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Effect of Structural and Physico-Chemical Features of Cellulosic Substrates on the Efficiency of Enzymatic Hydrolysis

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ABSTRACT

Effects of major physicochemical and structural parameters of cellulose on the rate and degree of its enzymatic hydrolysis were tested with cellulosic materials from various sources. Some different pretreatments were: mechanical (milling), physical (X-ray irradiation), and chemical (cadoxen, H_3PO_4, H_2SO_4, NaOH, Fe^{2+}/H_2O_2). The average size of cellulose particles and its degree of polymerization had little effect on the efficiency of enzymatic hydrolysis. For samples of pure cellulose (cotton linter, microcrystalline cellulose, α-cellulose), increase in the specific surface area accessible to protein molecules and decrease in the crystallinity index accelerated the enzymatic hydrolysis (the correlation coefficients were 0.89 and 0.92, respectively). In the case of lignocellulose (bagasse), a quantitative linear relationship only between specific surface area and reactivity was observed.

Index Entries: Cellulose; cellulases; hydrolysis; specific surface area; crystallinity index; degree of polymerization; particle size.

INTRODUCTION

Most investigations of enzymatic cellulose hydrolysis have featured enzymology with such factors as the composition of cellulase complex systems, the properties of cellulolytic enzymes, and the kinetics and mechanism of enzymatic cellulose conversion. Of equal interest is the elucidation

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of the role of structural and physicochemical parameters of the cellulosic substrate in the efficiency of enzymatic hydrolysis.

The discussion on the multiple structure of cellulose still goes on, but at present there are some undisputable principles supported by almost all researchers in this area (*1–3*). These are: (a) all samples of insoluble cellulose (both native and pretreated) are structurally nonuniform; (b) the structure of cellulose depends on pretreatment method and conditions; (c) native cellulose contains regions with highly ordered and disordered polymer chains (so-called crystalline and amorphous regions).

The crystalline regions of cellulose are less susceptible to enzymatic hydrolysis than are the amorphous regions (*4–8*). This is not surprising, since the crystalline, or highly ordered, cellulose regions are very compact and resistant not only to enzymes, but also to acids, swelling in water, and so forth. The amorphous regions are more susceptible. Of great importance would be the quantification of the relation between the degree of crystallinity of cellulose and the efficiency of its enzymatic hydrolysis. Some authors reported the correlation between these two parameters, but with a small number of cellulose samples (*6,9–11*).

In addition to the degree of crystallinity, a specific surface area (SSA) may play a great role in accessibility of cellulose to cellulolytic enzymes (*6,12–15*). Other physicochemical and structural parameters of cellulose, such as polymerization degree (*14,16,17*), particle size (*18,19*), and pore-size distribution (*11,20–22*), may also be important in susceptibility of cellulose to enzyme attack; however, the data published are rather at variance.

This paper describes the effects of the major physicochemical features of cellulose, such as the particle size, the degree of polymerization (DP), the crystallinity index (CI), and the specific surface area accessible to protein molecules (SSAP), on the rate and degree of its enzymatic hydrolysis. The role of these parameters in cellulose reactivity was estimated.

MATERIALS AND METHODS

Enzymes

Commercial cellulase preparations from *Trichoderma reesei* (NOVO, Denmark) and *Aspergillus niger* (Serva, FRG) were used for cellulose hydrolysis. The *T. reesei* preparation had filter-paper activity of 270 U/g (pH 4.8, 50°C) (*23*); endoglucanase activity determined by viscometrical method (*24*) of 3000 U/g (pH 4.5, 40°C); and cellobiase activity of 20 U/g (pH 4.5, 40°C) (*24*). The corresponding activities for the *A. niger* preparation were 11, 130, and 400 U/g.

Glucose oxidase (activity 380,000 U/g according to specification), a product of Vilnus Plant of Enzymic Preparations, and horseradish peroxi-

dase (activity 350,000 U/g according to specification) from Reanal (Hungary) were used for glucose analysis.

The highly purified horseradish peroxidase ($R_z = 2.8$), furnished by Professor N. N. Ugarova (Department of Chemistry, M. V. Lomonosov Moscow State University), and chymotrypsin from Reanal (Hungary) were used in experiments for determination of SSAP of cellulose samples.

Substrates and Pretreatment Methods

Cotton linter from Institute of Cotton (Tashkent), microcrystalline cellulose (Chemapol, Czechoslovakia), powdered cellulose and α-cellulose (Sigma, USA), sugar cane bagasse (CENIK, Cuba) were used as substrates for cellulases.

