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Development of a Generalized Phenomenological Model Describing the Kinetics of the Enzymatic Hydrolysis of NaOH-Treated Pine Wood

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ABSTRACT

Pinus pinaster wood samples were treated during 3 h with alkaline solutions (containing 1, 5.5, or 10 weight percent NaOH) at 100, 115, or 130°C using liquor/wood ratios of 6, 8, or 10 g/g. The solid residues obtained in treatments were used as substrates for enzymatic hydrolysis. In the hydrolysis assays, the reaction time (in the range 0–48 h) was considered as an operational variable. The cellulose conversion achieved at the end of assays were highest for samples pretreated at high temperature and high alkali concentration using low liquor/wood ratios. The experimental results obtained in each hydrolysis trial were fitted to an empirical model based on the assumption that the cellulose contained in substrates was composed by two fractions having different susceptibility to hydrolysis. The kinetic parameters obtained for the various experiments performed were correlated with the operational variables by means of empirical, statistically significant equations, which provided a generalized interpretation of the process.

Index Entries: Alkaline treatments; cellulose; enzymatic hydrolysis; kinetic modeling; pine wood.

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INTRODUCTION

It is well known that native lignocellulosic materials (LCM) are poor substrates for enzymatic hydrolysis, since the cellulose chains of the plant cell wall are embedded in a matrix of lignin and hemicelluloses that limits the accessibility of enzymes. In order to improve the kinetics and yields of hydrolysis, LCM must be subjected to physical and/or chemical pretreatments.

An ideal pretreatment should cause several effects, including:

1. delignification and hemicellulose hydrolysis, for rendering the cellulose accessible; and
2. modification of structural and physicochemical features of substrates (such as crystallinity, degree of polymerization, available surface area, water-holding ability, and pore-size distribution) for enhancing the enzymatic attack.

During the last decades, a variety of pretreatments for LCM have been proposed in literature. A comparative study on the feasibility of different pretreatments should be formulated on the basis of both technical and economic considerations. However, the evaluation of the feasibility of a given procedure requires extensive information, which is not available in the most part of cases. For this purpose, generalized kinetic models could provide a valuable help.

The modeling of the kinetics of enzymatic hydrolysis has been attempted from both theoretical and empirical approaches (*1*). The validity of theoretical models for describing the kinetics of the process is limited, because a rigorous approach should consider a variety of complex phenomena (for example, nature of feedstock, composition, particle size and physicochemical characteristics of the substrate and their evolution with the reaction course, interfacial mass transfer principles in the heterogeneous system, adsorption-desorption processes, synergistic effects among the enzyme components of cellulases, thermal and chemical deactivation of catalyst, product[s] inhibition, and so on).

On the contrary, empirical models provide an interesting alternative for describing the hydrolysis kinetics. For example, the hyperbolic model has been widely employed (*2–5*). Other models assumed the existence of substrates containing two cellulose fractions with different susceptibility to enzymatic hydrolysis, which are hydrolyzed to sugars with different rate following a first-order kinetics (*1,6*).

This work deals with the formulation and generalization of kinetic models describing the enzymatic hydrolysis of pine wood pretreated with NaOH solutions. With a reasonable amount of experimental deal, a generalized kinetic model has been derived. Wood samples were treated during 3 h with alkaline solutions under a variety of experimental conditions, and the solid residues obtained in treatments were used as substrates for enzymatic hydrolysis. The sugar concentration/time series of data obtained in each hydrolysis run were fitted to an empirical model based on the assumption that the substrates contained two cellulosic fractions, which reacted with the same hyperbolic kinetic pattern, but at different reaction rates. The set of kinetic parameters obtained by regression of data were correlated with the operational conditions used for pretreating the respective substrates, leading to equations useful for predicting the polysaccharide conversion.

MATERIALS AND METHODS

Composition and Processing of the Raw Material

Pinus pinaster wood chips were randomly taken from a local particleboard mill, milled to pass a 0.5-mm screen, air-dried, homogenized in a single lot, and stored. The chemical composition of untreated pine wood was measured by standard methods as it was reported elsewhere (*7*). The contents of wood in lignin, cellulose, and hemicelluloses were 30.2%, 42.9%, and 17.6%, respectively.

