

Microcrystalline-Cellulose Hydrolysis with Concentrated Sulphuric Acid

F. Camacho, P. González-Tello, E. Jurado* & A. Robles

Departamento de Ingeniería Química, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain

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Abstract: The effects of temperature (25–40°C), H₂SO₄ concentration (31–70% (w/v)) and the acid/substrate relationship (1–5 cm³ of H₂SO₄ per g⁻¹ of cellulose) on the solubilization rate of microcrystalline cellulose and on the glucose production rate have been analysed. The solubilization process was by determining reducing groups present in solution. For acid/substrate relationships of more than 1 cm³ g⁻¹ and H₂SO₄ concentrations of greater than 62% (w/v), the acid promoted the total solubilization of the cellulose in the form of chains with a low degree of polymerization within 4 h. The solubilization demonstrated zero-order kinetics in which the specific rate and time of total solubilization are a function of the variables in operation. Glucose was produced according to a mechanism of two consecutive first-order pseudo-homogeneous reactions. The values of the kinetic constants k_1 and k_2 have been correlated with temperature, the H₂SO₄ concentration and the acid/substrate relationship.

Key words: acid hydrolysis, pretreatments, cellulose, cellulose solubilization

1 INTRODUCTION

Enzymatic hydrolysis of cellulose contained in the lignocellulose residues takes place at a low rate and offers a meagre yield due to lignin, which represents a physical barrier in terms of its crystalline structure and the small area of substrate–enzyme contact. The pretreatments of the residues to eliminate this difficulty can be physical or chemical; the latter, more effective, involves of two types: acids and bases.

The pretreatments with dilute acids (usually HCl and H₂SO₄) at low temperatures alter the crystalline structure, making the substrate spongy (swelling effect) by facilitating the penetration of water molecules into the cellulose crystals, thereby expanding the specific surface area. Nevertheless, with expansion the acid concentration provokes firstly the individualization of the cellulose molecules, and secondly a breaking up of these

molecules, rendering products with a low degree of polymerization. This acid hydrolysis leads to the solubilization of the substrate and the formation of glucose as the end product.^{1–3} Thus, the acid pretreatments of cellulose can serve a dual aim: as preparation for enzymatic hydrolysis and as hydrolytic treatments to produce fermentable sugars.

The pretreatments with concentrated H₂SO₄ (\approx 70% (w/v)), at room temperature, solubilize the cellulose by breaking the hydrogen bonds between molecules; this cellulose can reprecipitate by adding water or methanol to the solution, thereby producing an amorphous cellulose with a large specific surface area. However, the rupture of the β -(1,4) bonds can also give rise to the formation of sugars of low polymerization which do not precipitate but perhaps would in large part be usable later in fermentation.^{2,3}

Acid hydrolysis of cellulose occurs at a faster rate and the acids used are cheaper than the enzymes; however, enzymatic hydrolysis is a cleaner and more selective

* To whom correspondence should be addressed.

process that takes place at moderate pressures and temperatures. Most authors report using acid hydrolysis with dilute acids and high temperatures, conditions which raise the cost of the equipment, increase the corrosion and, given that the process is not highly selective, the products can be difficult to ferment.⁴⁻⁶

2 MATERIALS AND METHODS

In all the experiments, a flask containing 1 g of microcrystalline cellulose Merck 2330 (Avice) in 100 cm³ precipitate vessel was placed in a thermostatic bath at a desired temperature. Simultaneously, a volume of H₂SO₄ was adjusted to the concentration desired. When the two substances reached working temperature, a given volume of H₂SO₄ was mixed vigorously with the cellulose, and the mixture was kept under thermostatic control for the treatment time desired. In all cases, the reaction was stopped by neutralizing the acid with a solution of 1.3 mol dm⁻³ NaOH. The resulting suspension at a known volume was vigorously stirred for 15 min and the concentrations of the reducing sugars, AR were determined (expressed as glucose equivalents), using the DNS method of Miller,⁷ as was the glucose, using the glucose-oxidase method of Werner *et al.*⁸

The temperatures investigated were 25, 30, 35 and 40°C; the H₂SO₄ concentrations (C_A) were varied from

31% to 70% (w/v) (31.0, 45.0, 58.5, 62.0 and 70.0%); and the acid/substrate relationships (D/S) were 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 cm³ of acid per g⁻¹ of substrate.

