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Empirical Assessment on the Cellulase Digestibility of Processed *Eucalyptus* Wood

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ABSTRACT

Samples of *Eucalyptus globulus* wood were delignified in HCl-catalyzed acetic acid medium under selected conditions and extracted with alkaline solutions to improve their susceptibility to enzymatic hydrolysis. The effects of three independent variables defining the operational conditions of the alkaline extraction stage on five dependent variables measuring both the chemical composition and the susceptibility of the solid residues to enzymatic hydrolysis were assessed using empirical models deduced from experimental data. The enzymatic conversion depended mainly on the NaOH concentration used in the alkaline extraction. Hydrolysis yields up to 76% were predicted for operational conditions within the range studied for the independent variables.

Index Entries: Acetic acid; delignification; enzymatic hydrolysis; NaOH; pretreatment; *Eucalyptus* wood.

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INTRODUCTION

Eucalyptus globulus is an excellent raw material for industry because of its high cellulose content and fast growth. The Northwest country of Spain produces over 1,000,000 m^3 of this type of hardwood each year, which is mainly used in making Kraft cellulose pulp.

Previous work (*1*) showed that the separation of the main *Eucalyptus* wood components into different streams can be performed using HCl-catalyzed acetic acid solutions: The lignin fraction is depolymerized and extracted with low alterations, hemicelluloses are hydrolyzed to sugars and cellulose remains in solid phase. After evaporation of the pulping liquors for solvent recovery, the sugars produced from hemicelluloses can be rediluted by water, leaving lignin as an insoluble residue (*2*). All the fractions thus obtained can be used for chemical purposes.

Eucalyptus wood is a particularly nonreactive substrate for enzymatic saccharification (*3*). HCl-catalyzed acetic acid extraction can be considered as a possible pretreatment to increase the cellulase digestibility of wood: It can be noted that the solid residue obtained using this process has a chemical composition fitting some characteristics required for good hydrolysis substrates, such as low lignin and hemicelluloses content. However, the resistance of lignocellulose to enzymatic hydrolysis depends on the combined effects of both chemical and physical-chemical properties of these materials (*4*). Besides the modification of chemical composition, a good pretreatment should reach some physical-chemical alterations of the substrate (reduction of crystallinity and polymerization index, increase of the available surface area, and so on) to render it digestible to cellulases. Since the acetic acid is essentially apolar, the physical-chemical features of substrate are not adequately modified during the extraction, giving a solid residue with poor potential as substrate for enzymatic saccharification.

The acetic acid delignified wood must be chemically treated to render it susceptible to cellulases. NaOH solutions have been selected for this second treatment, because of their ability for reaching the physical-chemical alterations cited above (*5*).

Figure 1 shows the principle of the two-step process (delignification/alkaline extraction) developed for wood utilization. Two-step processes have previously been used for the enzymatic saccharification of resistent lignocellulosic materials. Successive treatments with acid and oxidizing solutions have been reported to be an efficient way to improve the potential of *Eucalyptus saligna* wood as substrate for enzymatic saccharification (*6*).

In the work described here we report the results obtained on the chemical and biotechnological processing of *Eucalyptus globulus* wood. Samples of wood were delignified under experimental conditions chosen on the basis of our previous work, and the solid residues obtained were treated with alkaline solutions and hydrolyzed by a mixture of cellulases and β-glucosidase.

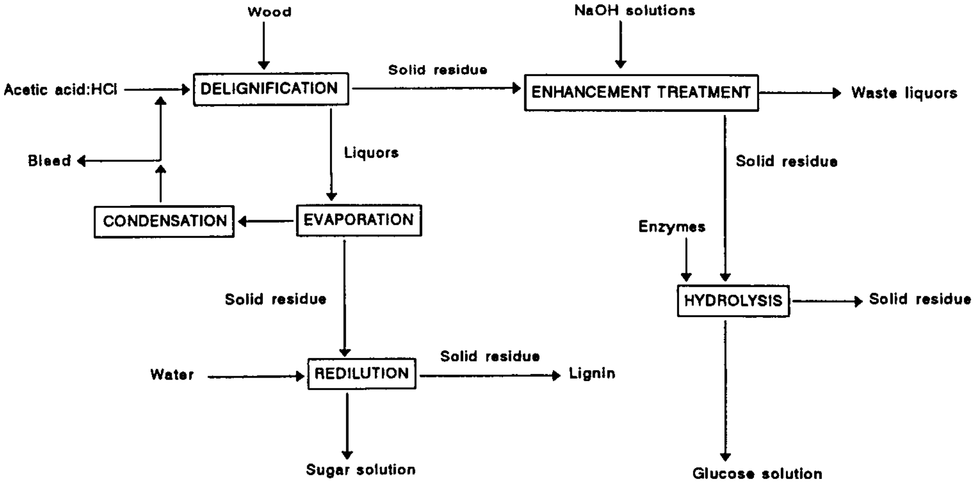

Fig. 1. Summary of the process studied for the utilization of *Eucalyptus globulus* wood.

The alkaline extraction and the enzymatic hydrolysis stages were studied using a 3×3 incomplete, centered design of experiments. Three experimental variables defining the operational conditions used in the NaOH-extraction step were considered as independent variables. Five dependent variables were used to measure the extraction yield, the changes in chemical composition of the solid residues caused by extraction, and the conversion reached when these residues were used as hydrolysis substrates.