Alkaline Pretreatment of Wood

The alkaline treatments were carried out during 3 h in a batch reactor. The duration of treatments was selected according to previous experiments (unpublished results). The operational variables chosen were temperature (100, 115, or 130°C), liquor/wood ratio (6, 8, or 10 g/g), and NaOH concentration (1, 5.5, or 10 g/100 g solution). The experimental plan was based on an incomplete, second-order, factorial design of experiments previously reported in literature (*8*). With a set of 16 experiments (exploring 14 different combinations of the experimental variables, and two additional replicates for evaluating the experimental error), empirical models including linear, interaction, and second-order terms can be derived. The experimental plan was completed with 8 additional experiments, selected for improving the reliability of the model predictions. Figure 1 shows the operational conditions assayed in our study. The residue yield of treatments and the chemical alterations caused by extraction were previously correlated with the operational conditions (*7*). In the present study, these reported results were used for estimating the ''potential sugar concentrations'' corresponding to the various hydrolysis media formulated from the pretreated wood samples, which were necessary for measuring the polysaccharide conversion.

Enzymatic Hydrolysis

Wood samples pretreated under the conditions of Fig. 1 were used as substrates for hydrolysis experiments. The reaction media contained 20 g

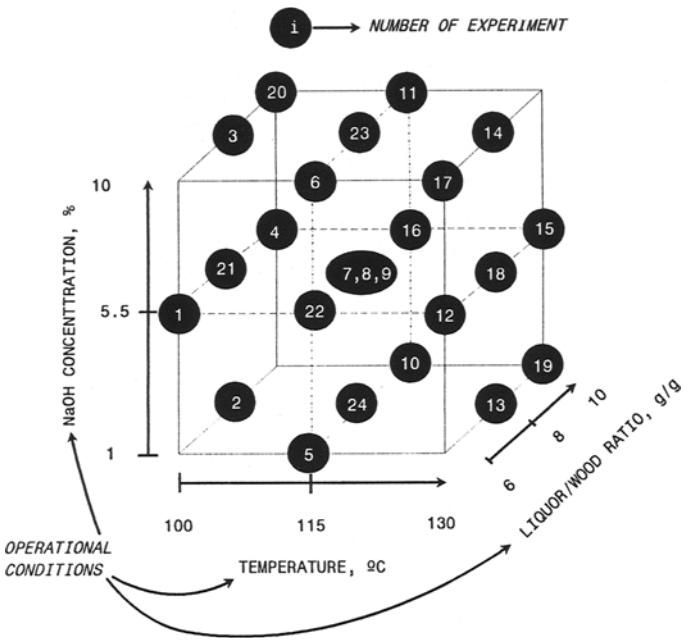

Fig. 1. Structure of the experimental design used in this work.

solution/g substrate (o. d. basis), 0.30 FPU/mL of cellulases from *Trichoderma reesei,* and 4 IU/mL of β-glucosidase from *Aspergillus niger.* Concentrated enzyme solutions were obtained from Novo Nordisk (Madrid, Spain). The reaction media were maintained at pH 4.85 during the hydrolysis runs using a citric acid-citrate buffer 0.05*N*. The hydrolyses were conducted at 48.5°C during 48 h in Erlenmeyer flasks with magnetic stirring. At given reaction times, samples were withdrawn from the reaction media, filtered, and analyzed for reducing sugars by the Somogyi-Nelson method. The sugar concentrations determined were used for estimating the polysaccharide conversions. These polysaccharide conversions were calculated as the percent of the "potential sugar concentration" corresponding to a given reducing sugar concentration.

Correlation of Data

The sugar concentration/time series of data corresponding to individual hydrolysis runs were fitted to hyperbolic models (considering one or two cellulose fractions) by nonlinear regression using commercial software (TableCurve from Jandel Scientific, Corta Madera, CA). The kinetic parameters were correlated with the operational conditions using statistical software (SPSS).

