowego w stężeniu 1-4%. Masa celulozowa otrzymana po powtórnym działaniu 1% roztworu NaOH w temp. 135°C w ciągu 1 h zawierała 74,7% celulozy i 18,9% ligniny, a charakteryzowała się powierzchnią właściwą 110,8 m^2/g.