Empirical models giving a quantitative description of the interrelationships between dependent and independent variables were deduced from the experimental data. The reliability of the model predictions was confirmed by further experimentation.

EXPERIMENTAL

Raw Material

Wood samples were collected in a local pulp mill (ENCE, Pontevedra), milled, screened to select the fraction of particles with a size between 0.2-0.5 mm, homogenized to ensure identical composition among the various aliquots taken from the wood lot, air-dried, and stored.

Analysis of Samples

The samples of wood were analyzed for moisture content and submitted to a quantitative acid hydrolysis with H_2SO_4 (*7*). The Klason lignin content was calculated from the oven-dry weight of the solid residue produced in this treatment. The polysaccharide content of samples was calculated from the sugar concentration of the hydrolyzates, which was measured by the spectrophotometric or HPLC methods reported elsewhere (*8*). The glucan content of samples was calculated from the glucose content of the same hydrolyzates, measured by the Sigma glucose-oxidase method or by HPLC.

Chemical Treatments of Wood

Samples of wood were treated with HCl-acetic acid solutions at atmospheric pressure under selected experimental conditions (*see below*). The solid residues from this treatment were submitted to extractions with NaOH solutions under a variety of experimental conditions. Aliquots of the solid phases produced in the acetic acid or alkaline treatments were subjected to moisture and yield determination, as well as to the above quantitative acid hydrolysis to measure their contents in Klason lignin, polysaccharides, and glucan.

Enzymatic Hydrolysis

The enzymatic hydrolysis of the solid residues were performed using cellulases from *Trichoderma reesei* (Celluclast, Novo, Denmark) and β-glucosidase from *Aspergillus niger* (Novozym, Novo, Denmark) (*8*). Enzymatic activities of commercial solutions were determined using reported methods (*9,10*). The operational conditions used for enzymatic hydrolysis were as follows: liquid/solid ratio = 20; temperature, 48°C; reaction time, 72 h; pH = 4.85 (acetic-acetate buffer 0.05*N*); cellulolytic activity = 0.30 FPU/mL; β-glucosidase activity = 4.0 UI/mL. Thymol was used as microbial preservative. At the end of the hydrolyses, samples from the reaction medium were filtered through 0.45 μ membranes and analyzed for total sugars and glucose by the above HPLC or spectrophotometric methods.

RESULTS AND DISCUSSION

Chemical Composition and Delignification of Wood

The samples of wood utilized for experimentation were taken from the same wood lot previously used to study the acetic-acid extraction stage (*1*). Table 1 shows the lignin, polysaccharide, and glucan content of untreated wood.

The operational conditions used in the catalyzed-acetic acid extraction of wood were chosen on the basis of our previous work (*1*). Table 1 shows the conditions selected for delignification. Several samples treated under these conditions were mixed to give a new homogenized lot that was used to measure the extraction yield and the solid residue composition. Table 1 includes the experimental results achieved.

Table 1
Data on the Composition and Delignification of *Eucalyptus* Wood

Composition of untreated wood (weight percent, oven-dried basis)
- Lignin: 25.8%
- Polysaccharides: 61.6%
- Glucan: 43.2%

Acetic acid treatment of wood
- Operational conditions
  - Temperature: 110°C
  - Liquor/wood ratio: 10/1
  - Time: 5 h
  - HCl concentration: 0.15 g HCl/100 g liquor
  - Acetic acid concentration: 95 weight percent of liquor
- Extraction yield and composition of the solid residue
  - Extraction yield: 50.8 g solid residue/100 g untreated wood, od basis
  - Solid residue composition (weight percent of residue, od basis)
    - Lignin: 7.5%
    - Polysaccharides: 84%
    - Glucan: 75.8%

The results from Table 1 show that an extensive delignification occurred during the acetic acid extraction. The percent of delignification achieved is far above the 30–60% of lignin removal that has been cited as the necessary level to significantly improve the enzymatic hydrolysis of lignocellulose (*5,11*). The low hemicellulose content of the solid residues (which can be estimated as the difference between the polysaccharide and glucan percents of samples) is also a favorable feature for the cellulase digestibility of samples. However, limited conversions (in the vicinity of 5%) were achieved using the delignified samples as hydrolysis substrates. This behavior was thought to be related to the physical-chemical characteristics of the solid residues: The acetic acid medium is essentially apolar, and it does not cause effects such as the swelling of the cellulosic material or reductions in crystallinity and polymerization index, which could promote the enzymatic hydrolysis. In order to reach these objectives, the solid residues from the delignification stage were submitted to extraction with NaOH solutions under a variety of experimental conditions.