Cotton linter was milled in a laboratory vibrational ball mill at 1300–1500 vibrations per min. The time of milling varied from 2 to 20 min. To prevent the overheating of cellulose on milling, the stainless steel housings were cooled with water (10°C) after each min of milling. The average diameter of milled particles varied from 17 to 32 μm.

The method of cotton linter pretreatment by cadoxen was the following. To 100 mL of cadoxen, obtained by method (*25*), 4 g of cellulose was gradually added on stirring. The mixture was stirred at room temperature for 1 h till the complete dissolution of cellulose. The viscous cellulose solution was added to 2 L of water–acetone mixture (1:1), and the formed precipitate of regenerated cellulose was separated and washed with water.

In the H_3PO_4 pretreatment, 5 g of the cellulosic material was added on stirring to 250 mL of 79% H_3PO_4 (cooled to 0°C). The mixture was stirred on cooling for 4 h and added to 2 L of water-acetone mixture (1:1). The precipitate was separated and washed with water till the neutral reaction of washings with litmus.

A similar procedure was used for H_2SO_4 (60%) pretreatment of cotton linter except the quantities of cellulose and acid were 3 g and 80 mL, and the time of stirring was 30 min.

In the pretreatment of cellulosic materials by 1% or 10% NaOH, 5 g of material was added to 400 mL of alkaline solution, the mixture was subjected to boiling for 1 h, and then the cellulose residue was separated and washed with water till the neutral reaction of washings with litmus. The pretreatment of cotton linter by 22% NaOH was carried out for 2 h at room temperature.

The procedure of cotton linter pretreatment by Fe^{2+}/H_2O_2 system was the following. Five g of the material was added to 300 mL of solution, containing $FeSO_4$ (0.5*M*) and H_2O_2 (1%). After stirring, the mixture was incubated at room temperature for 8 d. Then the substrate was washed with water.

Cellulosic materials were pretreated by γ-irradiation as described by Kovalev et al. (*26*).

Determination of Physico-Chemical and Structural Parameters

The particle size distribution was determined on a Quantimeter-720 Instrument (Cambridge Instruments, UK) with a resolution of 1 μm. The instrument consisted of an optical microscope equipped with a scanning video-display system and a Hewlett-Packard computer.

The DP was determined by the viscometric assay (*27–29*), based on the property of cellulose to form viscous solutions in cadoxen, using the formula:

$$[\eta] = 7 \cdot 10^{-3} DP^{0.9}$$

where $[\eta]$ is characteristic viscosity (mL/g), calculated as

$$[\eta] = (-1 + \sqrt{1 + 2\eta_{sp}}) / C$$

where η_{sp} is specific viscosity, C—cellulose concentration (g/100 mL). Specific viscosity was determined directly from experiments by the formula:

$$\eta_{sp} = \eta_r - 1 = \tau / \tau_0 - 1$$

where η_r—relative viscosity, τ—elution time for cellulose solution in cadoxen from the viscometer bulb, τ_0—elution time for cadoxen. The viscosity of solutions was determined at 25°C using an Ostwald viscometer having a 2.5 mL bulb. The elution time for cadoxen was 90–100 s.

The degree of cellulose crystallinity was determined by X-ray diffraction (*4*). The CI was calculated by the method proposed by Segal (*30*).

The SSAP was measured using the adsorption isotherm of highly purified peroxidase ($R_z = 2.8$) or chymotrypsin, based on the method reported previously (*25*). The SSAP (m^2/g) was calculated by the formula:

$$SSAP = [E]_{sat} \cdot N_A \cdot S_E / [S]$$

where $[E]_{sat}$ is the saturating concentration of peroxidase on cellulose surface when protein monolayer is formed (mol/L); N_A—Avogadro's constant ($6.02 \cdot 10^{23}$ mol^{-1}); S_E—area occupied by one peroxidase molecule ($9 \cdot 10^{-18}$ m^2); [S]—cellulose concentration (g/L).

The amount of adsorbed peroxidase was found by measuring the decrease in enzyme activity or the decrease in optical density at 403 nm of enzyme solution after adsorption. The peroxidase activity was determined according to (*31*).

In the case of chymotrypsin, the relative change of SSAP of cellulose samples was determined. The saturating concentration of chymotrypsin at the cellulose surface was found by measuring the decrease of optical density at 280 nm of the enzyme solution after adsorption. The SSAP was expressed by mg of adsorbed chymotrypsin per g of cellulose.

