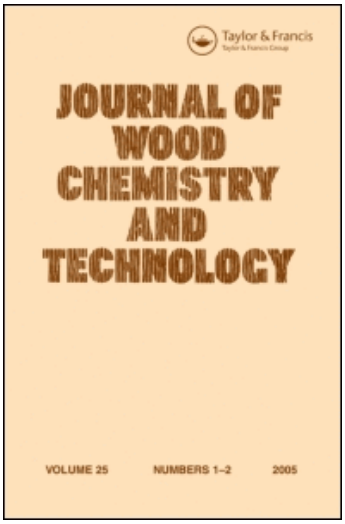

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EFFECT OF PRETREATMENT OF POPLAR WOOD UPON ENZYMATIC SACCHARIFICATION

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ABSTRACT

The wood of young poplar grown in short rotation coppices was used as a substrate for enzymatic saccharification. Several pretreatments of the wood, both physical and chemical, including delignification were applied to enhance the polysaccharide conversion into fermentable sugars. Comparing the yields obtained on a delignified material and on alkali treated material pointed out that lignin is not the major obstacle to saccharification. On the other hand, the swelling and dissolution effect of the potent cellulose solvent, N-methyl morpholine N-oxide, on wood brought about a nearly quantitative sugar recovery. This shows the importance of the ultrastructural organization of the plant cell wall over its enzymatic hydrolysis.

INTRODUCTION

Among the possible utilizations of lignocellulosic biomass, conversion into fermentable sugars constitutes an issue of commercial interest[1]. However the exploitation of woody wastes in view of their saccharification can be economically feasible only if high sugar yield can be obtained.

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In this respect acid hydrolysis can provide good yields of hexoses and pentoses from cellulose and hemicelluloses, but neutralization of the acid and its recycling are costly processes. The availability of efficient enzyme mixtures and the development of cellulose technology makes the enzymatic saccharification of lignocellulosic materials an attractive procedure. But, because of its structural composition made of a mixture of three main groups of polymers, namely, lignin, cellulose and hemicelluloses, wood offers a natural resistance to enzyme degradation[2]. This resistance is increased by the ultrastructural organization of the higher plant cell walls in which the tight interactions between the polymer components constitutes a barrier to the penetration of large protein molecules like enzymes. An additional factor which makes an obstacle to enzymatic hydrolysis of woody material at the macromolecular level is the crystallinity of cellulose. Therefore increasing the accessibility of polysaccharides constituents of the wood cell wall to enzymes has become a major problem.

Several pretreatments have been suggested by chemical, physical or biological techniques[3-11]. Recently the swelling effect of organic solvent has been studied[12-16].

In the present work the effect of the polysaccharide solvent N-methyl morpholine N-oxide (MMNO)[16] on enzymatic hydrolysis of young poplar wood from short rotation coppices has been investigated. The most complete saccharification of cellulose and hemicelluloses has been studied by adding different types of pretreatments to the action of the solvent.

EXPERIMENTAL

Lignocellulosic Substrates

The poplar wood used was a hybrid from *Populus trichocarpa* X *Populus deltoides* c.v. Raspalje. The two-year-old trees were obtained by the technique of short rotation coppices[17].

The mechanical pulp was prepared in a twin screw extrusion machine[18].

The sawdust (0.2-0.4 mm) was extracted with the mixture ethanol-toluene (1:2 v/v) in a soxhlet apparatus. The wood was treated with the delignifying reagent of Wise et al.[19], 1 h at room temperature (sample C) or 2 h at 70°C (sample H). The residual lignin was estimated according to Klason[20].

Pretreatments

Alkaline pretreatment was carried out with sodium hydroxide (0.1 M or 0.5 M, for 15 h at 20°C under nitrogen). The insolubles (NS) were dialyzed and freeze dried.

Acid hydrolysis was performed with sulfuric acid (2 M, 1 h, 120°C)[21].

Steam explosion was performed in a pilot flash autohydrolysis apparatus (EC 300 - CNRS built by Deltalab)[22].