Alkaline Treatments and Enzymatic Digestibility of the Residues

The duration of the NaOH-extractions was fixed in 1 h. Three operational variables (temperature, NaOH concentration, and liquor/solid ratio) were selected as independent variables in this study. Table 2 shows their definitions and variation ranges. The same Table includes the definitions

Table 2
Fixed, Independent and Dependent Variables Used in the Study of the Alkaline Extraction and the Enzymatic Hydrolysis Stages

Operational conditions
  Fixed variables
    Extraction time: 1 h
  Independent variables and variation ranges
    T = temperature (90–110 °C)
    C = NaOH concentration (1–9 g NaOH/100 g solution)
    LWR = liquor/wood ratio (6–12 g/g)
Dependent variables
  y_1 = residue yield, g residue recovered after alkaline treatment/100 g sample submitted to alkaline treatment, od basis
  y_2 = lignin content of alkali-treated samples, g lignin/100 g alkali-treated wood, od basis
  y_3 = polysaccharide content of alkali-treated samples, g polysaccharides/100 g alkali-treated wood, od basis
  y_4 = glucan content of alkali-treated samples, g glucan/100 g alkali-treated wood, od basis
  y_5 = conversion of enzymatic hydrolysis, g sugars produced in enzymatic hydrolysis/100 g potential sugars*

*Potential sugars: amount of sugars corresponding to the theoretical conversion of the polysaccharides contained in the substrate into monosaccharides.

of the five dependent variables ("effects") chosen to measure the extraction yield, the chemical composition of the treated residues, and their cellulase digestibility.

The interrelationship between the dependent and independent variables was assessed using empirical models derived from data obtained following an incomplete factorial design of experiments. The structure of the experimental design used in this work corresponds to an optimized design proposed by Box that has been used for the empirical modeling of the chemical or biotechnological processing of lignocellulosic materials (*12,13*). Table 3 shows the experimental conditions studied as well as the sequence followed in the experimentation, which was randomly established in order to limit the influence of systematic errors (*14,15*).

Prior to the mathematical calculations, the independent variables must be converted into dimensionless ones, having normalized variation limits (−1, 1). These dimensionless normalized variables were defined as:

x_1 = dimensionless temperature = (T-100)/10
x_2 = dimensionless NaOH concentration = (C-5)/4
x_3 = dimensionless liquor/wood ratio = (LWR-9)/3

Table 3
Structure of the Incomplete, Centered,
Second-order Experimental Design Used in this Work

Experiment	Run	T*	C*	LWR*
1	12	100	1	6
2	5	90	1	9
3	1	110	1	9
4	10	100	1	12
5	3	90	5	6
6	14	110	5	6
7	7	100	5	9
8	15	100	5	9
9	11	100	5	9
10	2	90	5	12
11	9	110	5	12
12	8	100	9	6
13	13	90	9	9
14	6	110	9	9
15	16	100	9	12
16	4	100	5	12

*T, C, and LWR as in Table 2.

where T, C, and LWR are the independent dimensional variables shown in Table 2.

Table 4 shows the experimental conditions studied (defined using the dimensionless variables x_1, x_2, and x_3) as well as the results determined in each experiment for the effects $y_1 \ldots y_5$. This information was utilized to develop second-order models following the generalized form:

$$y_j = b_{0j} + \Sigma_i b_{ij} x_i + \Sigma_i \Sigma_k b_{ikj} x_i x_k$$

where: y_j (j:1 to 5) are the dependent variables, x_i or x_k (i or k: 1 to 3, $k \geq i$) are the independent variables, and $b_{0j} \ldots b_{ikj}$ are the regression coefficients, calculated from the experimental data by the least-squares method.

Table 5 shows both the set of coefficients obtained by regression analysis and the parameters measuring the correlation and statistical significance of the models.

The calculated coefficients have been tested for statistical significance by applying a *t*-test at the 95% confidence level (*14,15*). The nonsignificant terms were dropped from the equations, and the new coefficients included in Table 6 were calculated by performing new regression calculations. The reduced models thus obtained (*see* Table 6) showed decreased R^2 values in comparison with those shown in Table 5, but their statistical significances

Table 4
Operational Conditions Studied
and Experimental Results Achieved for the Dependent Variables[a]

		Independent variables			Dependent variables				
Experiment	Run	x_1	x_2	x_3	y_1	y_2	y_3	y_4	y_5
1	12	0	−1	−1	86.0	3.0	95.8	89.2	39.1
2	5	−1	−1	0	87.8	3.4	93.6	87.7	44.5
3	1	1	−1	0	84.7	2.0	94.9	89.8	62.7
4	10	0	−1	1	81.6	1.8	95.7	91.4	49.2
5	3	−1	0	−1	75.8	3.3	96.7	91.8	52.1
6	14	1	0	−1	67.8	1.0	98.9	95.4	72.1
7	7	0	0	0	68.5	0.6	98.9	92.7	65.4
8	15	0	0	0	69.6	1.2	99.3	93.0	66.0
9	11	0	0	0	69.5	1.6	98.9	92.9	68.4
10	2	−1	0	1	75.6	1.0	95.5	93.0	61.3
11	9	1	0	1	66.6	0.8	101.2	100.1	73.8
12	8	0	1	−1	73.8	0.5	98.1	91.2	69.1
13	13	−1	1	0	72.6	2.4	98.1	95.5	61.3
14	6	1	1	0	65.5	0.2	99.2	98.5	74.4
15	16	0	1	1	67.4	0.3	100.9	97.3	65.1
16	4	0	0	1	68.3	0.3	99.2	95.8	66.1

[a] Data used to perform the regression and statistical calculations.