Reaction Conditions and Analysis of Products

Enzymatic hydrolysis of cellulose was performed with a combined preparation, consisting of cellulases from *T. reesei* (0.4 mg/mL) and *A. niger* (0.4 mg/mL), at 40°C and pH 4.5 (0.1*M* acetate buffer) in 50 mL thermostatically controlled glass cells equipped with a magnetic stirrer. The concentration of cellulosic substrates was 10 g/L in all cases.

Glucose was analyzed by the glucose oxidase—peroxidase method (*24,32*). Reducing sugars were determined by the modified Nelson-Somogyi method (*24*).

RESULTS AND DISCUSSION

The initial rate of hydrolysis of cellulosic substrates by the mixture of cellulase preparations from *T. reesei* and *A. niger* as well as glucose yield after 24 h of hydrolysis were taken as criteria of the susceptibility of cellulose to enzyme attack. The hydrolysis product was mostly glucose (cellobiose was 3 to 5% of the glucose concentration). So, glucose alone was a satisfactory index for comparing processes.

Glucose at elevated concentrations (>20 g/L) markedly inhibits the catalytic activity of cellulases (*33*). For this reason, we used thin suspensions of cellulosic substrates (10 g/L) to minimize the inhibition by glucose even with a high degree of conversion.

Effect of Particle Size on the Efficiency of Hydrolysis

The average size of cellulose particles does not influence, to any noticeable extent, the efficiency of hydrolysis (Table 1). This is supported by poor correlation coefficients in the relationship between the average particle size and the initial rate of hydrolysis and between the particle size and glucose yield after 24 h (the correlation coefficients are 0.29 and 0.28, respectively). Shewale and Sadana (*18*) also found no difference between the hydrolysis rates for two samples of microcrystalline cellulose with average particle size 38 and 90 μm. The authors noted that "this effect seems unexpected" since from a general point of view the hydrolysis rate should be proportional to the particle external surface area and, hence, to the particle size. The fact that particle size is not the major factor determining accessibility of lignocellulosics to cellulolytic enzymes was also reported by Rivers and Emert (*19*).

We presume, however, that the results obtained are not unexpected since the particle size of the powdered cellulose sample, including the microcrystalline cellulose, varies in a wide range (Table 2 and Fig. 1). The

Table 1
Reactivity and Physico-Chemical Features of Cellulose After Different Kinds of Pretreatment

Sample	Average particle size (μm)[1*]	DP	SSAP (m^2/g)[2*]	CI		Reactivity	
				dry samples	moistened and dried	hydrolysis rate (g/l·h)	glucose yield after 24 h (g/l)
Cotton linter	fibers	1100	0.17	85	86	0.09	1.8
Ball milled linter, time of milling:							
2 min	32	870	0.27	65	69	0.16	2.0
3 min	23	510	0.29	-	-	0.25	3.0
5 min	19	440	0.28	-	-	0.41	5.2
10 min	19	430	0.30	30	54	0.61	6.6
15 min	18	320	0.45	-	-	0.57	6.5
20 min	17	280	0.54	25	52	0.56	6.2
Linter after treatment by cadoxen	fibers	-	1.20	-	32	0.96	9.0
Linter after ball milling and treatment by cadoxen	fibers	380	1.10	-	16	1.15	10.0
Linter after treatment by 79% H_3PO_4	fibers	980	1.05	-	35	0.84	9.0
Linter after ball milling and treatment by 79% H_3PO_4	fibers	800	0.90	-	30	1.05	9.6
Linter after treatment by:							
22% NaOH	fibers	700	0.69	-	38	0.62	10.0
10% NaOH	fibers	2220	-	-	-	0.54	5.0
1% NaOH	fibers	2700	0.30	-	80	0.40	4.6
Linter after treatment by 1% NaOH and ball milling (5 min)	fibers	870	0.85	-	-	1.00	9.5
Linter after treatment by 60% H_2SO_4	fibers	220	0.32	-	-	0.60	7.5
Linter treated by Fe^{2+}/H_2O_2	fibers	1070	0.54	-	80	0.18	2.5
Microcrystalline cellulose [3*]	23	170	0.31	65	65	0.41	6.3
Microcrystalline cellulose [4*]	24	160	0.30	68	70	0.39	5.2
Powdered cellulose	27	260	0.45	-	70	0.33	5.1
α-cellulose	30	840	0.69	55	64	0.45	6.8

[1*] For powdered cellulosic materials.
[2*] Accessible to peroxidase molecules.
[3*] Microcrystalline cellulose for column chromatography.
[4*] Microcrystalline cellulose for thin-layer chromatography.