RESULTS AND DISCUSSION

Kinetic Modeling

The experimental [sugar concentration]/time series of data obtained with the substrates pretreated under the conditions shown in Fig. 1, were fitted to the hyperbolic model suggested by Holtzapple et al. (*3*):

$$X = X_{\infty} . t / (t + \tau) \quad (1)$$

where X is the polysaccharide conversion achieved at time t, X_{∞} is the conversion predicted for infinite reaction time and τ is the reaction time necessary to achieve the 50% of X_{∞}. The correlations obtained were satisfactory for goodness of fit, with Fischer parameters F_{exp} in the range 94–150 and correlation coefficients R^2 in the range 0.91-0.99. However, it was observed that for short reaction times, the hyperbolic model systematically predicted conversions lower than the experimental results (*see below*). Owing to this, the parameter τ (which is inversely proportional to the initial reaction rate) did not show a coherent variation pattern with the operational conditions.

On this basis, a modified kinetic model was studied. In the same way proposed in literature (*1,6*), it was thought that the behavior observed could be owing to the existence of two cellulose fractions in the substrates, having different susceptibility to hydrolysis. Assuming that α is the percent of ''easily hydrolyzable'' cellulose, and that both fractions react according to an hyperbolic kinetics, the following equation was assayed for fitting the experimental data:

$$X = \alpha . t / (t + \tau_1) + (100\text{-}\alpha) . t / (t + \tau_2) \quad (2)$$

where X is as above, and τ_1 and τ_2 are the reaction times necessary for achieving 50% conversion of the ''easy'' and ''difficult'' fractions of cellulose, respectively. It can be noted that, after infinite reaction time, this model predicts 100% polysaccharide conversion (according to the predictions of theoretical models) instead of value X_{∞} predicted by the hyperbolic model (3).

A preliminary analysis of data was carried out according to Eq. 2, taking α, τ_1, and τ_2 as regression parameters. It was observed that the model provided an excellent correlation, giving a very close interpretation of the experimental results (particularly, at short reaction times). Furthermore, the values obtained for α were distributed in a narrow variation range, with a mean value of 20. This result agrees with the lower limit of the easily hydrolyzable fraction reported by Chum et al. (*6*) in studies of the saccharification of pretreated poplar wood. New regression calculations were done assuming that $\alpha = 20$, and it was also observed an excellent fitting of data for individual experiments. As a basis for comparison, Fig. 2 shows some representative experimental data and the predictions

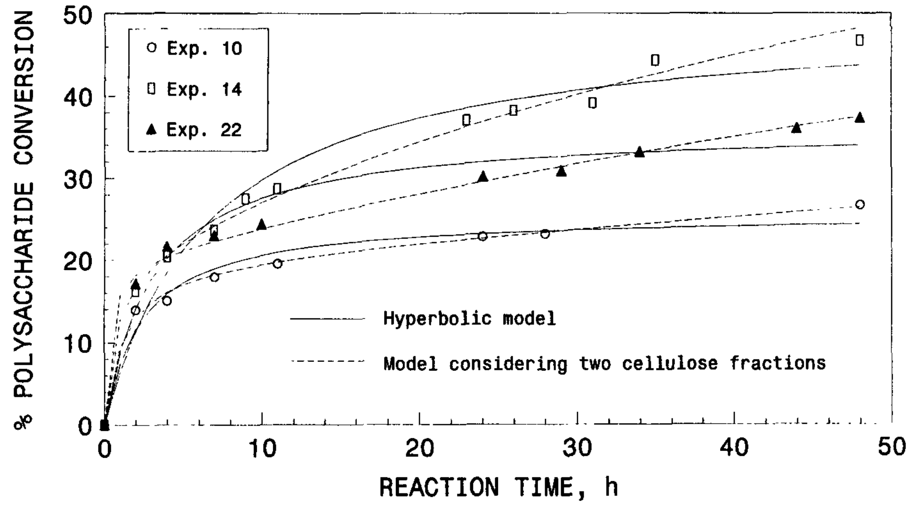

Fig. 2. Experimental and predicted results corresponding to representative hydrolysis assays.