(measured by the "F" values) were satisfactory because of the increase in the degrees of freedom caused when dropping some terms in the models.

In order to obtain additional information allowing an estimation of the reliability of the empirical models, a new set of experiments was performed under experimental conditions different from those considered in Table 4. Table 7 includes the set of experimental conditions considered and the experimental values determined for the effects. The results calculated from the empirical equations are also included to allow an easy estimation of their predictive capacity.

Little influence of the liquor/wood ratio (measured by x_3) on the extraction yield (y_1) was observed. The main variations of yield were related to the NaOH concentration (measured by x_2). Under the mildest studied conditions ($x_1 = x_2 = x_3 = -1$, experiment 4 of Table 7), the yield was 92.5%. Severe operational conditions resulted in decreased yields. The lowest yield ($y_1 = 65.5\%$) was determined when both NaOH concentration and temperature were fixed in their upper limits ($x_1 = x_2 = 1$, experiment 14 of Table 4). It can be seen from Figure 2 that marked decreases in yield were predicted when x_2 increased from -1 to 0.

The lignin content of samples (variable y_2) showed low values and a limited variation range (0.2–4.2), leading to increased relative errors in both experimental and predicted values. However, the influence of the

Table 5
Regression Coefficients and Statistical Parameters

Coefficient	Variable (y_j) y_1	y_2	y_3	y_4	y_5
Coefficients					
b_{0j}	69.17	1.04	99.00	92.90	66.70
b_{1j}	-3.40	-0.76	1.29	1.98	7.98
b_{2j}	-7.60	-0.85	2.04	3.05	9.30
b_{3j}	-1.55	-0.51	0.44	1.81	2.20
b_{12j}	-1.00	-0.20	-0.05	0.23	-1.28
b_{13j}	-0.25	0.55	0.88	0.88	-1.88
b_{23j}	-0.50	0.25	0.73	0.98	-3.53
b_{11j}	1.38	0.55	-1.01	1.37	1.58
b_{22j}	7.13	0.43	-1.46	-1.43	-7.62
b_{33j}	0.08	-0.88	0.10	0.83	-3.38

Variable	R^2	Corrected R^2	F_{exp}*	Prob[$F_{exp} > F_{st}$]*
Statistical parameters				
y_1	0.9690	0.9224	20.81	<0.01
y_2	0.9481	0.8703	12.18	<0.05
y_3	0.9365	0.8412	9.83	<0.01
y_4	0.9202	0.8006	7.69	<0.05
y_5	0.9716	0.9220	22.80	<0.01

*F_{exp} defined as the ratio between the mean squares of model and error.

*F_{st} defined as the statistical value of F for the degrees of freedom of model and error.

residual lignin content of samples after successive treatments with acetic acid and NaOH solutions is not influential on the overall process studied for wood processing, and the variable y_2 can be considered only at a qualitative level.

The experimental values determined for the polysaccharide content (variable y_3) varied from 92.6 (experiment 4 of Table 7) up to values slightly higher than 100% (experiments 11 and 15 of Table 4). The excess of polysaccharide recoveries observed in some cases is owing to experimental errors. The liquor/wood ratio caused only limited effects on y_3. Figure 3 shows the predicted influence of x_1 and x_2 on y_3 when $x_3 = 0$. It can be seen from this Figure that the main variations of the polysaccharide content were in relation to x_2.

A new variable ("precent of polysaccharide recovery," PPR) can be defined to measure the combined effects of yield and polysaccharide content. This variable can be defined as:

$$\text{PPR} = 100\ (\text{g polysaccharides in alkali-treated wood}) \ / \ (\text{g polysaccharides in wood submitted to alkaline treatment})$$

Table 6
Regression Coefficients and Statistical Parameters
Obtained for the Models Including Only the Significant Terms

Coefficient	Variable (y_j)				
	y_1	y_2	y_3	y_4	y_5
Coefficients					
b_{0j}	70.41	1.20	99.10	90.34	67.61
b_{1j}	−3.40	−0.76	1.29	1.98	7.98
b_{2j}	−7.60	−0.85	2.04	3.05	9.30
b_{3j}	−1.59	−0.53		1.85	2.11
b_{12j}					
b_{13j}		0.53			
b_{23j}					−3.52
b_{11j}		0.56	−1.08		
b_{22j}	7.01		−1.53		−7.66
b_{33j}					−3.57

Variable	R^2	Corrected R^2	F_{exp}*	Prob[$F_{exp} > F_{st}$]*
Statistical parameters				
y_1	0.9512	0.9335	53.65	<0.01
y_2	0.8808	0.8212	14.78	<0.01
y_3	0.8400	0.7818	14.44	<0.01
y_4	0.7751	0.7289	13.79	<0.01
y_5	0.9531	0.9218	30.45	<0.01

* F_{exp} and F_{st} as in Table 5.