Table 2
Average Distribution of Particles of Microcrystalline Cellulose by Sizes and External (Two-Dimensional) Particle Area

Particle size (μm)	Number of particles		Area, occupied by particles	
	absolute number in a sample	%	absolute value (μm^2)	%
5-10	97	12	4290	1.2
10-15	80	10	9800	2.8
15-20	174	22	41900	12
20-25	233	30	92600	26
25-30	109	14	64700	19
30-35	52	6.6	43100	12
35-40	20	2.5	22100	6.3
40-45	12	1.5	17000	4.9
45-50	6	0.7	10600	3.1
50-55	1	0.13	2160	0.7
55-60	2	0.25	5190	1.5
60-65	0	0	0	0
65-70	0	0	0	0
70-75	0	0	0	0
75-80	2	0.25	9430	2.7
80-85	1	0.13	5350	1.5
85-90	0	0	0	0
90-95	2	0.25	13400	3.9
95-100	0	0	0	0
100-105	1	0.13	8250	2.4
Total	791	100	350000	100

notion "average size" is rather relative. If the particles of some samples of cellulose were monodisperse, the sample with a smaller size could be more susceptible for hydrolysis since it has a larger geometric (external) SSA. However, with a wide distribution of particle sizes, the surface area and size appear to be in a more complicated relationship, which is illustrated by the results of Table 2 and Fig. 1. The area occupied by cellulose particles (to be more precise, the two-dimensional particle projection visible in the optical microscope, see Materials and Methods) can be used for a rough estimation of the substrate geometric (external) surface area. This is characterized by a notably wider distribution than that for one-dimensional particle sizes (Fig. 1). It is evident that the smallest and largest particles make a minimum contribution to the area (projection). A major portion of the overall area, occupied by cellulose particles, is made by those particles, which size is somewhat greater than the average size in the sample. It is misleading to consider the particle size as a single parameter determining the efficiency of enzymatic hydrolysis since the smallest

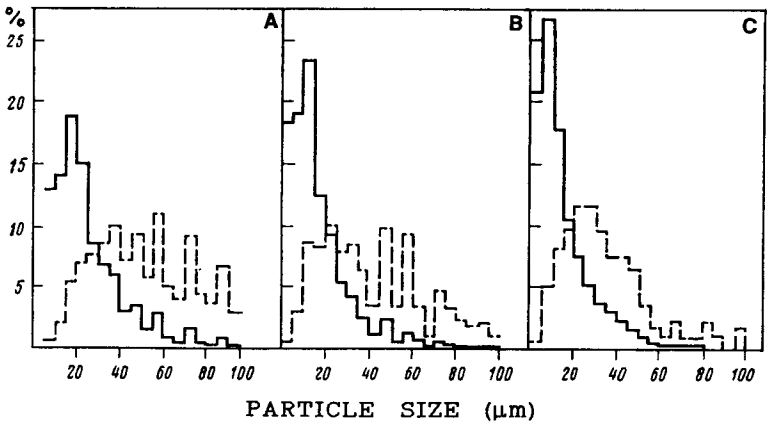

Fig. 1. Size distribution of ball milled cotton linter particles by a relative number of particles (solid line) and by a relative geometric surface area occupied by the particles (broken line). Time of milling: A, 2 min; B, 2.5 min; C, 15 min.

Table 3
Effect of γ-irradiation on the Structure and Reactivity of Cotton Linter

Dose (Mrad)	DP	CI (%)	Reactivity	
			hydrolysis rate (g/l·h)	glucose yield after 24 h (g/l)
0	1100	85	0.09	2.3
1.7	680	86	0.09	2.2
3.2	560	86	0.10	2.0
12	250	86	0.11	2.2
29	140	85	0.10	2.3
43	110	84	0.09	1.7
118	19	84	0.11	1.6

and the largest particles may distort the size measurement while playing minor roles in hydrolysis.

The fact that cellulose particle size does not noticeably influence the efficiency of enzymatic hydrolysis is also evident from comparison of hydrolysis rates of fibrous and powdered cellulosic materials. The sizes of cellulose fibers (native cotton linter and the linter after pretreatment by the cadoxen or phosphoric acid) are much higher than the particle sizes of powdered cellulose (this is clear under light microscope and by direct observations). Nevertheless, the untreated linter has the minimal susceptibility, whereas the linter after cadoxen and acid treatment—the maximal susceptibility to hydrolysis among all cellulosic substrates we studied (Tables 1,3,4).