Treatment with N-methyl morpholine N-oxide (MMNO)

The solvent MMNO contained 13 % water (w/w)[23]. The wood or pulp samples (1 g) were mixed in MMNO (25 ml) and kept at 120°C for 20-25 min. with stirring.

Water (25-50 ml) was then added and the mixture dialyzed and freeze dried. In each case the recovery of sample was nearly quantitative[23].The enzymatic digestion was then carried out on the whole mixture.

Enzymatic hydrolysis

Samples (1 g) were treated with 50 mg of the commercial enzyme mixture (Gist Brocades) in 100 ml of water at 40°C for 48 h under gentle shaking. The yields of saccharification were determined on the soluble sugars estimated in g.l.c. as their alditol acetate derivatives after hydrolysis[16].

RESULTS AND DISCUSSION

The wood sawdust used in this study was prepared from short rotation coppices of Populus. The trees were two years old when harvested. The commercial enzyme mixture of cellulases and hemicellulases was inefficient on unpretreated wood (0). Therefore pretreatments of different natures were applied to the wood, which consisted of two delignification treatments with sodium chlorite, the first at room temperature and the second at 70°C according to Wise et al.,[19] giving respectively samples C and H. Another chemical pretreatment was carried out with sodium hydroxide at two concentrations, 0.1 M and 0.5 M, giving two insoluble residues which were used in this study, (NS 0.1 and NS 0.5 respectively). For comparison, a pretreatment intended to remove specifically the hemicelluloses was performed with 1 M sulfuric acid[21] and gave the cellulosic residue NS H^+. Finally a steam explosion pretreatment in mild conditions of pressure[22] afforded sample NS EX. On the other hand, another set of samples was prepared from a mechanical pulp obtained by subjecting the young poplar wood from coppices to a twin-screw extrusion procedure[18]. Here again pretreatments were applied to the pulp, namely sodium hydroxide at two concentrations (0.1 M and 0.5 M) and 1 M sulfuric acid giving respectively pulps NS 0.1, NS 0.5 and NS H^+ samples.Analytical data of the different substrates used are reported in Table 1.

The twin screw extrusion process is in itself a pretreatment which is both mechanical and chemical. This double action made the original pulp (0) more susceptible to enzymes than wood since saccharification amounted to 20 % (Table 2). It is important to note that the pulp (0) had a composition nearly identical to that of the original wood (0) (Table 1). Therefore the improvement of saccharification brought about by the pulping process shows that the partial internal disorganization of the wood seems to facilitate the action of the enzymes. Disorganization of the cell walls

TABLE 1

Analytical Data of the different Substrates used

Fractions used	% recovered from wood	Lignin %	mg/100 mg* Neutral sugar %	Xyl	Glc
Wood					
0	100	22	70	16.5	49.5
C	91.5	16	70	15.5	51
H	82	7	80	16	60
NS 0.1	93	20	67.5	14	50
NS 0.5	90	19	67	13	50
NS H^+	78.5	28	65	6	57
NS EX	70	30	64.5	7.5	55
Pulp					
0	95	21	71	16.5	52
NS 0.1	94	20	78	15.5	51
NS 0.5	87.5	19	70.5	12.5	55
NS H^+	76.5	28	70.5	5.5	62.5

* mg/100 mg of each fraction used. Neutral sugars % includes xylose, glucose and other neutral sugars ; rhamnose, arabinose, mannose and galactose, less than 4 % have been omitted from these data[23].

TABLE 2

Yields of Enzymatic Saccharification

Fractions treated	Saccharification* hydrolysis (%)	XYL.s/XYL.t**	GLC.s/GLC.t***
Wood			
C	11	13	10
H	52.5	51.5	53
NS 0.1	53	58	52
NS 0.5	56	63	54
NS H	35	30	37
NS EX	40	37.5	40
Pulp			
0	22.5	30	20
NS 0.1	52	65	48.5
NS 0.5	57	70	54
NS H^+	41.5	60	40

* Percent saccharification relative to the total polysaccharides in the fraction.

