Pretreatment of Eucalyptus Wood with Sodium Hypochlorite and Enzymatic Hydrolysis by Cellulases of *Trichoderma viride*

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Synopsis

Eucalyptus saligna meal and chips have been treated with HClO-NaClO in order to increase their accessibility to cellulases from *Trichoderma viride* and hence to increase the yield of hydrolysis of cellulose into glucose. Different experimental variables determine the efficiency of the pretreatment. These are the pH and temperature of the pretreatment, the granulometry and concentration of the substrate, the oxidant concentration and prior elimination of hemicelluloses by prehydrolysis with dilute acid. An incomplete factorial design has been used to study the effect of these experimental variables on the weight loss during the pretreatment, the composition of the pretreated substrate, and the quantity of glucose formed as function of time in the subsequent enzymatic hydrolysis. Delignification can reach 70% using 5 mol HClO-NaClO per kilogram of wood.

INTRODUCTION

A pretreatment has been developed to increase the accessibility of lignocellulosic materials to chemical and biochemical reagents.[1] It involves competitive oxidation of cellulose and lignin by sodium hypochlorite at controlled pH and has been applied to wheat and barley straw, bagasse, spruce bark, spruce, and Eucalyptus saligna wood.[2,3] This pretreatment is particularly efficient to increase the rate and yield of enzymatic hydrolysis of the cellulosic fraction of the substrate into glucose. When 9.4 mol of oxidant per kg of Eucalyptus wood are consumed[3] at pH 8, a high yield of hydrolysis can be obtained for this particularly nonreactive type of hardwood. It has been demonstrated that the characteristics of the pretreated substrate (lignin, hemicelluloses, and cellulose content, reactivity in enzymatic reactions) depend on several experimental variables. The most significative of them are the pH, the time, and temperature of the pretreatment, the concentration and granulometry of the substrate, the quantity of oxidant consumed during the reaction, and the prior elimination of the hemicelluloses by prehydrolysis with dilute acid.[2,3] Even if only two or three different values are given to each of these variables, a systematic study of the effect of these variables is impossible owing to the large number of experiments which would be required. Another method has thus to be used to screen and optimize the most important variables as efficiently as possible. Therefore, a design of 15 experiments based on statisti-

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cal analysis has been derived. Using this series of experiments, it was possible to determine the effect of the seven previously cited experimental variables on the weight percent of substrate recovered, on the percent lignin, glucan pentosan, and other hexosans recovered in the pretreated substrate and on its initial rate and maximum yield of enzymatic hydrolysis. Each of these properties is considered to be a sum of factors which are the contributions of the different experimental variables. Statistical analysis of the experimental results as a function of this model allows quantitative determination of the relative importance of each variable on a given property which can then be predicted in any other set of conditions. Optimization of the experimental conditions of the pretreatment for a given application is then possible. In the present work, Eucalyptus saligna wood has been used as substrate. Particular attention has been paid to the effect of prehydrolysis with dilute acid which eliminates the hemicelluloses, to the use of reduced quantity of oxidant, and to the effect of pH in the narrow range of 7–9 which was shown previously to correspond to an optimum efficiency.

EXPERIMENTAL METHODS

Prehydrolysis

Prehydrolysis is performed by refluxing the initial Eucalyptus saligna meal (± 0.2 mm) or chips (± 5 mm) for two hours with either 2% H_2SO_4 or 2% HCl.

Pretreatment

The pretreatments are performed in a thermostatted glass reactor equipped with a pH-meter electrode, a thermometer, a cooling coil, and an inlet for gaseous chlorine. The pH is maintained at a constant value (7, 8, or 9) using a ITT 80 Radiometer Titrator equipped with an ABU 80 Autoburet and a PHM 82 Standard pH meter which delivers NaOH 10*N*. The flow rate of Cl_2 is 0.6 mol/h.