Thus, it is evident that the average geometric (external) size of substrate particles (especially having a wide size distribution) cannot be the dominant parameter in the efficiency of enzymatic cellulose hydrolysis.

Table 4
Reactivity and Physico-Chemical Features
of Lignocellulose (Bagasse) After Different Kinds of Pretreatment

Sample	DP	SSAP[1*] (relative units)	CI[2*] (%)	Reactivity	
				hydrolysis rate (g/l·h)	glucose yield after 24 h (g/l)
No pretreatment	800	4.7	80	0.11	0.5
Delignification (1% NaOH)	700	18.0	82	0.70	6.5
Treatment by 79% H_3PO_4	650	9.2	72	0.51	5.3
Ball milling:					
0.5 min	750	-	73	0.11	1.1
1 min	-	-	-	0.20	3.6
2 min	290	8.1	50	0.40	5.3
5 min	300	-	-	0.51	5.4
γ-irradiation:					
25 Mrad	220	-	80	0.06	0.4
100 Mrad	170	-	-	0.14	0.8
150 Mrad	150	-	-	0.19	2.2
250 Mrad	140	-	70	0.20	2.6
Treatment by 10% NaOH	-	-	80	0.98	7.1
Delignification (1% NaOH) and ball milling (2 min)	440	10.1	49	0.46	6.2
Ball milling (2 min) and delignification (1% NaOH)	600	15.7	77	0.96	7.5
Delignification (1% NaOH) and treatment by 79% H_3PO_4	-	20.1	-	0.90	9.1
Treatment by 79% H_3PO_4 and delignification (1% NaOH)	690	20.3	85	0.86	8.0
Ball milling (2 min) and γ-irradiation (25 Mrad)	40	-	73	0.19	1.1
γ-irradiation (25 Mrad) and ball milling (2 min)	560	-	-	0.20	1.1

[1*]SSAP was determined by adsorption of chymotrypsin. One relative unit corresponds to $1 \cdot 10^{-6}$ mg of chymotrypsin/g of bagasse.
[2*]CI was determined after premoistening of cellulosic samples.

Effect of Polymerization Degree

Effect of cellulose DP on the efficiency of enzymatic hydrolysis has had little attention. Knappert et al. (*16*) have reported the influence of DP of three cellulosic samples, pretreated with sulfuric acid, on the degree of conversion. They saw no correlation between DP and cellulose susceptibility to cellulases. Puri (*17*) has found some difference in cellulose reac-

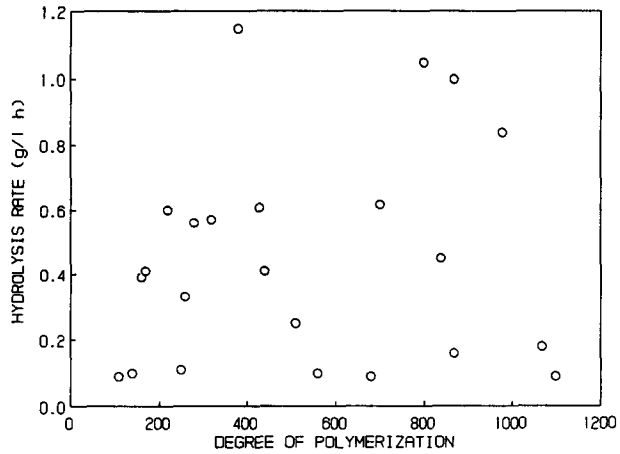

Fig. 2. Effect of DP on the initial rate of hydrolysis of cellulosic substrates (based on results presented in Tables 1 and 3).

tivity with pretreated lignocellulosic samples having different DP; however, other physico-chemical parameters of the substrates were also altered during the pretreatment.

Tables 1, 3, and 4 list the values of DP for various native and pretreated cellulose samples. There was a very poor correlation between the DP values and the initial rate of hydrolysis. The relevant correlation coefficients for cellulosic and lignocellulosic samples were 0.22 and 0.27 (see Fig. 2 and 3). Similarly, linear correlation between DP and glucose yield after 24 h of hydrolysis was not observed. In fact, the cellulose samples having the highest DP (native cotton linter—1100, recovered cellulose—980 and 800, *a*-cellulose—840) differed in reactivity almost by 10 times. The initial rates of hydrolysis were 0.09 g/L·h for native linter, 0.84 and 1.05 g/L·h for recovered cellulose, and 0.45 g/L·h for α-cellulose. Such hydrolysis rates overlap with the rates for cellulose samples having the minimal DP (280–320 for ball milled linter, 160–170 for microcrystalline cellulose with the initial rates of hydrolysis 0.4–0.6 g/L·h). A similar situation was observed in the case of bagasse (Table 4).