3 RESULTS AND DISCUSSION

3.1 Cellulose solubilization

Figures 1, 2 and 3 present the yield of soluble reducing sugars against hydrolysis time. In most of these curves, two periods are clearly differentiated: the first (dissolution phase), in which the production rate of the reducing sugars is constant and appears not to depend on the quantity of undissolved substrate; a second phase (stabilization phase), in which the velocity abruptly decreases, becomes nil, and in some cases slightly negative (Figs 1 and 2), due to the possible degradation reactions of the solubilized sugars. It was experimentally verified that the total dissolution of the cellulose by conversion to reducing sugars practically coincided with the end of the dissolution phase. However, in the experiments corresponding to a D/S relationship of 1 cm³ g⁻¹ (Fig. 3) and for acid concentrations of less than 62% (Fig. 2), it was confirmed that only a small fraction of the initial cellulose was solubilized (between 8% and 15% at 20 h).

These results indicate that at any temperature, for $C_A \leq 62\%$, the H₂SO₄ served fundamentally as a swell-

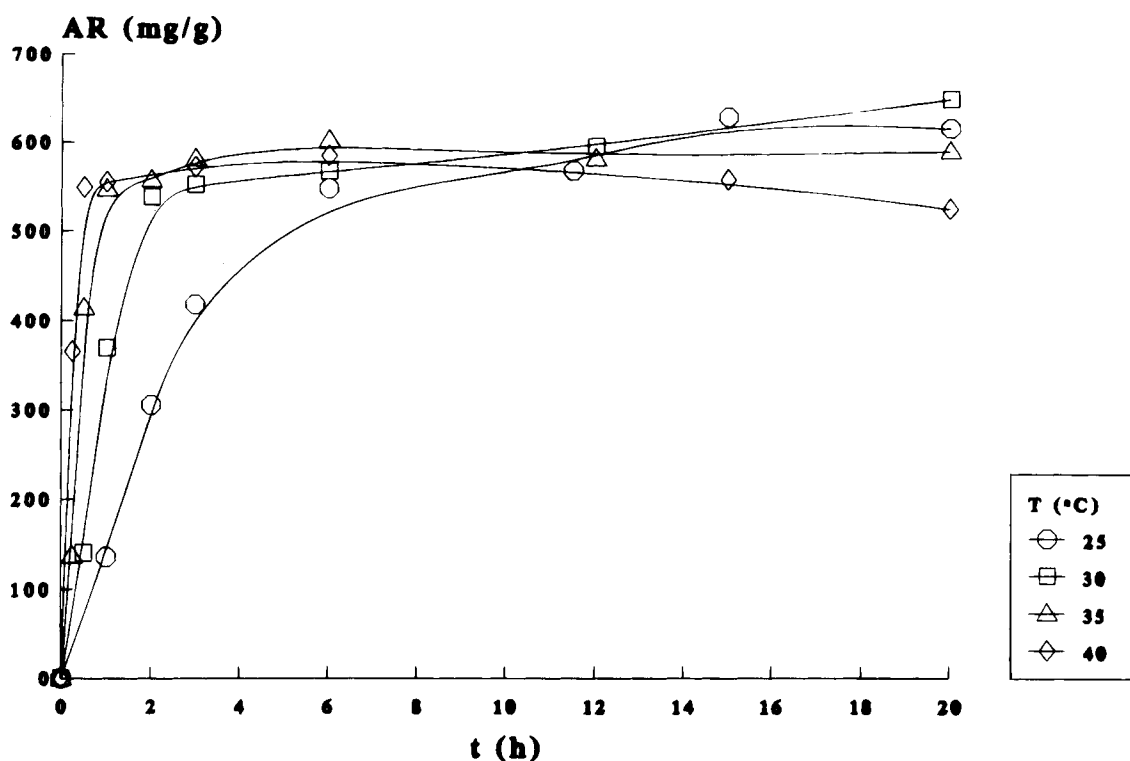


Fig. 1. Influence of temperature on the cellulose-dissolution rate (H₂SO₄ at 70% and $D/S = 3 \text{ cm}^3 \text{ g}^{-1}$).