After delivery of the exact amount of chlorine the liquid phase is evacuated using a stopcork situated at the bottom of the reactor. The pretreatment is performed using a quantity of substrate which corresponds either to 70 or to 140 g of initial dry unprehydrolyzed and untreated substrate in 700 mL liquid phase (100 or 200 g/L). Consumption of the hypochlorite is quantitative in all cases. The pretreated solid is washed three times with 350 mL water with stirring and dried to constant weight at 60°C.

Weight Loss

Pretreated substrate was dried to a constant weight (24 hours in aerated oven at 105°C) and the value subtracted from the original weight.

Analysis of the Initial and Pretreated Substrates

The lignin content is obtained by the K number method.[4] The substrate is milled and the obtained particles (dimension < 200 μm) are suspended in a known amount of $KMnO_4$. After 10 min, the oxidation reaction is stopped by adding an excess KI which is titrated with $Na_2S_2O_3$. The K number is

proportional to the $KMnO_4$ consumed. This index, multiplied by 0.13, gives the percent of native lignin in the substrate. This factor of 0.13 has been determined for the untreated Eucalyptus meal (nonextractive free) used in the present work by comparison with the lignin content obtained by the Klason method.[5]

The pentosan and hexosan content are obtained by quantitative saccharification of milled substrate followed by high performance liquid chromatography (HPLC) analysis. The quantitative saccharification is performed by dissolving the substrate (250 mg) in 3 mL H_2SO_4 72% (1 h at 30°C). The obtained solution is then diluted with 84 mL water and kept in the oven for 2 h at 150°C in septum vials. The acid solution is then neutralized with solid $Ba(OH)_2$. The 25 cm length and 0.46 cm internal diameter HPLC column, is filled with the anion exchange resin Aminex A-28 in the borate form. A boric acid-potassium borate pH 8.8 buffer is used as mobile phase with a flow rate of 0.4 mL/min. Spectrophotometric detection is made at 570 nm, using the *in situ* prepared complex of the sugar with copper 2.2′ bicinchoninate. The glucose content is also determined by the glucose-oxidase method (Boehringer GOD-perid glucose).[6]

Enzymatic Hydrolysis

The substrate (250 mg) is first soaked for 24 h at room temperature with 9 mL citrate buffer. Cellulases (10 mg) from *Trichoderma viride* (Onozuka R-10 from Kinki Yakult M6F Co. Ltd; Specific activity: 0.07 IFPU/mg) are then dissolved in 1 mL citrate buffer and added to the substrate. The hydrolysis is performed at 45°C in shaken flasks. A volume of 10 μL is taken after convenient time intervals and analyzed for glucose by the glucose-oxidase method.[6]

EXPERIMENTAL DESIGN

The experimental variables x considered are given in Table I. An incomplete factorial design[7] has been obtained by minimization of the volume of the confidence region associated with the parameters of the considered models. The experimental design is given in Table II in standardized variables. Each measured property R can be considered to be the sum of different terms which are the contributions of the different variables:

$$R = k_0 + LC_j + k_1x_1 + k_2x_2 + k_3x_3 + k_4x_4 + k_5x_5 + k_6x_4^2 + e \quad (1)$$

where $x_1, \ldots, x_5$ are the different variables expressed in standardized values
k_0 is the general mean
$k_1, \ldots, k_6$ are the parameters associated with the quantitative variables
LC_j are the parameters associated with the levels of the qualitative variable ($\Sigma_i LC_j = 0$)
e is a random error distributed with zero mean and variance σ^2.

In matrix notation, (1) is of the form

$$\mathbf{Y} = X\mathbf{k} + \mathbf{e} \quad (2)$$

TABLE I
Experimental Variables Considered

Qualitative variable (LC_j)		Reduced variable
Nature of the substrate	nonprehydrolyzed	$LC_0 = 1$
	prehydrolyzed HCl	$LC_{HCl} = 2$
	prehydrolyzed H_2SO_4	$LC_{H_2SO_4} = 3$
Quantitative variable (x_n)		
Granulometry	meal	$x_1 = -1$
	chips	$x_1 = 1$
HClO-NaClO concentration	2	$x_2 = -1$
(mol/kg substrate)	5	$x_2 = 1$
Substrate concentration	100	$x_3 = -1$
(g/L)	200	$x_3 = 1$
pH	7	$x_4 = -1$
	8	$x_4 = 0$
	9	$x_4 = 1$
Temperature (°C)	30	$x_5 = -1$
	50	$x_5 = 1$