The fact that DP does not noticeably influence the efficiency of hydrolysis was particularly evident from the susceptibility to cellulases of cotton linter irradiated by various X-ray doses (Table 3). On increasing the irradiation dose from 0 to 43 Mrad, the DP value decreased more than tenfold, whereas the rate of hydrolysis did not actually change. It should be noted that such parameters as CI and SSAP were not significantly altered during such kind of pretreatment, i.e., in these experiments the influence of other structural and physico-chemical parameters was minimized. Irradiation of bagasse also led to decrease in DP more than fivefold (under irradiation dose 150 Mrad) but no significant change of substrate susceptibility was observed.

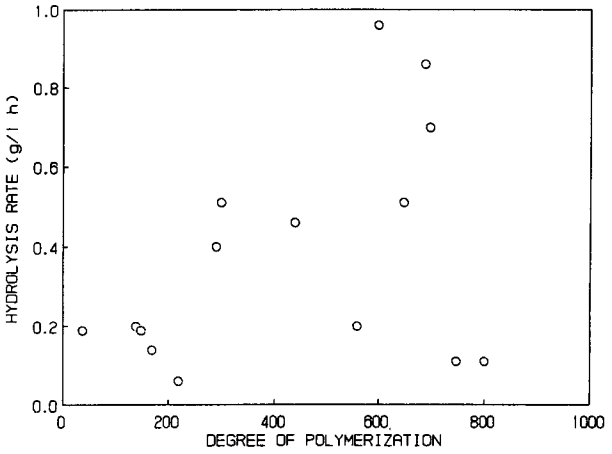

Fig. 3. Effect of DP on the initial rate of hydrolysis of pretreated bagasse (based on results presented in Table 4).

Effect of the Specific Surface Area Accessible to Protein

It would be reasonable that the reaction rate should be a function of the surface area of cellulose because direct physical contact between cellulose and cellulase molecules is a prerequisite to hydrolytic reaction (*34*). Some correlation has been shown between the amount of adsorbed enzyme and the rate of enzymatic reaction (*20*). The amount of adsorbed enzyme should, in turn, be dependent on the internal surface area of insoluble substrate. The effect of the SSA of cellulose on the susceptibility of cellulose to enzymatic hydrolysis was discussed in some papers (*6,12,15,20,22*). In the most cases authors made a statement like "the higher is surface area—the higher is hydrolysis rate." But in some cases (*6*) no good enough correlation between SSA and hydrolysis rate was found. The problem in such research is that the SSA was measured by the BET method using the adsorption of gaseous nitrogen (or the adsorption of such small molecules like water). These methods are traditional and well developed for determination of the internal surface area of cellulose. But, on the other hand, for large molecules of enzymes not all pores and capillaries of cellulose fibers are accessible. Hence, another method of the SSA determination should be used to relate to molecules of enzymes. The method of nitrogen adsorption is the best for the determination of "fine" internal surface area, but it is formal and not essential for the determination of "rough" surface area.

In some papers (*11,20–22*) a relationship between pore size (pore volume) distribution and hydrolysis rate has been investigated. It has been shown that the rate-limiting size of pores is equal or greater than the size of cellulase molecules, which has been estimated to be 40–90 Å (*11,20,22*).

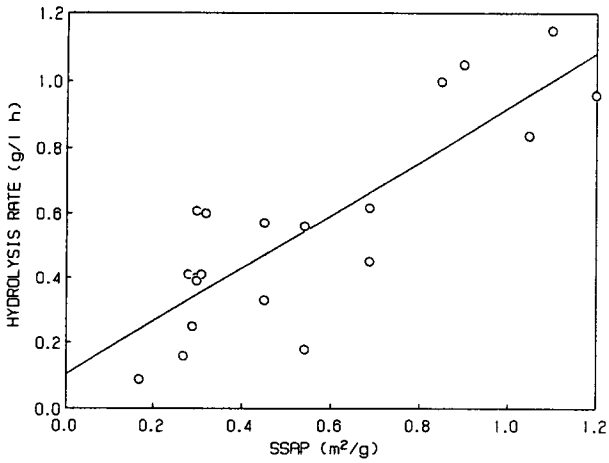

Fig. 4. Effect of SSAP on the initial rate of hydrolysis of cellulosic substrates (based on results presented in Table 1).