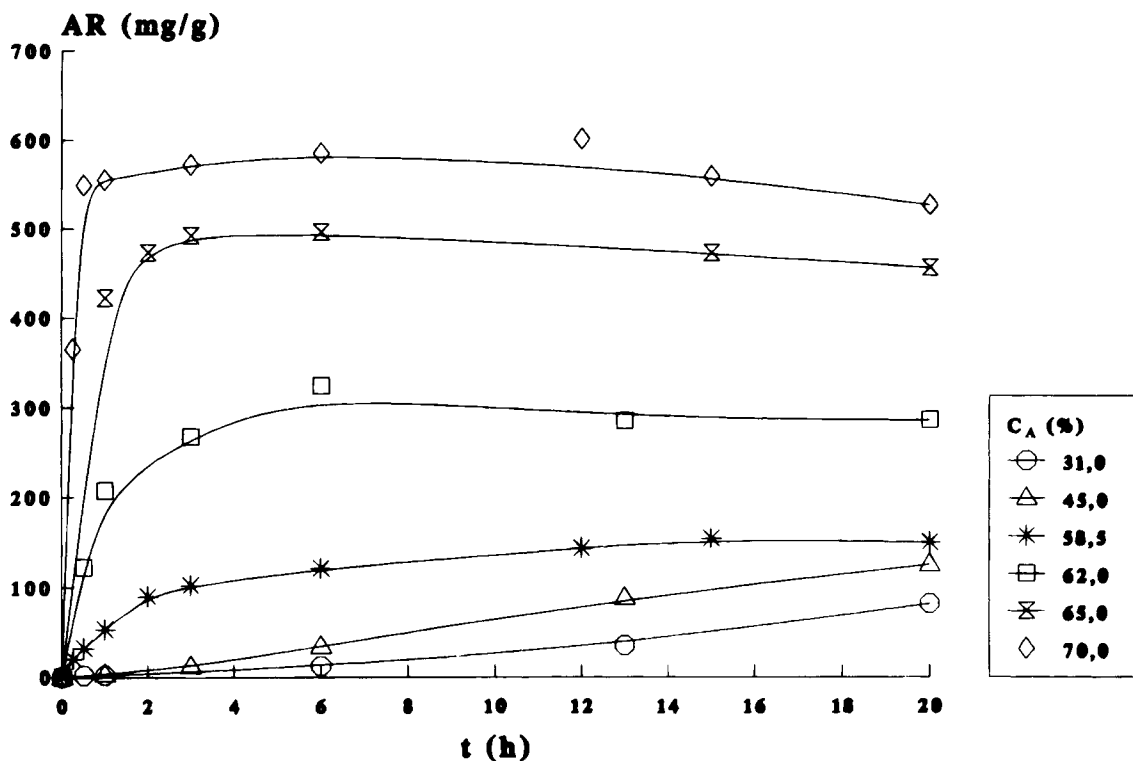


Fig. 2. Influence of H_2SO_4 concentration on the cellulose-dissolution rate ($T = 40^\circ\text{C}$ and $D/S = 3 \text{ cm}^3 \text{ g}^{-1}$).

ing agent, given that no appreciable cellulose solubilization was observed; this fact became evident during the experimentation and was confirmed by comparing the yields obtained for reducing sugars at the different acid concentrations tested (Fig. 2).

The scant solubilization obtained for $1 \text{ cm}^3 \text{ g}^{-1}$ (Fig. 3) appears to be due to the slowness of the heterogeneous acid-substrate reaction, since the liquid phase in our experimental system was not adequate to thoroughly soak the cellulose.