The least-squares estimate for $\mathbf{k}$ is provided by

$$\hat{\mathbf{k}} = (X'X)^{-1}X'\mathbf{Y} \tag{3}$$

where X' denotes the transpose of X. The covariance matrix of $\hat{\mathbf{k}}$ is provided by

$$\text{var}(\hat{\mathbf{k}}) = (X'X)^{-1}s^2$$

where

$$s^2 = (\mathbf{Y} - X\hat{\mathbf{k}})'(\mathbf{Y} - X\hat{\hat{\mathbf{k}}})/(N - p)$$

TABLE II
Experimental Design in Reduced Variables

Exp.	LC	Variable 1	2	3	4	5
1	2	−1	−1	−1	0	−1
2	2	−1	1	−1	1	1
3	2	1	−1	1	0	1
4	3	−1	−1	−1	−1	1
5						
6	1	1	1	−1	0	1
7						
8	2	1	1	1	−1	−1
9						
10	1	1	−1	−1	−1	−1
11	1	−1	−1	1	1	−1
12	1	−1	1	1	−1	1
13	3	1	1	−1	1	−1
14	3	1	−1	1	1	1
15	3	−1	1	1	0	−1

The mean square for error s^2 is an unbiased estimate of σ^2; N is the number of experiments and p is the number of parameters.

The statistic $\mathcal{F}$ used for all the tests is defined by

$$\mathcal{F} = \frac{(S_0 - S)/(\nu_0 - \nu)}{S/\nu}$$

where $S = (N - p) \cdot s^2$

$\nu = N - p$

$\nu_0 = N - p_0$ (p_0 is the number of parameters under the hypothesis tested)

$S_0 = (N - p_0) \cdot s_0^2$ (s_0^2 is the mean square for error under the hypothesis tested).

If the hypothesis tested is true, $\mathcal{F}$ has a Snedecor's distribution with $\nu_0 - \nu$ and ν degrees of freedom.

All the models used in the next sections were found to be acceptable using Snedecor's test, at the significance level of 5%.

RESULT AND DISCUSSION

Analysis of the Initial and Prehydrolyzed Substrates

The analysis of the initial and prehydrolyzed substrates are given in Table III, which shows that the prehydrolysis removes very efficiently the hemicelluloses with negligible loss of lignin or cellulose. There is no good method for the absolute determination of lignin in the large diversity of samples used in the present work (initial nonextracted, acid-treated, or NaClO-treated substrates). The Klason lignin method is not suitable for samples treated with NaClO.[8] Indeed, it is based on the insolubilization of lignin by condensation reaction involving the aromatic rings which are selectively oxidized in the presence of NaClO. The K number method is less selective since $KMnO_4$ in acidic medium degrades lignin completely.[8] It is however rather empirical since the oxidation is performed during a short constant time; the results

TABLE III
Analysis of the Initial and Prehydrolyzed Substrates: Composition in g/kg or in % of the Initial Value

Substrate	Substrate recovered (%)	Li	Xy	Gl	Ma	Ga	Ar	$C_5 + C_6$
Meal	100	158	105	419	15	17	7	563
Meal HCl	78.6	248 (123%)	32 (24%)	527 (99%)	11	6	2[a]	577
Meal H_2SO_4	80.5	242 (123%)	35 (27%)	492 (94%)	16	14	3	560
Chips	100	187	120	375	12	13	8	529
Chips HCl	79.1	254 (107%)	28 (18%)	396 (83%)	11	5	2[a]	442
Chips H_2SO_4	80.7	240 (103%)	33 (22%)	392(84%)	10	5	2[a]	441

[a] Limit of detection.