So, in the determination of surface area accessible to cellulolytic enzymes (proteins), molecular probes with similar size must be used.

In the present work, the SSAP was measured using the adsorption of peroxidase or chymotrypsin. These enzymes were chosen because they are inert to cellulose and their molecular sizes and weights are fairly close to the relevant parameters of cellulolytic enzymes. The diameter of peroxidase molecule has been estimated to be 50 Å (*35*), the size of chymotrypsin—40×40×50 Å (*36*).

The SSA of cellulose measured by Fan et al. (*6*) using nitrogen adsorption varied in a little extent (within ±12%) after milling. Conversely, we found that SSAP of cotton linter after ball milling was markedly increased (Table 1). Measuring the "fine" surface area of microcrystalline cellulose by nitrogen gave a value of 2.2 m^2/g (close to 1.84 m^2/g determined in (*6*)). SSAP measured by peroxidase was 0.30 m^2/g (Table 1). This discrepancy evidently results from structural nonuniformity of cellulose in respect to adsorption of low-mol-wt gases, on the one hand, and of high-mol-wt proteins, on the other hand.

Having determined the SSAP of a number of cellulosic and lignocellulosic samples (Tables 1 and 4) and having compared the results with the efficiency of enzymatic cellulose hydrolysis, we found a definite quantitative relationship (Figs. 4, 5). The correlation coefficient between the SSAP of the pure cellulosic substrates and the initial rate of their hydrolysis was 0.89. The correlation coefficient between the SSAP and the glucose yield after 24 h of hydrolysis was 0.84. In the case of lignocellulosic samples, the correlation coefficients were 0.84 and 0.87, respectively.

Thus, the SSAP (or "rough" internal surface area) of cellulosic and lignocellulosic substrates significantly influenced the efficiency of hydrolysis.

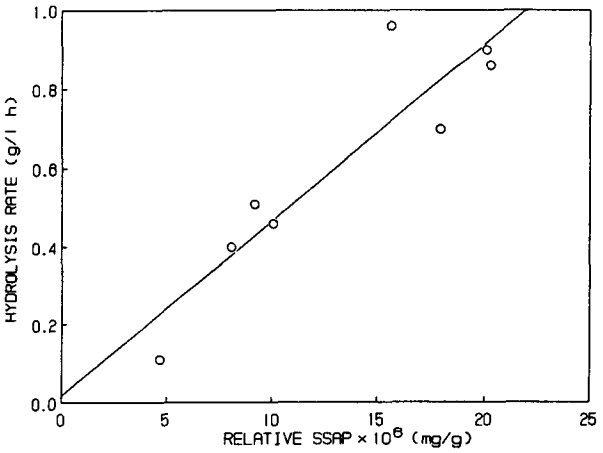

Fig. 5. Effect of SSAP on the initial rate of hydrolysis of pretreated bagasse (based on results presented in Table 4).

Effect of Crystallinity Index

When discussing effect of CI on cellulose reactivity, attention should be payed to a possible partial recrystallization of amorphous dry cellulose under the action of water (*25,37*). For cellulose, treated by cadoxen, H_3PO_4, H_2SO_4, and NaOH, the method of preparation required water. To standardize all the other cellulose samples, their CI were also determined after a moistening and subsequent drying of cellulose. Table 1 shows that for cellulose featuring a sufficiently high content of ordered regions (cotton linter, microcrystalline cellulose) the moistening influences the CI values insignificantly, but markedly increases the CI of cellulose after dry ball milling.

Figure 6 shows a fairly distinct linear relationship between the initial rate of hydrolysis and CI of cellulosic samples (determined after preliminary moistening and drying) with the correlation coefficient 0.92. The correlation coefficient 0.88 was found in the relationship between the CI and glucose yield after 24 h of hydrolysis. Basically the same result has been observed by other research groups (*6,9–11,38*).

Of some interest is a linear relationship between CI of cellulose samples and the SSAP, the correlation coefficient of which is 0.86 (Fig. 7). In fact, almost all samples of high-crystalline cellulose listed in Table 1 (cotton linter, microcrystalline cellulose) feature a fairly small SSAP (0.17–0.31 m^2/g) whereas most samples of amorphous cellulose (ball milled linter, recovered cellulose, and so on) have a fairly large SSAP (0.5–1.2 m^2/g). This is not unexpected in terms of morphological features of cellulose. Disordering the structure may increase the surface area accessible for binding protein molecules.