Table 7
Operational Conditions, Experimental and Calculated Results
Obtained for the Dependent Variables in the Experiments
Performed to Check the Reliability of the Mathematical Models

Independent variables				Dependent variables									
				Experimental values					Calculated values				
Exp	x_1	x_2	x_3	y_1	y_2	y_3	y_4	y_5	y_1	y_2	y_3	y_4	y_5
1	0	1	0	69.6	0.5	99.4	93.9	68.0	68.7	0.6	99.5	94.5	68.3
2	1	−1	1	82.7	1.8	95.2	93.5	65.0	82.4	2.0	96.5	94.1	61.0
3	−1	1	1	72.5	1.5	98.2	96.2	62.2	73.5	1.2	97.7	96.4	60.4
4	−1	−1	−1	92.5	4.2	92.6	86.5	34.1	89.3	4.7	94.4	88.9	31.1
5	1	1	−1	65.7	0.2	98.8	97.9	74.0	68.8	0	97.8	95.2	76.4
6	0	−1	0	85.8	2.2	94.5	89.0	46.8	83.8	2.3	95.5	88.4	49.7
7	1	0	0	67.3	0.8	98.9	97.1	71.7	67.1	0.8	99.2	96.3	76.2
8	−1	0	0	75.9	2.6	95.4	90.6	60.5	73.9	2.3	96.7	92.3	60.3

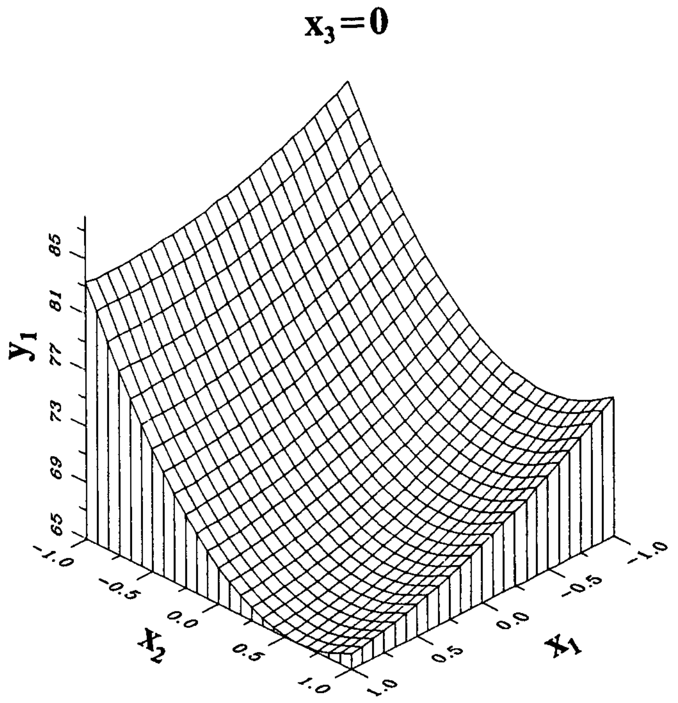

Fig. 2. Dependence of the extraction yield (y_1) on the dimensionless temperature (x_1) and on the dimensionless NaOH concentration (x_2) for extractions carried out using liquor/wood ratio = 9 ($x_3 = 0$).

Such a variable provides an easy understanding of the behavior of the polysaccharides contained in the wood samples during the extractions with NaOH solutions. The values of PPR for each experiment can be calculated from the following expression:

$$PPR = y_1 \cdot y_3 / y_{30}$$

where y_{30} is the poolysaccharide content of samples subjected to alkaline extraction (*see* Table 1).

The dependence of PPR on the experimental variables $x_1 \ldots x_3$ can be calculated using the empirical models deduced for y_1 and y_3. Figure 4 shows the dependence of PPR on x_1 and x_2 when $x_3 = 0$. The variations of PPR were most marked when x_2 increased from -1 to 0. The influence of x_1 was low in all its variation range. In the central point of the experimental design ($x_1 = x_2 = x_3 = 0$) the percent of polysaccharides retained in solid phase accounts for the 82% of the amount contained in the samples subjected to alkaline extraction.

The behavior of the glucan content (variable y_4) is closely related to that observed for y_3. Figure 5 shows the dependence of the glucan content y_4 on the dimensionless independent variables x_1 and x_2 when $x_3 = 0$.

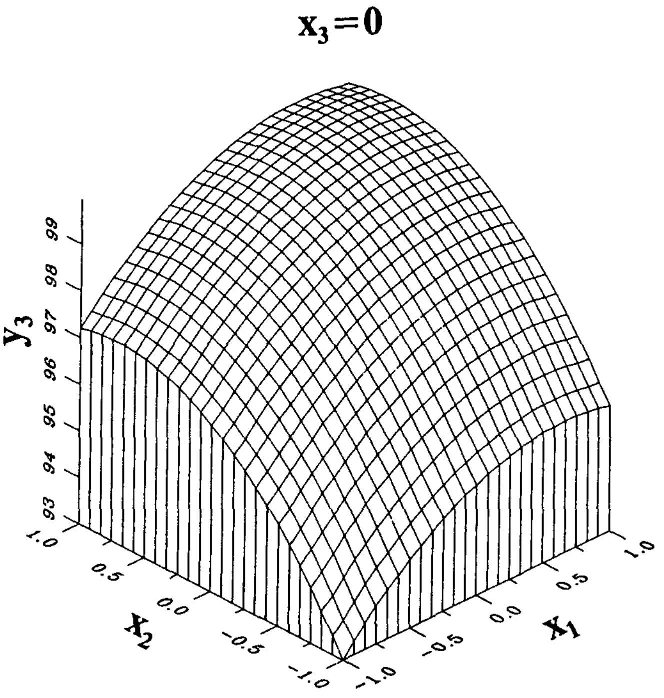

Fig. 3. Dependence of the polysaccharide content of alkali-extracted wood (variable y_3) on the dimensionless temperature x_1 and on the dimensionless NaOH concentration x_2 for extractions carried out using liquor/wood ratio = 9 ($x_3=0$).