TABLE IV
Experimental Conditions

Experiment[a]	Prehydrolysis	Granulometry[b]	ClO^- (mol/kg substrate)	Substrate (g/L)	pH	Temperature (°C)
1	HCl	M	2	100	8	30
2	HCl	M	5	100	9	50
3	HCl	C	2	200	8	50
4, 5	H_2SO_4	M	2	100	7	50
6, 7	—	C	5	100	8	50
8, 9	HCl	C	5	200	7	30
10	—	C	2	100	7	30
11	—	M	2	200	9	30
12	—	M	5	200	7	50
13	H_2SO_4	C	5	100	9	30
14	H_2SO_4	C	2	200	9	50
15	H_2SO_4	M	5	200	8	30

[a] Experiments 5, 7, 9 = confirmation experiments of 4, 6, 8.
[b] Abbreviations: M = meal; C = chips.

could thus depend on the accessibility of the lignin and hence on the efficiency of pretreatments. Large differences in lignin content (from 57 to 250 g/kg) being obtained for the various samples considered in this work, the last method was chosen as the least objectionable and used for comparative purposes. Satisfactory agreement with the theoretical model is obtained for the samples oxidized by NaClO, as will appear in the next paragraphs. Too large quantities of lignin are however obtained for the acid-treated samples as indicated by the yields of recovered lignin exceeding 100% reported in Table III (formation of oxidizable groups by hydrolysis of ethers and/or esters). These results do however not change anything to the general conclusions of this work.

Effect of the Experimental Conditions on the Yield of Lignocellulosic Substrate Recovered after the Pretreatment (% Substrate Recovered)

The experimental conditions corresponding to the 15 experiments selected in the previous section are summarized in Table IV. The experimental results are given in Table Vb. The calculated coefficients LC_j and k_0 to k_6 are given in Table VI. Examination of these coefficients shows that most of the experimental variables are without effect (substrate concentration, pH) or have only a weak influence (granulometry and temperature) on the % substrate recovered. The concentration of HClO-NaClO increases this yield by 6.2% when passing from 5 to 2 mol/kg substrate. Prehydrolysis has an important influence through the removal of hemicelluloses. Using Eq. (1), and the coefficients LC_j and k_0 to k_6, the yield of recovered substrate can be calculated for the 15 experiments (Table VI). Agreement between the experimental and calculated values demonstrates the accuracy of the model. The same conclusion has been obtained for the other properties discussed below.

TABLE Va
Analysis of the Pretreated Substrates—Composition in g/kg[a]

Experiment	Li		Xy	Gl	Ma	Ga
	Exp	Calcd[b]				
1	195	174	30	540	12	0
2	119	121	23	532	11	0
3	189	205	36	524	11	0
4	183	178	27	524	9	0
5	175		27	504	13	0
6	84	81	84	401	11	9
7	98		91	391	7	11
8	131	123	39	590	9	0
9	113		37	584	17	0
10	122	133	98	390	8	6
11	116	116	102	423	9	11
12	57	64	86	402	7	9
13	117	112	29	572	6	0
14	200	195	40	479	7	0
15	83	95	26	525	11	0

[a] The content of arabinose was 2 g/kg in all cases.
[b] Calculated using Eq. (1) and the coefficients LC_j and k_0 to k_6 of Table VI.

TABLE Vb
Analysis of Pretreated Substrates—Composition in Weight Percent of the Initial Value

Exp.	Substrate		Li		Xy	Gl	Ma	Ga	C_5		$C_5 + C_6$[a]	
	Exp	Calcd[b]	Exp	Calcd[b]					Exp	Calcd[b]	Exp	Calcd[b]
1	77.7	75	96	91	23	100	61	0	21	21	98	97
2	70.8	71	53	55	16	90	52	0	15	16	89	92
3	74.0	75	75	83	22	103	65	0	21	21	99	101
4	81.3	78	94	85	21	102	51	0	20	19	99	94
5	76.1		84		19	91	68	0	18		91	
6	91.0	89	41	39	63	97	80	63	59	62	87	87
7	87.0		46		66	91	48	65	65		86	
8	65.6	66	54	48	18	103	50	0	17	18	98	96
9	65.7		47		20	102	92	0	19		99	
10	95.1	93	62	74	78	99	59	43	72	74	90	92
11	94.6	96	69	67	92	95	60	69	86	85	92	88
12	94.1	92	34	32	77	90	44	46	72	72	84	83
13	68.3	67	43	50	16	104	35	0	15	15	96	93
14	77.1	75	82	78	26	98	45	0	24	25	95	98
15	68.7	70	36	42	17	86	50	0	16	17	86	89