of both the hyperbolic model (Eq. 1) and the model with two fractions assuming the above considerations, i. e.,

$$X = 20.t/(t + \tau_1) + 80.t/(t + \tau_2) \qquad (3)$$

Table 1 lists the regression parameters τ_1 and τ_2 calculated from the experimental data obtained in hydrolysis assays performed with substrates pretreated in the conditions of Fig. 1. The same Table includes the R^2 and F_{exp} parameters measuring the correlation and statistical significance of the equations.

Generalization of the Kinetic Model

In order to obtain a generalized model for describing the process, the dependence of the kinetic parameters listed in Table 1 with the operational conditions was studied. For this purpose, empirical models (including linear, interaction, and second-order terms in the independent variables) were assayed. The same type of correlation between independent and dependent variables has been previously used by authors in the study of the enzymatic hydrolysis of alkali-pretreated wood (*9*). The operational conditions used for pretreating the substrates (temperature, alkali concentration, and liquor/wood ratio) were considered as independent variables, and the dependent ones were the kinetic parameters τ_1 and τ_2. This procedure for generalization of data is conceptually related with the ideas suggested by Koullas et al. (*5*), dealing with the enzymatic hydrolysis of straw: these authors found a dependence between the kinetic parameters and the physicochemical characteristics of substrates, which are dependent on the conditions used for pretreatments.

Table 1
Kinetic and Statistical Parameters Obtained From the Experimental Results

Exper.	τ_1 (h)	τ_2 (h)	R^2	F_{exp}[a]
1	0.43	132	0.9886	608
2	1.07	443	0.9900	593
3	1.95	259	0.9894	658
4	1.02	522	0.9770	297
5	0.36	574	0.9872	310
6	0.77	98	0.9968	2214
7	0.69	234	0.9975	2392
8	0.34	200	0.9890	449
9	0.76	271	0.9948	1359
10	1.18	512	0.9948	1159
11	0.54	114	0.9777	308
12	0.49	177	0.9974	2703
13	0.84	534	0.9968	2219
14	0.70	87	0.9940	1489
15	1.12	196	0.9926	1208
16	0.85	270	0.9818	487
17	0.69	87	0.9958	1916
18	0.75	210	0.9995	1738
19	0.76	581	0.9990	8822
20	1.55	337	0.9993	1198
21	1.02	331	0.9978	3598
22	0.32	172	0.9962	2099
23	1.07	134	0.9955	1788
24	0.75	827	0.9939	1297

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

According to the above considerations, the kinetic parameters were related with the operational variables by the equation:

$$\tau_i = b_{oi} + b_{1i} \,.\, N_T + b_{2i} \,.\, N_L + b_{3i} \,.\, N_c + b_{12i} \,.\, N_T \,.\, N_L + b_{13i} \,.\, N_T \,.\, N_C + b_{23i} \,.\, N_L \,.\, N_C + b_{11i} \,.\, N_T^2 + b_{22i} \,.\, N_L^2 + b_{33i} \,.\, N_C^2 \tag{4}$$

where τ_i (i: 1 or 2) denotes τ_1 or τ_2, b_{oi}...b_{33i} are regression coefficients, and N_T, N_L, and N_C are the independent variables expressed in normalized, dimensionless form *(10)*, which were related with the operational variables (temperature T, liquor/wood ratio LWR, and NaOH concentration C) by the equations:

$$N_T = (T\text{-}115)/15 \tag{5}$$

$$N_L = (LWR\text{-}8)/2 \tag{6}$$

$$N_C = (C\text{-}5.5)/4.5 \tag{7}$$

Table 2
Regression Coefficients and Statistical Parameters

Coefficient	Depend. Variables τ_1	Depend. Variables τ_2
b_{0i}	0.688	262.2
b_{1i}	-0.195	-40.4
b_{2i}	0.204	44.8
b_{3i}	0.116	-222.6
b_{12i}	-0.077	-86.3
b_{13i}	-0.182	-62.7
b_{23i}	-0.170	-0.2
b_{11i}	0.215	-3.97
b_{22i}	-0.170	-24.6
b_{33i}	-0.190	111.3