** Ratio of solubilized xylose (Xyl.S) to the total xylose (Xyl.t) available in the fraction.

*** Ratio of solubilized glucose (Glc.S) to the total glucose (Glc.t) available in the fraction.

along with improved penetration of the enzymes are even more important after sodium hydroxide pretreatment since the saccharification yields were of 55 %. It is clear that the action of sodium hydroxide alone is more efficient than mechanical pulping and that there is no additive effect of pulping and alkali since the yield of saccharification on Pulp NS 0.1 was the same as wood NS 0.1 (Table 2). It can be concluded that the major factors for enzymatic hydrolysis of wood are the swelling and the chemical changes (de-acetylation of the xylan, alteration of lignin) of the cell walls. However, these factors show a certain limitation since about 45 % of the potential polysaccharides remained unhydrolyzed, even when higher alkali concentrations were used. In all the results of saccharification it is apparent that the ratios of solubilized sugars to the total available sugars in the polysaccharides are the same for xylose and glucose. This means that there is no preferential hydrolysis of hemicelluloses over that of cellulose.

The protective effect of lignin upon enzymatic saccharification was investigated on delignified wood. Delignification by sodium chlorite at room temperature (sample C) and at 70°C (sample H) afforded samples having 16 % and 7 % lignin respectively. Saccharification was very limited on sample C (11%) whereas 52 % of the available polysaccharides of sample H were hydro-lyzed (Table 2). The fact that the same yield of saccharification was obtained when the wood had been treated with sodium hydroxide, that is with samples still having 20 % or 19 % residual lignin, shows that lignin is not the limiting factor in wood saccharification. This again points out the importance of the ultrastructural organization of the substrates and to the necessity of reaching a high level of disorganization of the cell wall structure in order to improve the penetration of the enzymes.

Improve penetration of the enzymes was achieved by subjecting all the pretreated samples to the organic solvent of polysaccharides N-methyl-morpholine-N-oxide (MMNO). The

TABLE 3

Yields of Enzymatic Saccharification after Treatment with MMNO.

Fractions treated	Saccharification* hydrolysis (%)	XYL.s/XYL.t**	GLC.s/GLC.t***
Wood			
O	55	50	56
C	56	52	57
H	87.5	81	89
NS 0.1	87	88	87
NS 05	90	90	90
NS H^+	91	84.5	92
NS EX	92.5	91	93
Pulp			
0	73	67	75
NS 0.1	88	87	88
NS 0.5	92	87	93
NS H^+	96	95	96

* Percent saccharification relative to the total polysaccharides in the fraction.

** Ratio of solubilized xylose (Xyl.S) to the total xylose (Xyl.t) available in the fraction.

*** Ratio of solubilized glucose (Glc.S) to the total glucose (Glc.t) available in the fraction.

action of this tertiary amine was intended to dissolve most of the cell wall constituents[23]. As was shown previously[16] the solubilizing action of MMNO was greatly improved after delignification but also after sodium hydroxide pretreatment. The results given in Tables 2 and 3 demonstrate the positive action of MMNO upon enzymatic saccharification. Even on the unpretreated starting material,wood (0), the yield of saccharification was brought