[a] Total balance including the hemicelluloses degraded in the prehydrolysis.
[b] Calculated using Eq. (1) and the coefficients LC_j and k_0 to k_6 of Table VI.

Lignin Content of the Pretreated Substrate

The experimental results are given in Table Va. The calculated coefficients LC_j and k_0 to k_6 are given in Table VI. They indicate that prehydrolysis and concentration of HClO-NaClO both considerably influence the lignin content. The granulometry and the temperature have a weak influence while the other

TABLE VI
Values of the coefficients $k_0, \ldots k_6$ and LC_j in Eq. (1) for Different Properties of the Pretreated Substrate

	Property					Enzymatic hydrolysis	
Coefficient	Yield of substrate (%)	Li content (g/kg)	Li recovered (%)	C_5 recovered (%)	C_6 recovered (%)	V_0 (10^{-3} g/L h)	Gl formed in 4 days
k_0	79.1	133.3	62.1	34.5	96.6	4.8	0.67
LC_0	13.7	−34.4	−8.9	36.4	—	—	—
LC_{HCl}	−7.3	22.7	7.3	−17.6	—	—	—
$LC_{H_2SO_4}$	−6.3	11.7	1.6	−18.8	—	—	—
k_1[a]	−1.3	8.3	—	−1.34	3.0	—	—
k_2[b]	−3.1	−33.7	−17.6	−3.77	—	0.40	0.56
k_3[c]	—	—	−3.7	2.64	—	—	—
k_4[d]	—	—	—	1.7	—	—	—
k_5[e]	1.1	7.6	—	−1.34	—	—	—
k_6[d]	—	—	—	3.6	—	—	—

[a] Granulometry.
[b] HClO-NaClO concentration.
[c] Substrate concentration.
[d] pH.
[e] Temperature.

factors are not significant. The lowest lignin content (45 g/kg substrate) can be obtained for nonprehydrolyzed meal pretreated with 5 mol/kg substrate at 30°C. The unfavorable effect of a prehydrolysis with dilute acid probably results from the crosslinking of the lignin network which is well known to occur in these conditions[8] and probably results in a lower degradability in the presence of hypochlorite.

Percent of the Initial Lignin Recovered in the Pretreated Sample

The experimental results and the calculated coefficients LC_j and k_0 to k_6 are given in Tables Vb and VI. The important experimental variables are the same as in the preceding case. The lowest lignin recovery (30%) as calculated from Eq. (1) can be obtained for unprehydrolyzed samples using 5 mol oxidant per kg substrate (cf. Lignin Content of the Pretreated Substrate).

Percent Pentoses Recovered after Pretreatment

The percent pentoses recovered after the pretreatment is given in Table V. The arabinose content lies near the detection limit of the analytical method; the xylose content is a significant indicator of the degradation of the hemicellulose. The LC_j and k_0 to k_6 values (Table VI) indicate that the determining factor for the removal of hemicelluloses is, as expected, the prehydrolysis. HCl and H_2SO_4 have comparable efficiencies. The lowest percent pentoses recovered (4%) can be obtained for H_2SO_4 prehydrolyzed chips pretreated using 5 mol oxidant per kg substrate, the substrate concentration being 100 g/L at pH 7 and 50°C. The highest percent pentoses recovered (85%) corresponds to unprehydrolyzed meal, pretreated with 2 mol HClO-NaClO/kg substrate, the substrate concentration being 200 g/L at pH 9 and 30°C.