It can be observed from this Figure that the most important relative increases of y_4 occurred when x_2 increased from −1 to 0. Little changes in y_4 were observed when the temperature was increased from 90 to 100°C ($x_2=-1$ to 0), but the glucan content of samples was significantly enhanced when temperature was increased from 100 up to 110°C.

In the same way previously considered for the "percent of polysaccharide recovery," the "percent of glucan recovery" (PGR) can be defined to measure the combined effect of yield and glucan content on the extraction, according to the expression:

PGR = 100 (g glucan in alkali-treated wood) /
(g glucan in wood submitted to alkaline treatment)

PGR can be calculated according to the following equation:

$$\mathrm{PGR} = y_1 \cdot y_4 / y_{40}$$

where y_{40} is the glucan content of samples subjected to alkaline extraction (*see* Table 1).

Figure 6 shows the dependence of PGR on x_1 and x_2 when $x_3=0$. It can be noted that for extractions carried out under mild conditions (x_1 and x_2 near −1), the glucan fraction is almost quantitatively recovered. Small effect of temperature (measured by x_1) on PGR was observed if $x_2<0$.

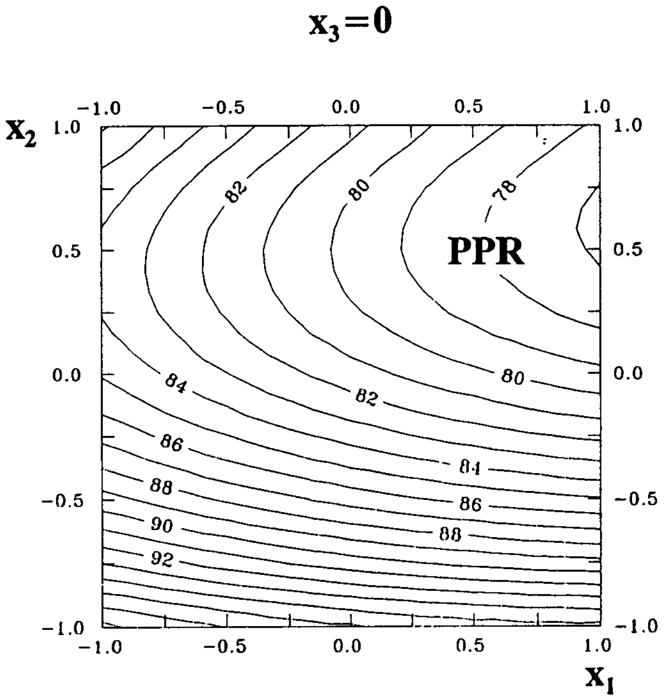
$x_3=0$

Fig. 4. Dependence of the percent of polysaccharide recovery (PPR) on the dimensionless temperature x_1 and on the dimensionless NaOH concentration x_2 for extractions carried out using liquor/wood ratio = 9 ($x_3 = 0$).

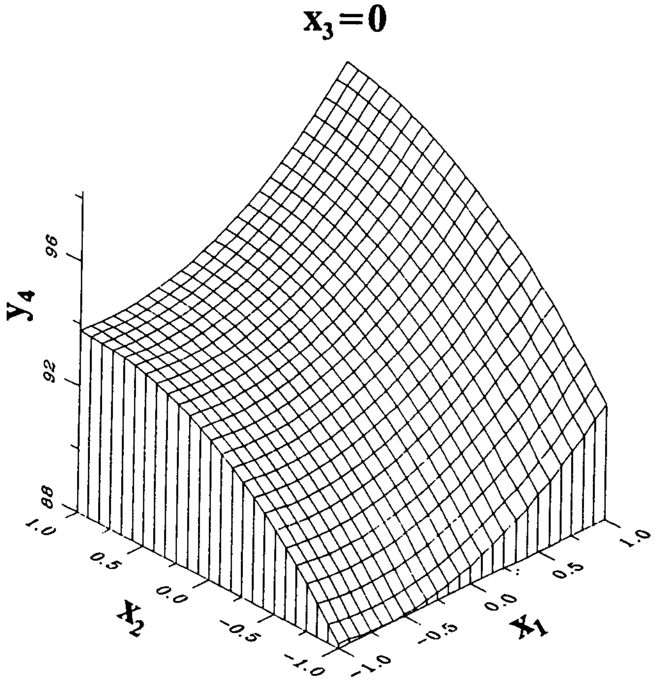
$x_3=0$

Fig. 5. Dependence of the glucan content of processed samples (y_4) on the dimensionless temperature x_1 and on the dimensionless NaOH concentration x_2 for extractions carried out using liquor/wood ratio = 9 ($x_3 = 0$).