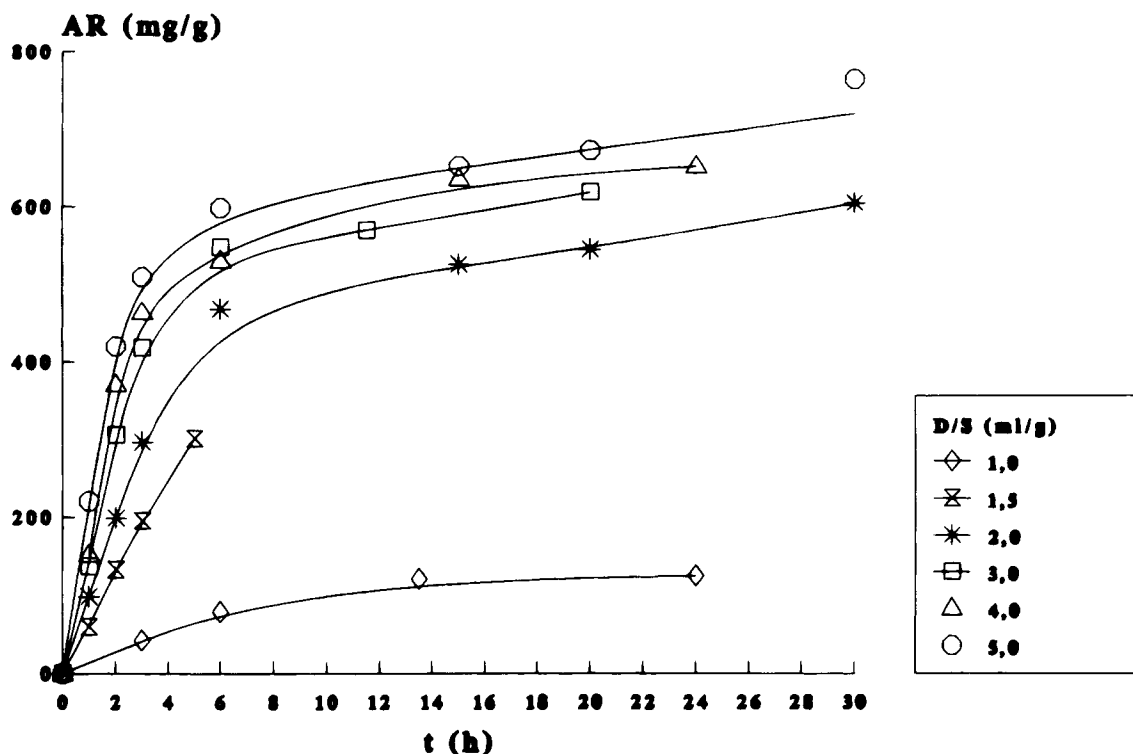


Fig. 3. Influence of the acid/substrate relationship on the cellulose-dissolution rate ($T = 25^\circ\text{C}$ and $C_A = 70\%$).

The results in the experiments in which total cellulose solubilization was achieved ($C_A \geq 62\%$ and $D/S \geq 1.5 \text{ cm}^3 \text{ g}^{-1}$) were fitted to a zero-order kinetic equation:

$$\frac{dAR}{dt} = k \quad 0 < t < t_d \quad (1)$$

where AR is in mg of reducing sugar per gram of initial cellulose; which, integrated, gives:

$$AR = kt \quad 0 < t < t_d \quad (2)$$

Table 1 shows the values obtained for the specific dissolution rate, k , under the different experimental conditions. This parameter was correlated with temperature (T), the acid concentration (C_A) and the acid/substrate relationship (D/S), using the expression:

$$k = 6.73 \times 10^{-4} (D/S) C_A^{1.3 \cdot 41} \exp\left(\frac{-113.6}{RT}\right) \quad (3)$$

where D/S is expressed in $\text{cm}^3 \text{ g}^{-1}$, C_A in %, the apparent activation energy in kJ mol^{-1} and k in mg of reducing sugars (such as glucose equivalent) per gram of initial cellulose and duration of reaction in hours. In all cases, the deviations between the k values that best reproduced the experimental data and the calculations with eqn (3) were less than $\pm 10\%$. In addition, we also estimated the times necessary for total dissolution of the cellulose, t_d , for $C_A \geq 62\%$ and $D/S \geq 1.5 \text{ cm}^3 \text{ g}^{-1}$. These times are recorded in Table 1 and correlated with the experimental variables by the expression:

$$t_d = 5.35 \times 10^{-12} (1 - 0.125(D/S)) C_A^{-2.6} \exp\left(\frac{89.6}{RT}\right) \quad (4)$$

This expression gives the minimum time required to solubilize a given quantity of substrate and these values

TABLE 1
Influence of the Operation Values on the Specific Cellulose-Dissolution Rate (k) and Total Dissolution Time (t_d)

T (°C)	C_A (%)	D/S ($\text{cm}^3 \text{ g}^{-1}$)	k ($\text{mg g}^{-1} \text{ h}^{-1}$)	t_d (h)
25	70	3.0	142.5	3.0
30	70	3.0	305.8	1.5
35	70	3.0	641.9	1.0
40	70	3.0	1278.7	0.5
40	65	3.0	422.0	1.0
40	62	3.0	255.8	1.0
25	70	1.5	62.6	—
25	70	2.0	98.7	4.0
25	70	4.0	163.7	2.6
25	70	5.0	215.1	2.0

are useful in fixing the experimental conditions that minimize the degradation of the reducing sugars by the action of the acid, since this activity increases with contact time.