Percent Cellulose Recovered Measured by the Quantity of Glucose Obtained by Quantitative Saccharification

Cellulose is almost quantitatively recovered (Table Vb). The only significant k value (Table VI) corresponds to granulometry. The calculated yield is either 99.7% for chips or 93.6% for meal.

Initial Rate and Quantity of Glucose Formed after 4 Days of Hydrolysis

The results are given in Table VII. The linear model (1) is not verified for these properties. A logarithmic model

$$\log_e Y = k_0 + LC_j + k_1x_1 + k_2x_2 + k_3x_3 + k_4x_4 + k_5x_5 + k_6x_4^2 \qquad (8)$$

was found to be obeyed. The only significant parameter was found to be the quantity of HClO-NaClO per kg substrate. Using the k values of Table VI, the maximum values of the initial rate and the quantity of glucose formed after 4 days can be calculated to be, respectively, 0.187 g/L · h and 3.43 g/L. In the same way, the minimum values of the same variables would be 0.083 g/L · h and 1.2 g/L. Examination of Table V shows that most of the results lie in this range except for experiments 8 and 9 and for experiment 2 which exhibit, respectively, too high and too low reactivity. This indicates that the experimental variables are not independent as they are considered to be in the model. For experiments 8 and 9, the initial rate and the quantity of glucose formed after 4 days are ~ 0.27 g/L · h and 8.6 g/L. Combination of some of the experimental variables (HCl prehydrolysis, chips, and 5 mol oxidant/kg substrate) leads thus to very reactive substrates. In contrast, yields which are

TABLE VII
Glucose Formation in the Enzymatic Hydrolysis of Pretreated Eucalyptus Wood

Experiment	Initial rate[a] (g/L h)	Glucose formed[b] after 4 days (g/L)	Yield of glucose after 4 days (%)
1	0.055	0.90	5.4
2	0.133	1.96	12.1
3	0.102	1.08	6.7
4	0.051	0.83	5.2
5	0.094	2.06	13.4
6	0.166	2.47	20.2
7	0.294	3.17	26.6
8	0.277	8.90	49.4
9	0.265	8.25	46.3
10	0.105	0.85	7.1
11	0.121	0.86	6.7
12	0.107	1.19	9.7
13	0.135	3.08	17.6
14	0.078	1.19	8.1
15	0.221	3.08	19.2

[a] Hydrolysis time: 1, 2, and 3 h.
[b] Similar quantities of glucose and yields are measured after 3, 4, and 6 days.

about two times lower than predicted by the model are obtained for experiment 2 using a combination of the more drastic conditions used in the present work (meal, prehydrolysis with HCl, and pretreatment with NaClO at 50°C). This can be due to the fact that the model does not take into account morphological changes of the substrate which could be a consequence of the combination of these conditions. Indeed, it has been shown previously,[9] that refluxing spruce sawdust pretreated by NaClO, with boiling water, dilute NaOH, or HCl decreases the yield of glucose although further elimination of lignin occurs in the first two cases. This has been assigned to a rearrangement of the fibrillar structure of cellulose which accompanies the treatment at 100°C. Such a rearrangement with loss of accessibility of the cellulose could