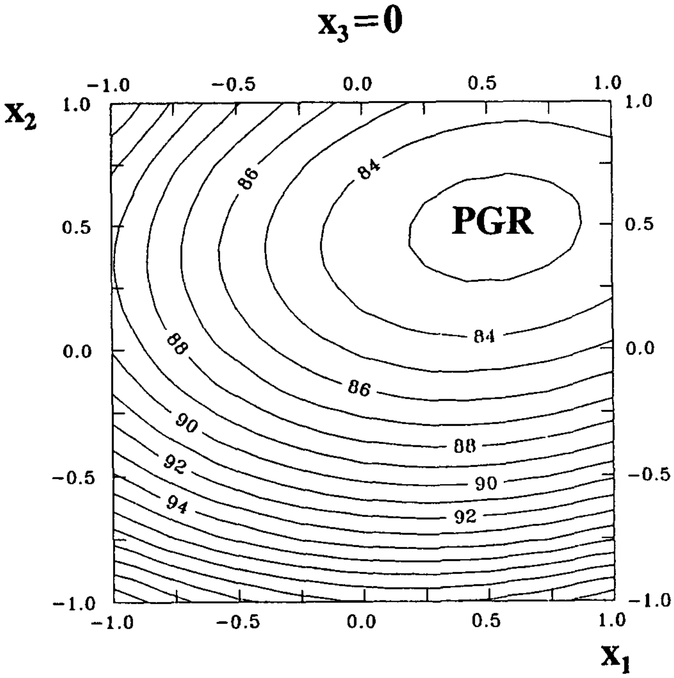

Fig. 6. Dependence of the percent of glucan recovery (PGR) on the dimensionless temperature x_1 and on the dimensionless NaOH concentration x_2 for extractions carried out using liquor/wood ratio = 9 ($x_3 = 0$).

Under experimental conditions defined by x_1 and $x_2 > 0$, the glucan fraction recovered accounts for the 83–85% of its initial amount.

The potentiality of the process proposed for the utilization of *Eucalyptus* wood depends mainly on the conversion reached in the enzymatic saccharification step. The experimental values determined for the enzymatic conversion (variable y_5) lie in the range 34.1–74.4%. These results are far above the values achieved using samples without alkaline treatment. Figure 7 shows the contour lines of y_5 dependent on x_1 and x_2 when $x_3 = 0$. It can be seen that both x_1 and x_2 significantly affected the cellulolytic digestibility of substrates. High hydrolysis yields (66–76%) were predicted for operational conditions defined by x_1 and $x_2 > 0$.

A new variable, the "percent of polysaccharide utilization" (PPU) can be defined to measure the combined effect of alkaline and enzymatic stages of the studied process. This variable can be defined as the percent of sugars produced in the enzymatic hydrolysis on the basis of the sugars that could be obtained by the quantitative saccharification of the solids before the alkaline extraction; and it can be calculated using the following expression:

$$PPU = y_1 \cdot y_3 \cdot y_5 / 100 \cdot y_{30}$$

where the several variables involved in this equation are as above.

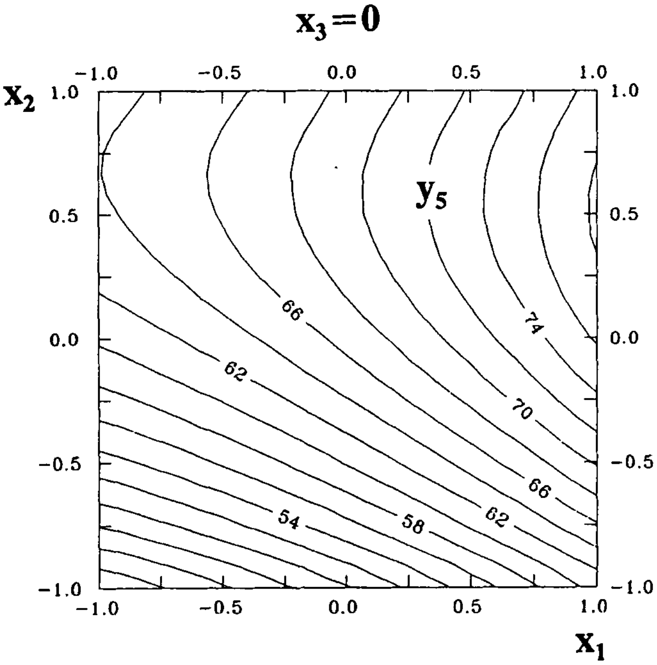

Fig. 7. Dependence of the enzymatic hydrolysis yield (y_5) on the dimensionless temperature x_1 and on the dimensionless NaOH concentration x_2 for extractions carried out using liquor/wood ratio = 9 ($x_3 = 0$).

It can be seen from Figure 8 that the predicted dependence or PPU on x_1 and x_2 is similar to that already seen for y_5. The highest percents of polysaccharide utilization were reached if x_1 and x_2 were near their upper limits. The variations observed in PPU were most important when x_1 and x_2 increased from -1 to 0, whereas PPU was not enhanced when x_2 was increased from 0 to 1.

CONCLUSIONS

Delignification of *Eucalyptus globulus* wood with HCl-catalyzed acetic acid solutions gives solid residues having high cellulose content and limited percents of lignin and hemicelluloses, but their potential as substrates for enzymatic hydrolysis is low. The cellulase digestibility of delignified wood can be notably increased by performing an alkaline treatment able to alter some physical-chemical features of solid as crystallinity, available surface area, and polymerization index.