Parameter	Variable τ_1	Variable τ_2
F_{exp}[a]	4.1	9.8
Prob $F_{st} > F_{exp}$[a]	<0.01	<0.01
R^2	0.7268	0.8633

Significant coefficients at the 95% confidence level: b_{11}, b_{21}, b_{111}, b_{32}, b_{122}, b_{332}. Significant coefficients at the 90% confidence level: b_{131}, b_{331}, b_{22}, b_{132}.

[a] 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.

Regression analyses were performed according to the empirical model (eq. 4) using both the values of dependent variables listed in Table 1 and the correspondent values of the dimensionless, independent variables calculated from Fig. 1 by equations 5-7. The regression coefficients obtained, as well as the statistical parameters R^2 and F_{exp} are shown in Table 2. Additional information on the statistical significance of coefficients (based on a *t*-test) is shown in the same Table.

From the data listed in Table 2, it can be observed that for τ_2 the dominant effect was N_C, while all three effects were significant for τ_1. For all these four effects, both linear and quadratic terms were significant, but signs of the linear and quadratic terms were opposite in each case. The equations assayed for both τ_1 and τ_2 showed a high level of statistical significance.

In order to provide an easier understanding of the model predictions, Figs. 3 and 4 show the dependence of the kinetic parameters τ_1 and τ_2 on the most influential operational variables.

It can be seen from Fig. 3 that, for samples treated with 5.5% NaOH, both decreases in temperature and increases in liquor/wood ratio resulted

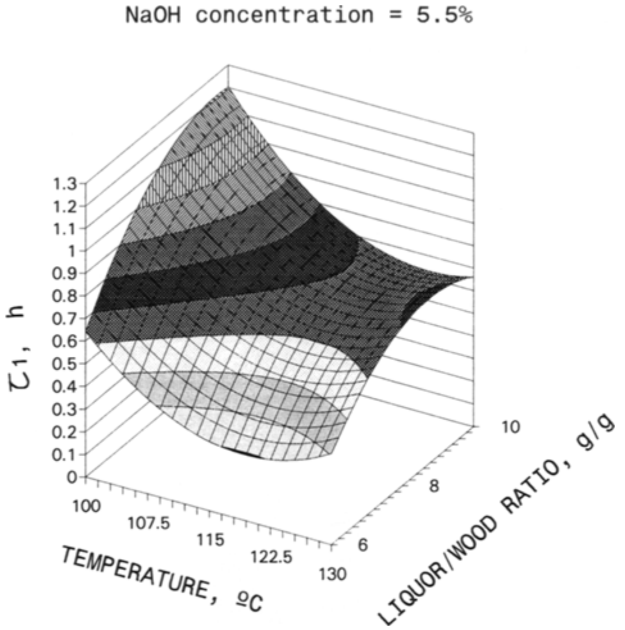

Fig. 3. Predicted dependence of the kinetic parameter τ_1 on temperature and liquor/wood ratio for samples treated with 5.5% NaOH.

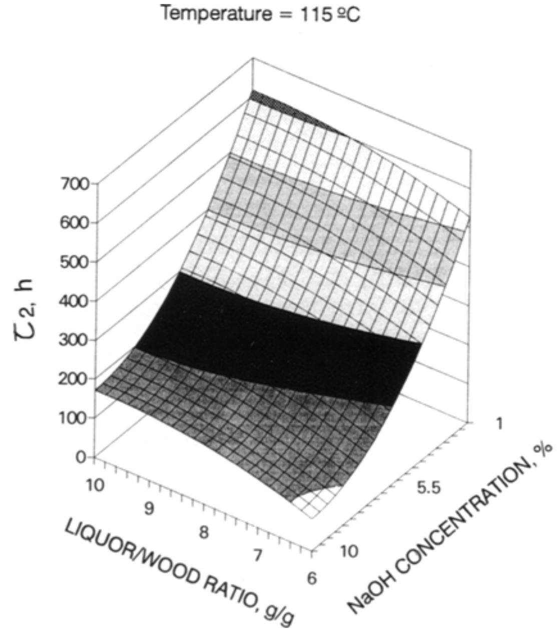

Fig. 4. Predicted dependence of the kinetic parameter τ_2 on liquor/wood ratio and NaOH concentration for samples treated at 115°C.