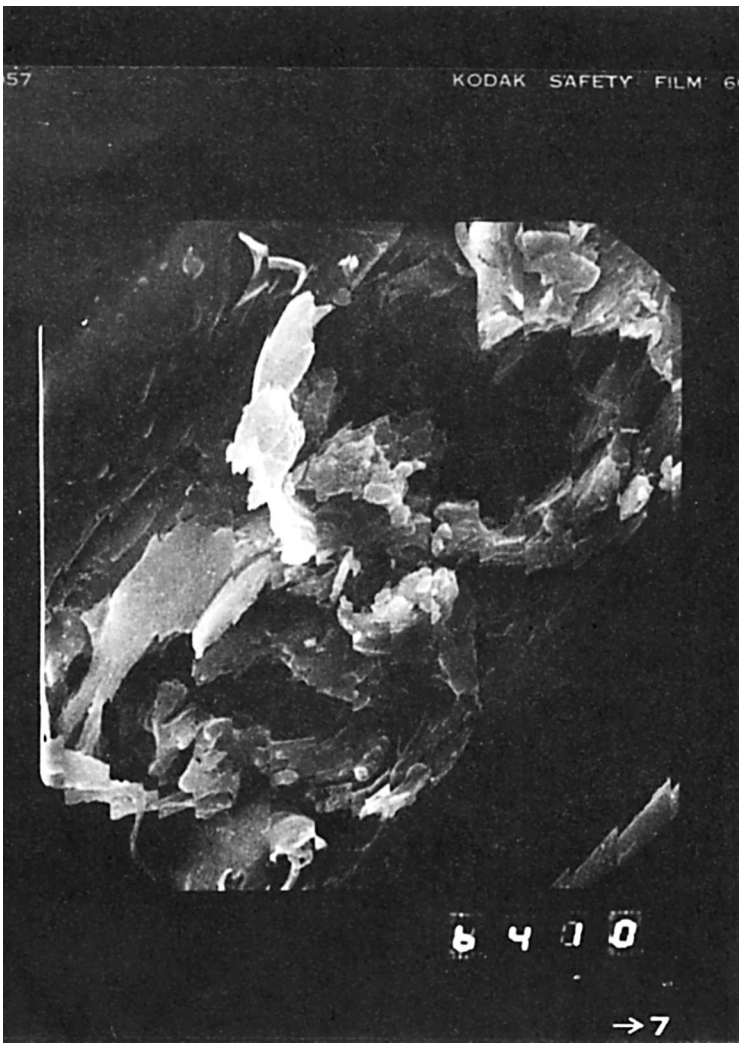

Fig. 1a. Ray-cells of untreated Eucalyptus chips.

also occur by the combination of the drastic conditions used in the present work.

The yields of glucose calculated using as 100% the potential glucose contained in the sample, are 49.4 and 46.4%, respectively, for experiments 8 and 9. These results obtained at pH 7 compare very favorably with those reported in a previous paper and obtained for Eucalyptus saligna meal[3] pretreated with a higher initial quantity of HClO-NaClO (16.6 mol/kg). In the latter case, yields of 25, 41, and 26% were obtained at pH 7, 8, and 9, the quantity of HClO-NaClO consumed being, respectively, 12.7, 9.4, and 6.5 mol/kg, whereas only 5 mol/kg was required in the present work.

It must be emphasized that the experimental conditions of hydrolysis used in the present work (enzyme and substrate concentration) have been chosen for comparative purposes and do not correspond to the highest yields and rate. Comprehensive investigation of the kinetics of the enzymatic hydrolysis of spruce sawdust pretreated with HClO-NaClO at pH 8 has shown that high rate and yields can be obtained if substrate and enzyme concentrations are