The empirical character of eqns (3) and (4) make their use appropriate only within the limits of temperature, acid concentration and D/S relationship used in their deduction. Thus, for example, it was confirmed that treatment with H_2SO_4 (75% (w/v)) gave a higher hydrolysis rate, but at the expense of high mineralization of the substrate. Equations (3) and (4) proved useful for optimizing the T , C_A and D/S values to minimize the cost of pretreatment.

3.2 Glucose-production kinetics

Figures 4, 5 and 6 indicate, respectively, the influences of temperature, acid concentration and the acid/substrate relationship on the glucose yields. Figure 4 indicates that increased temperature boosts the rate of glucose production and degradation; a similar effect appears in Fig. 5 on varying the acid concentration. In Fig. 6, the presence of degradation products is not obvious, due possibly to the low temperature used (25°C). Comparisons between the glucose yields obtained with H_2SO_4 at 58.5% (w/v) and 62% (w/v) (8.2% and 21.3%, respectively at 20 h, Fig. 5) indicate against that for $C_A \geq 62\%$, the acid behaves fundamentally as a hydrolytic agent, whereas for $C_A \leq 58.8$, the acid performs more as a swelling agent.

In addition, it is important to emphasize that under the conditions used in the present work (low temperatures and high acid concentrations), glucose yields resembled those obtained by other workers using high temperatures (over 200°C) and low acid concentrations (around 1%).^{4,6,9,10} The yields of these other processes ranged from about 35%, whereas the present work yielded 32% of glucose, in addition to soluble saccharides which, in principle, are easily hydrolysed by cellulases and β -1,4-glucosidase.

To reproduce the results obtained during the acid hydrolysis (for $D/S \geq 1.5 \text{ cm}^3 \text{ g}^{-1}$ and $C_A \geq 62\%$), a model of two consecutive first-order pseudohomogeneous reactions was used:



In accordance with eqn (5), the glucose-production rate is

$$\frac{dG}{dt} = k_1 C - k_2 C \quad (6)$$

which, integrated, leads to

$$G = G_0 \frac{k_1}{k_1 - k_2} [\exp(-k_2 t) - \exp(-k_1 t)] \quad (7)$$

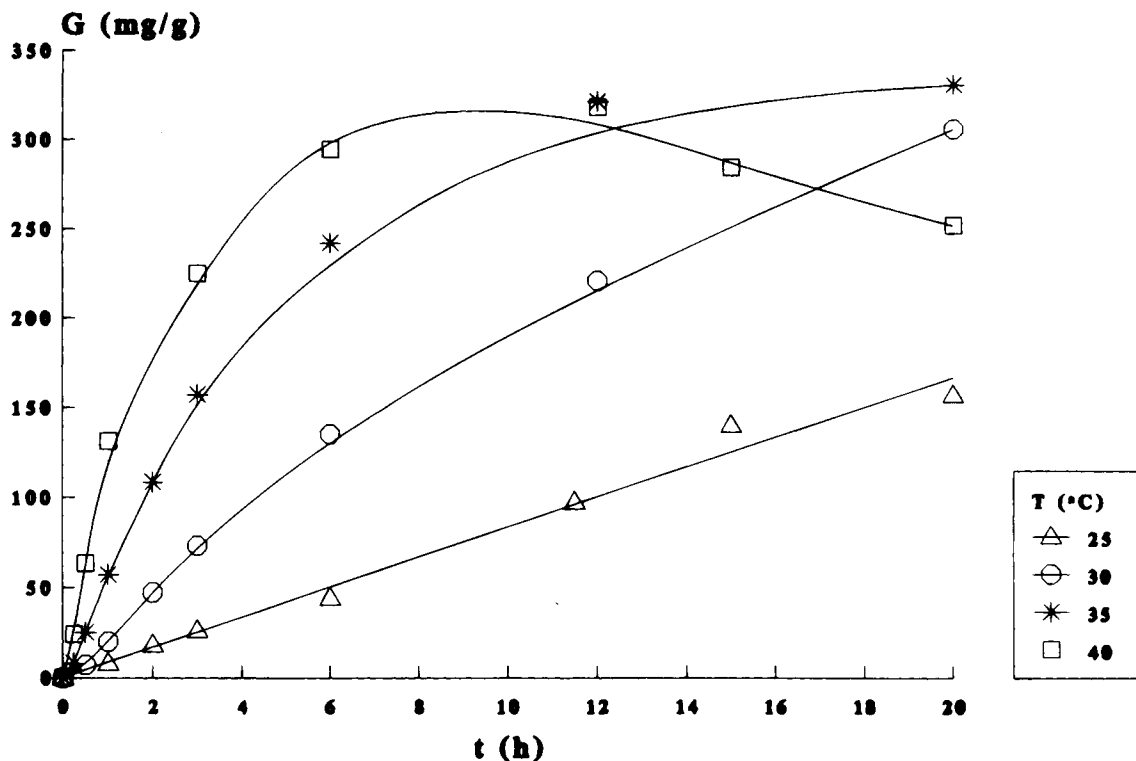


Fig. 4. Influence of temperature on the conversion of the cellulose to glucose (H_2SO_4 at 70%, $D/S = 3 \text{ cm}^3 \text{ g}^{-1}$).

The values of the kinetic constants k_1 and k_2 were obtained by the simplex method, and the values are presented in Table 2. In all cases, it was verified that, by these values, 95% of the experimental results are repro-

ducible with deviations of less than $\pm 10\%$. Nevertheless, the deviations obtained for the initial periods ($t < 0.5 \text{ h}$) were higher. This fact appears to indicate that the hypothesis of consecutive pseudohomogeneous