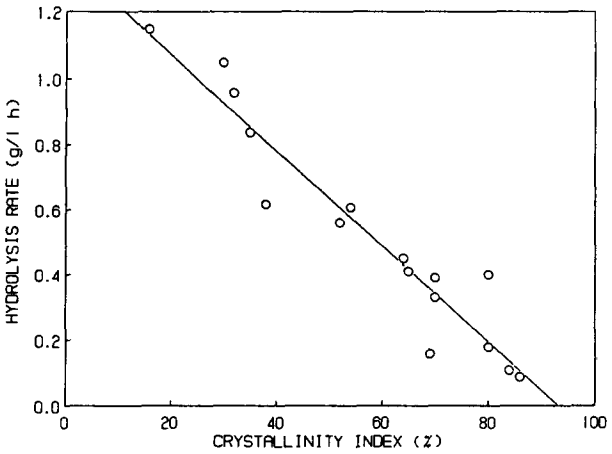

Fig. 6. Effect of CI on the initial rate of hydrolysis of cellulosic substrates (based on results presented in Tables 1 and 3).

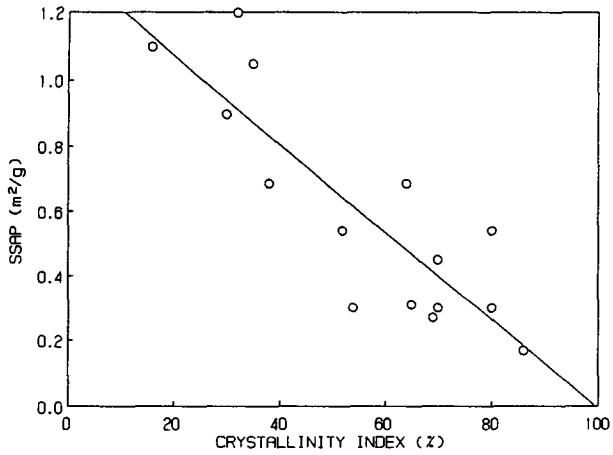

Fig. 7. Relationship between CI and SSAP of cellulosic substrates (based on results presented in Table 1).

For lignocellulosic samples no strong relationship between CI and reactivity was observed (Fig. 8). The reasons may be the following. As can be seen from Table 4, delignification of lignocellulose does not change CI, but leads to increase in SSAP and, hence, to increase in reactivity. On the contrary, such pretreatment procedures as ball milling or soaking with H_3PO_4 lower the CI, but do not remove the lignin. In these cases lignin, on the one hand, may protect cellulose surface from cellulolytic enzymes and, on the other hand, may adsorb cellulases (*39*), so reactivity of lignocellulosic samples does not increase very much.

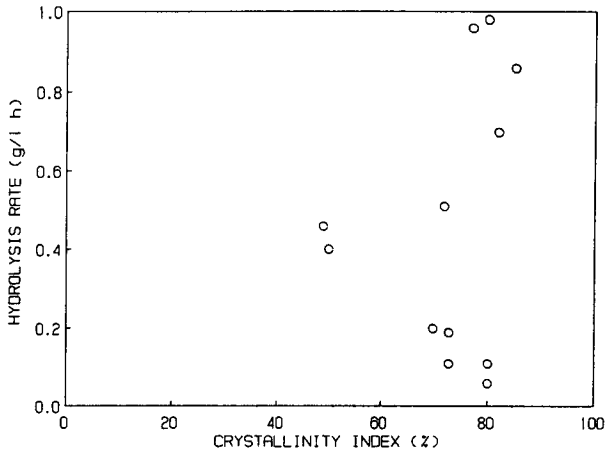

Fig. 8. Effect of CI on the initial rate of hydrolysis of pretreated bagasse (based on results presented in Table 4).

CONCLUSIONS

1. Cellulosic substrates may be characterized by four major physicochemical and structural parameters: the geometric particle size, the degree of polymerization, the internal specific surface area accessible to protein molecules, and the crystallinity index.
2. In the case of pure cellulose, the latter two parameters provide a linear relationship with the initial rate of enzymatic hydrolysis and glucose yield after 24 h of the reaction. This enables to quantify and predict the substrate reactivity in enzymatic hydrolysis. Moreover, a quantitative linear correlation between CI and SSAP was found, affording a one-test analysis of cellulose, i.e., CI determination by X-ray diffraction, which is a more simple, convenient, and standardizeable test compared to the determination of SSAP.
3. In the case of lignocellulose, a quantitative linear correlation only between SSAP and reactivity was observed.

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