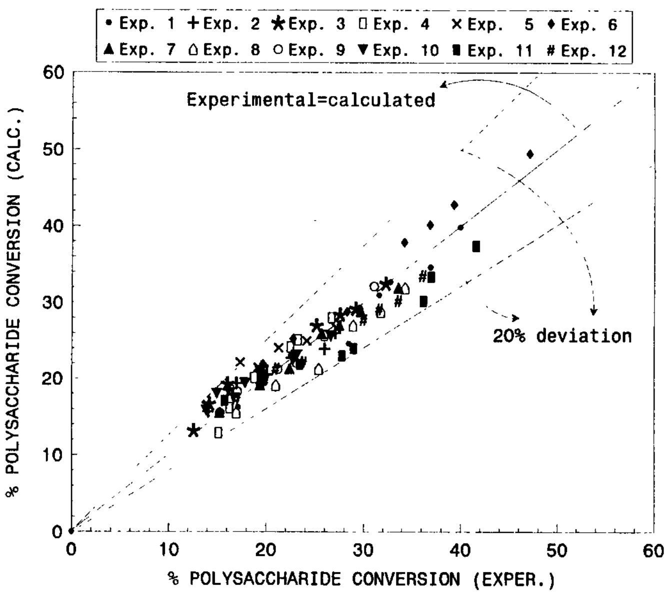

Fig. 5. Agreement between experimental and calculated polysaccharide conversions.

in increased values of τ_1 (the kinetic parameter corresponding to the "easily-hydrolyzable" fraction). The values predicted for τ_1 lied in a narrow variation range (0.3–1.2 h). The effects caused by both liquor/wood ratio and temperature were most important in the intervals 6–8 g/g and 100–115°C, respectively, and their influence decreased markedly under operational conditions within the ranges 8–10 g/g and 115–130°C.

Figure 4 shows the predicted behavior of the kinetic parameter τ_2 (corresponding to the "difficult" fraction) on NaOH concentration and liquor/wood ratio for samples treated at 115°C. τ_2 depended strongly on the NaOH concentration used, being the major effects associated to increases in alkali concentration from 1 up to 5.5%. Values of τ_2 higher than 600 h were predicted for samples treated at 115°C with the lowest alkali concentration (1% NaOH) using the highest liquor/wood ratio assayed (10 g/g).

For given operational conditions, the above equations allow the prediction of the correspondent polysaccharide conversion. In order to assess the reliability of the predictions derived from this procedure, Fig. 5 presents a comparison between the experimental and predicted results corresponding to representative experiments. The generalization of the kinetic parameters by Eq. 4 was responsible for the most part of the deviations observed between experimental and calculated results, but the ability of the method for reproducing the experimental data was satisfactory, with an error lower than ±20% for the 94% of data.

On the basis of the above results, it can be concluded that the empirical modeling of enzymatic hydrolysis and the further generalization of kinetic parameters can be an interesting strategy for describing the process. The [sugar concentration]/time series of data obtained under the various experimental conditions assayed were fitted to a modified hyperbolic model that provided a close interpretation of the experimental data. The set of kinetic coefficients calculated from the proposed model showed a coherent variation pattern with the experimental variables. On the basis of a reasonable amount of experimental deal, the analysis of data proposed in this work allowed the generalization of the kinetic parameters, leading to an easy evaluation of the influence of selected operational variables on the course with time of the hydrolysis reaction. The correlations between dependent and independent variables deduced were characterized by a wide range of applicability, providing a valuable help for simulation and optimization purposes.

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