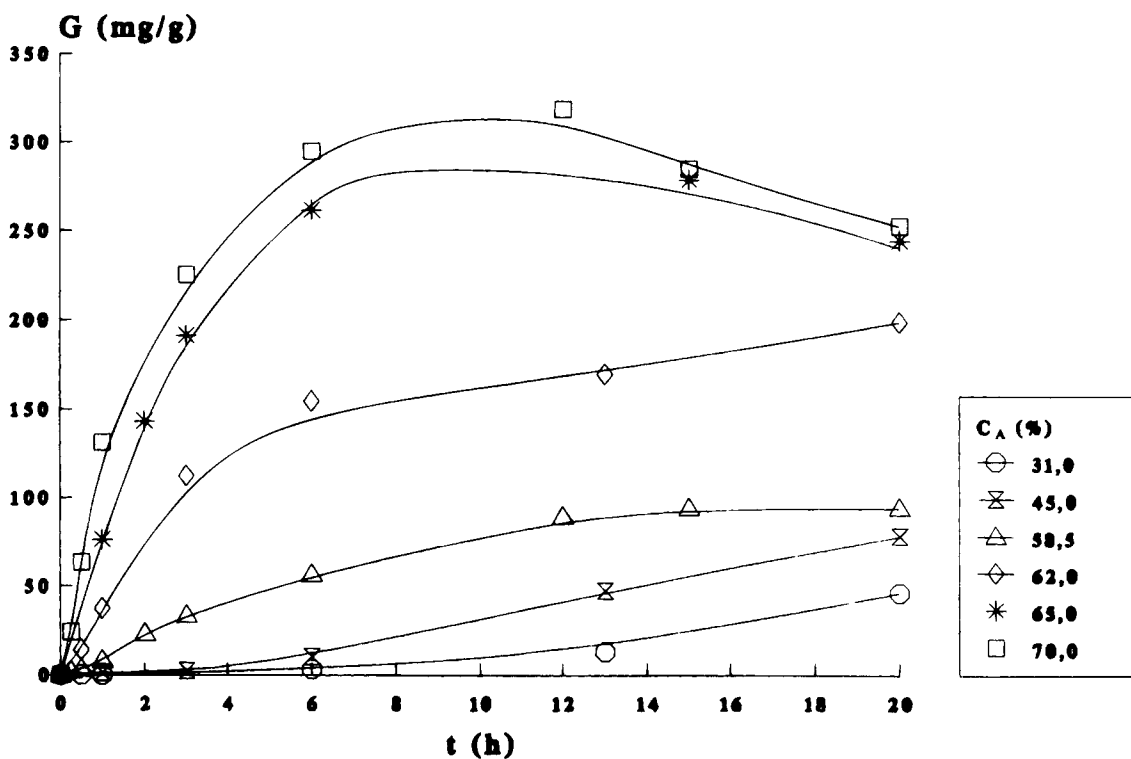


Fig. 5. Influence of the H_2SO_4 concentration on the conversion of cellulose to glucose ($T = 40^\circ\text{C}$ and $D/S = 3 \text{ cm}^3 \text{ g}^{-1}$).

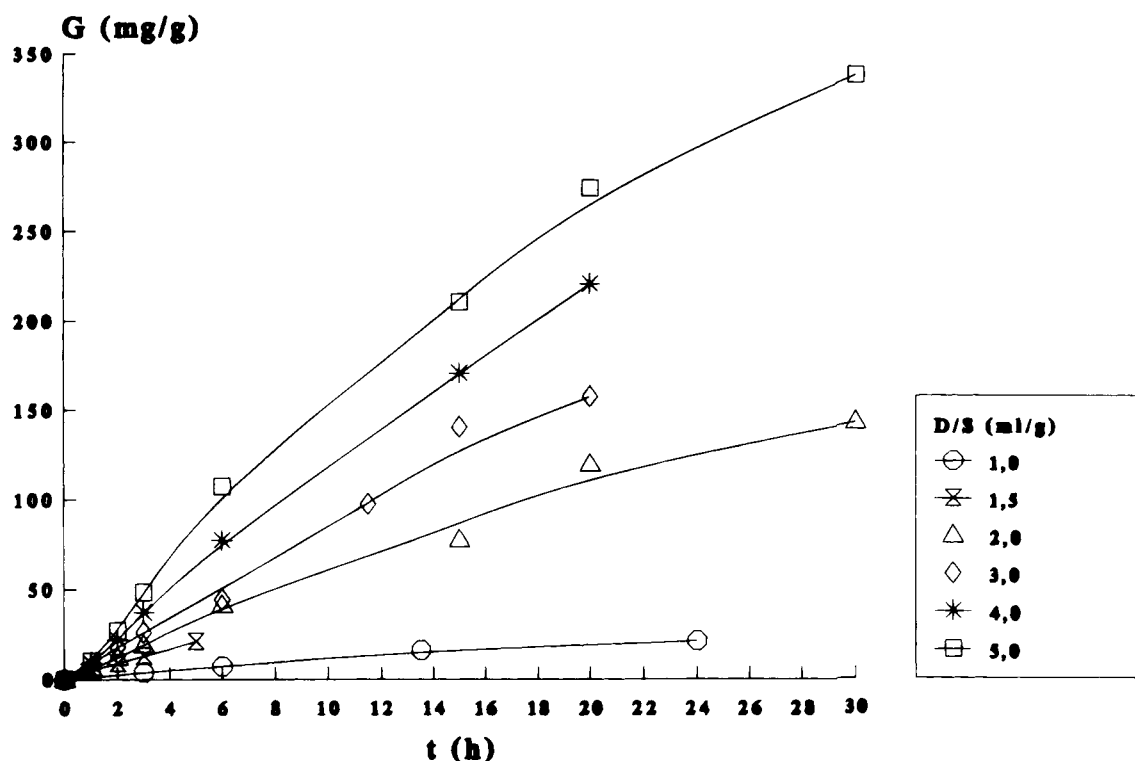


Fig. 6. Influence of the D/S relationship on the cellulose to glucose conversion ($T = 25^\circ\text{C}$ and H_2SO_4 at 70%).

reactions is not valid under these circumstances in which the controlling phenomena may be the external diffusion of the acid and the solubilization of the sugars of $\text{DP} > 2$.