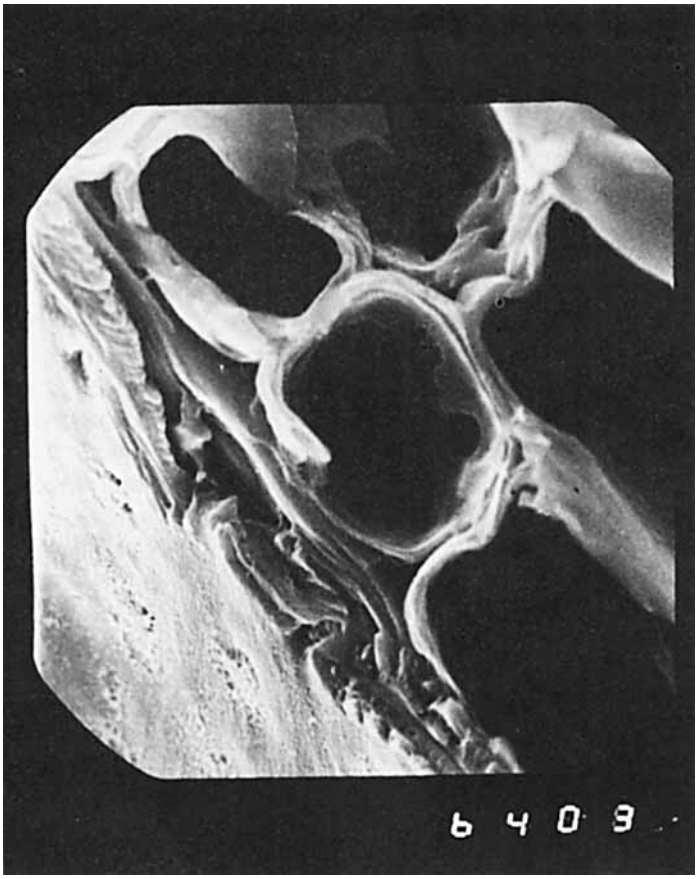

Fig. 1b. Ray-cells of Eucalyptus chips pretreated with HClO-NaClO.

suitably optimized.[10] The present work thus opens the possibility of obtaining high yields of glucose at lower oxidant consumption.

Electron Scanning Microscope Examination of the Pretreated and Untreated Substrate

Ray cells of the initial chips are shown in Figure 1(a). Their accessibility is seen to be limited by complex cellular material including polyphenols. The same cells are completely freed from this material in pretreated chips [Fig. 1(b)]. Furthermore, the cell wall region rich in lignin which joins the ray cells together is eliminated. Similar observations can be made on any kind of wood cells, pits, and vessels.

CONCLUSION

Pretreatment with HClO-NaClO has been shown previously[1-3] to increase efficiently the accessibility of ligno-cellulosic materials to chemical and biochemical reactants. The composition and reactivity of the pretreated substrate is strongly dependent on the pH of the pretreatment in the pH range 2–11.5, an optimum being obtained between pH 7 and 9.[2,3] The present work extends the former one at lower oxidant concentration in the pH range 7–9. The determining factors are oxidant concentration, prior elimination of the hemicelluloses with dilute acid, and, to a lesser extent, granulometry of the substrate. The other factors (pH between 7 and 9, substrate concentration and temperature) have a moderate or negligible effect. Delignifying factors of the order of 70% can be obtained when Eucalyptus saligna meal or sawdust is pretreated with 5 mol HClO-NaClO per kg wood at pH 7–9. Cellulose recovery is almost quantitative. The percent hemicelluloses recovered lies in the range 60–80% in the absence of any prior hydrolysis with dilute acid; such a prehydrolysis lowers the percent of hemicellulose recovered to 20%. Hemicelluloses can thus be retained or eliminated at will. The present work also shows that the properties of meal and chips are comparable. The reactivity of the pretreated substrate however decreases when the consumption of the oxidant decreases unless a prehydrolysis with dilute HCl is performed.

The value and limitations of the pretreatment by HClO-NaClO at pH 8 can now be discussed taking into account the present results and those obtained previously using higher amounts of HClO-NaClO (9.4 mol/kg). In this last case, the pretreatment compares very favorably in efficiency with other chemical pretreatments when the enzymatic hydrolysis is performed under standard conditions.[9] Indeed, the yield of glucose after 4 days is 61% for the present pretreatment using HClO-NaClO (pH 8 for 0.5 h) and 41 and 62%, respectively, for NaOH (18%, 2 h) and Cadoxen (24 h) when the substrate is spruce sawdust. Yields up to 41% have been obtained using the same standard conditions for the hydrolysis of Eucalyptus meal.[3] The yields can be increased up to 90% of the theoretical value if a twofold increase of either the enzyme concentrations or of the time of hydrolysis is used.[10] The yields of glucose obtained from spruce sawdust and Eucalyptus meal are, respectively, 12 and 0% in the absence of pretreatment.[2,3] The pretreatment with HClO-NaClO at pH 8 thus efficiently promotes the enzymatic hydrolysis. Its drawbacks are the rather high consumption of HClO-NaClO ($\simeq$ 9.4 mol/kg in the previous

results) and the loss of degraded lignins. Recent results have shown that the oxidized lignin is degraded to small nonaromatic fragments. Assimilation of these degraded fragments by micro-organisms could be possible.

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