The changes caused by NaOH-extractions in both chemical composition and enzymatic digestibility of delignified wood can be studied using empirical models deduced from experimental data. The residue yield of alkaline extractions depended mainly on the NaOH concentration of

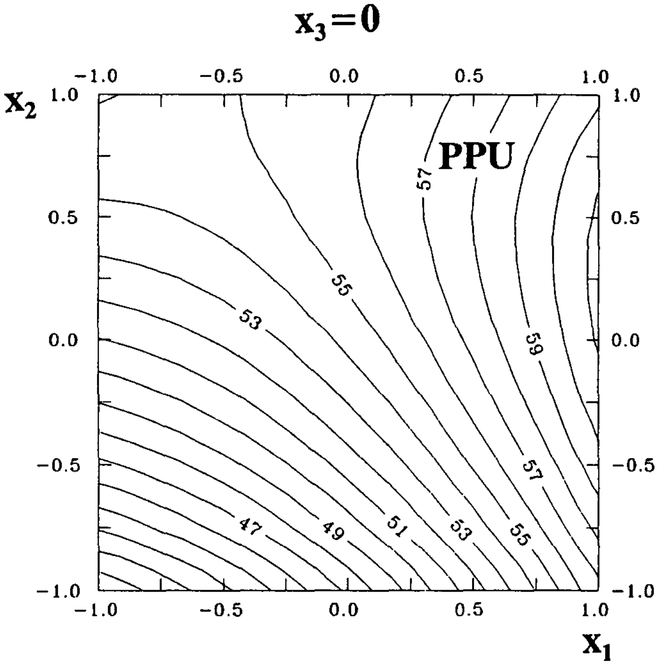

Fig. 8. Dependence of the percent of polysaccharide utilization (PPU) on the dimensionless temperature x_1 and on the dimensionless NaOH concentration x_2 for extractions carried out using liquor/wood ratio = 9 ($x_3 = 0$).

solutions. Marked decreases in yield were predicted when the NaOH concentration was raised from 1 to 5%.

The lignin content of samples showed low values and a limited variation range (0.2–4.2%). Little effect of the liquor/wood ratio on the polysaccharide content of solid residues was observed. The main effects on the polysaccharide content of solid residues were associated with variations in the concentration of the alkaline solutions.

Little influence of temperature on the percent of polysaccharide recovery (PPR) was observed. The variations of PPR were most marked when the NaOH concentration of solutions was increased from 1 to 5%. The same type of behavior was observed for the glucan content of samples. The glucan recovered in solid phase after alkaline extraction decreased with the vigor of treatments from 100 to 83%.

The potentiality of the process proposed for the utilization of wood depends mainly on the conversion reached in the enzymatic saccharification step. The experimental conversions achieved were in the range 34.1–74.4%. High hydrolysis yields (66–76%) were predicted for operational conditions defined by temperatures higher than 100°C and NaOH concentrations higher than 5%.

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REFERENCES

1. Vázquez, D., Lage, M. A., and Parajo, J. C. (1992), *Biores. Technol.* **40,** 131.
2. Nimz, H. H. and Casten, R. (1986), *Holz Roh und Werk.* **44,** 207.
3. David, C., Fornasier, R., Greindl-Fallon, C., and Vanlautem, N. (1985), *Biotechnol. Bioeng.* **27,** 1591.
4. Tanaka, M., Song, G., Matsuno, R., and Kamikubo, T. (1985), *Appl. Microbiol. Biotechnol.* **22,** 13.
5. Gharpuray, M. M., Fan, L. T., and Lee, Y. H. (1983), *Wood Agric. Residues* **20,** 369–389.
6. David, C., Fornassier, R., Lejong, W., and Vanlautem, N. (1988), *J. Appl. Polym. Sci.* **36,** 29.
7. Browning, B. L. (1967), *Methods of Wood Chemistry,* John Wiley & Sons.
8. Vazquez, G., Parajó, J. C., Antorrena, G., Thonart, Ph., and Paquot, M. (1987), *Wood Sci. Technol.* **21,** 167.
9. Mandels, M., Andreotti, R., and Roche, C. (1976), *Biotechnol. Bioeng. Symp.* **6,** 21.
10. Paquot, M. and Thonart, Ph. (1982), *Holzforschung* **36,** 177.
11. Sudo, K., Matsumura, Y., and Shimizu, K. (1976), *Mokuzai Gakkaishi* **22,** 670.
12. Poirier, M. G., Ahmed, A., Grandmaison, J. L., and Kaliaguine, S. C. F. (1987), *Ind. Eng. Chem. Res.* **26,** 1738.
13. Parajó, J. C., Alonso, J. L., Lage, M. A., and Vázquez, D. (1992), *Bioprocess Eng.* in press.
14. Box, G. E. P., Hunter, W. G., and Hunter, J. S. (1988), *Estadistica para investigadores.* Ed. Reverté.
15. Akhnazarova, S. and Kafarov, V. (1982), *Experiment optimization in Chemistry and Chemical Engineering.* MIR Publishers.