The values of k_1 and k_2 were correlated with the operation variables by the expressions:

$$k_1 = 4.91 \times 10^8 (D/S) C_A^{6.0} \exp(-127.2/RT) \quad (8)$$

$$k_2 = 1.33 \times 10^{25} (D/S) C_A^{0.77} \exp(-166.9/RT) \quad (9)$$

The use of these expressions adequately reproduces the experimental values of k_1 and k_2 (in 95% of the cases the deviations were less than $\pm 10\%$), but it should be taken into account that application is

restricted to the intervals of temperature, C_A and D/S investigated.

The values of the activation energies show that increased temperature would favour more the degradation rate than glucose formation, the opposite of the situation with dilute acids at high temperatures.^{4,6,9-11} Also notable is the great influence of the acid concentration on the glucose-formation rate.

4 CONCLUSION

Cellulose hydrolysis with concentrated H_2SO_4 at room temperature gave total solubilization of the cellulose and appreciable glucose yields. This process may be useful as a pretreatment for a subsequent enzymatic hydrolysis. Therefore, the attainment of hydrolysed products from glucose and other saccharides of $\text{DP} \leq 6$, free of degradation products and with a low salt content, at reasonable economic cost, implies the optimization of C_A and D/S .

REFERENCES

1. Millet, M. A., Baker, A. J. & Satter, L. D., Pretreatments to enhance chemical, enzymic and microbiological attack of cellulosic materials. *Biotechnol. Bioeng. Symp.*, **6** (1976) 125-9.
2. Camacho-Rubio, F., González-Tello, P., Jurado, A. E. & Robles, M. A., Influencia de distintos pretratamientos químicos sobre la hidrólisis enzimática de celulosa microcristalina. *Anales de Química, Serie A*, **84** (1988) 236-40.

TABLE 2

Experimental Values of the Kinetic Constants k_1 and k_2

T ($^\circ\text{C}$)	C_A (%)	D/S ($\text{cm}^3 \text{g}^{-1}$)	$k_1 \times 10^3$ (h^{-1})	$k_2 \times 10^3$ (h^{-1})
25	70	3	8.0	5.6
30	70	3	20.3	18.8
35	70	3	50.0	65.0
40	70	3	91.3	134.1
40	65	3	74.0	127.0
40	62	3	42.5	122.0
25	70	1.5	4.0	2.8
25	70	2	5.4	3.8
25	70	4	11.1	7.5
25	70	5	14.1	9.4

3. Tsao, T. G., Ladisch, M., Ladisch, C., Hsu, T. A., Dale, B. & Chou, T., Fermentación substrates from cellulosic materials: production of fermentable sugars from cellulose materials. In *Annual Reports on Fermentation Processes*, ed. Perlman. Academic Press, New York, 1978, Vol. 2, pp. 1-44.
4. Bhandari, N., McDonald, D. G. & Bakhshi, N. N., Kinetic studies of corn stover saccharification using sulphuric acid. *Biotechnol. Bioeng.*, **26** (1984) 320-7.
5. Chung, I. S. & Lee, Y. Y., Ethanol fermentation of crude acid hydrozate of cellulose using high-level yeast inocula. *Biotechnol. Bioeng.*, **24** (1985) 308-15.
6. Church, J. A. & Wooldridge, D., Continuous high-solids acid hydrolysis of biomass in a 1 1/2 in. plug reactor. *American Chemical Society*, **20** (1981) 371-8.
7. Miller, G. L., Dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, **31**(3) (1959) 426-8.
8. Werner, W., Rwy, G. & Wielinger, H., Properties of a new chromogen for the determination of glucose in blood according to the GOD/POD method. *Z. Analyt. Chem.*, **252** (1970) 224-8.
9. Thompson, D. R. & Grethelein, H. E., Pretreatments to enhance chemical, enzymic and microbiological attack of cellulosic materials. *Ind. Eng. Chem. Prod. Res. Dev.*, **18** (1979) 166-70.
10. Ranganathan, S., Macdonald, D. G. & Bakhshi, N. N., Kinetic studies of wheat straw hydrolysis using sulphuric acid. *Can. J. Chem. Eng.*, **63** (1985) 840-4.
11. Rubio, T. M., Abatzoblou, N. & Chornet, E., Consideraciones Cinéticas sobre la hidrolisis acida de la celulosa: Influencia de distintos parámetros sobre la conversión y la selectividad. *Afinidad*, **42** (1985) 305-11.