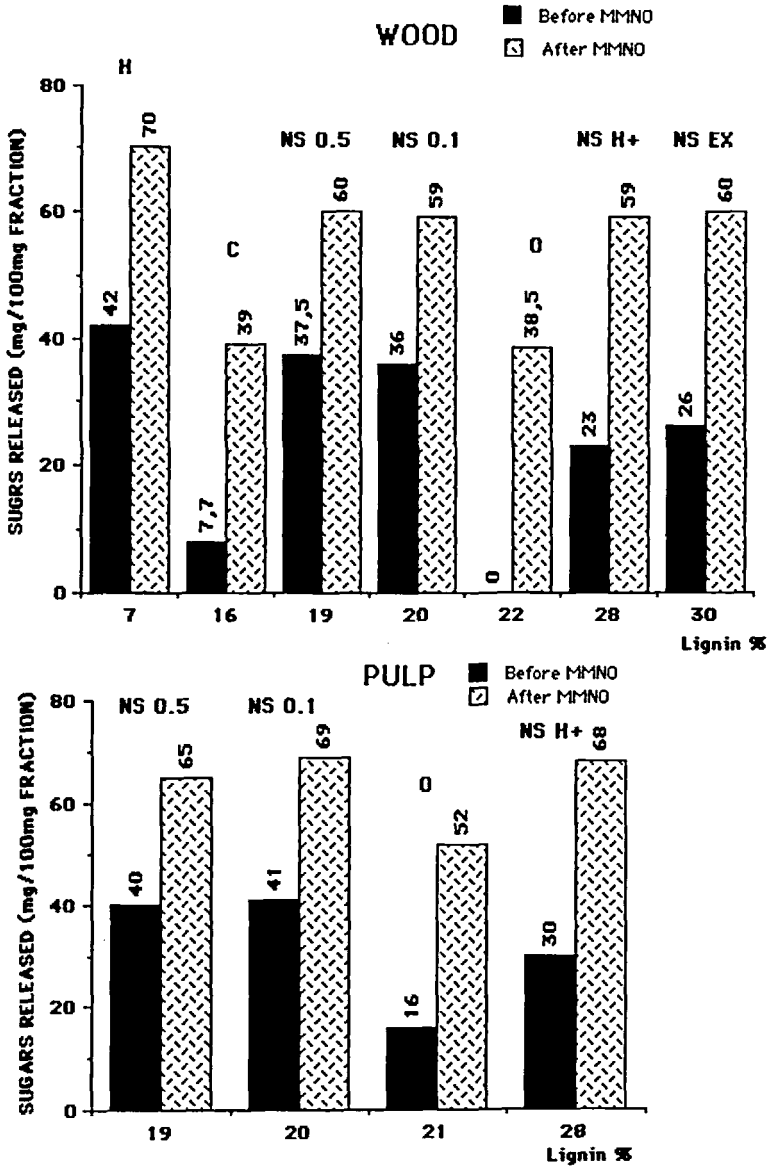

FIGURE 1-Enzymatically released sugars from differently pretreated samples.

up to 55 % of the potential sugars. This can be explained by the fact that after the swelling and solubilizing action of MMNO, the enzymes can diffuse more deeply into the lignocellulosic material and find a better contact with their polysaccharide substrates. This phenomenon became even more obvious when Pulp (0) was pretreated with MMNO. The subsequent saccharification afforded a yield of 73 % of the available polysaccharides in the material.

The effective action of MMNO is well illustrated by the enhancement of saccharification observed on the differently pretreated samples. Especially the yield obtained on sample C compared to Pulp (0) shows that both lignification and ultrastructure of the woody tissues impair hydrolysis of the polysaccharides constituents by enzymes.

The lower saccharification obtained on C is an indication that although the overall lignin content was inferior to that of Pulp (0) the latter could give a much better enzymatic conversion of its polysaccharides. Therefore the main factor to facilitate the penetration of the enzymes is the complete disruption of the cell wall ultrastructure by MMNO and not the simple removal of lignin. This explain why nearly quantitative sugar recovery could be achieved on all the samples which had undergone an important internal disorganization (NS 0.5, NS H^+, NS EX) and the corresponding pulps (Table 3 and Fig. 1)

CONCLUSIONS

In the actual conditions used it can thus be concluded that physical and chemical pretreatments applied to young poplar short rotation coppices had only limited effects upon enzymatic saccharification. These effects became more important when they were combined with a partial swelling and dissolution brought about by the organic solvent MMNO. A complete recovery of the sugars present as hemicelluloses and cellulose in the wood cell walls could be obtained without previous delignification ; as previously reported[24],

these results show that lignin content is not the major obstacle to enzymatic conversion of polysaccharides in wood. It appears therefore that a great advantage of the organic solvent is not only to perform an efficient disorganization between the cell wall constituents but also, by its solubilizing action, to overcome the resistance of crystalline cellulose to enzymatic hydrolysis.

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