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THE CORRELATION BETWEEN THE REACTIVITY AND THE SUPRAMOLECULAR STRUCTURE OF ALLOMORPHS OF CELLULOSE

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Although cellulose is arranged in variable and complex ways within plant material, relatively little attention has been paid to the relationship between this "fine-structure" of cellulose and its biodegradation. The variety of physical structures taken by cellulose molecules in their different crystalline forms is one structural feature of cellulose that has not been examined systematically for its effect on biodegradation. The aim of this paper is to study the enzymatic hydrolysis of the allomorphs forms of the microcrystalline cellulose. The biodegradation of cellulose was evidenced by the X-ray diffraction method, by the degree of polymerization, etc. The initial maximum rate can be considered as a measure of the reactivity of the substratum. The action of the endo- and exocellulasic components is evidenced by the decrease in the degree of polymerization of hydrolyzed samples. The study of X-ray diffractograms of residues resulting from enzymatic hydrolysis shows the fact that after biodegradation, the crystalline structure of allomorphic forms I and II does not suffer significant modifications, while for the polymorphic form of cellulose III, a partial return to the crystalline structure of cellulose I was observed.

INTRODUCTION

Since the middle of this century enzyme-catalyzed cleavage of glycosidic bonds by cellulolytic enzyme systems has received considerable attention, promoted by the anticipation of an eco-compatible alternative choice for degrading cellulose, especially waste cellulosics, to useful products and justified later by significant contributions to elucidation of cellulose structure as well as characterization of cellulose derivatives.[1-3] In this paper was demonstrated that the enzymatic degradation reaction of cellulose is adequate not only to demonstrate the influence of the physical structure of the initial material on the heterogeneous reaction, but also to determine the influence of the modifications induced by the chemical treatments of cellulose activation upon the enzymatic hydrolysis.

EXPERIMENTAL PART

Materials

Three kinds of cellulose were prepared:

Cellulose I (AI) – Avicel HP-101 (Fluka).

Cellulose II (AII*)* - was prepared from microcrystalline cellulose by soaking it in 17.5% NaOH for 24 hours at 15°C, followed by washing thoroughly with distilled water and dried in air.

Cellulose III (AIII) – was prepared from microcrystalline cellulose by soaking in organic amine (100% ethylenediamine) for 24 hours at room temperature. The cellulose amine complex was washed with anhydrous methanol and was air-dried.

Methods and measurements

1. Method of hydrolysis. The allomorphs were subjected to enzymatic hydrolyses using enzymes produced by fungus *Trichoderma reesei.* After preswelling of 0.5 g cellulosic substrate, in the recipient was added 16.5 mL of 1 M citrate buffer (pH 4.8) and 0.5 mL of enzyme [i]solution. The flask was placed in a 50°C incubator. Samples were withdrawn at different time periods, 2, 4, 6, 8 and 10h, centrifuged and the supernatant was refrigerated. The samples were analyzed for reducing sugar content by the dinitrosalicylic acid (DNS) method after appropriate dilution.[4] The residue obtained after enzymatic hydrolysis of cellulose were encoded with R1, R2, R3, R4 and R5, were R represents the allomorph form of cellulose.

2. Degrees of polymerization of cellulose (DP) were measured by the viscosity method in 0.5 mol Cuen.[5]

3. X-ray diffraction method. X-Ray diffraction patterns of the samples were collected on a RIGAKU RINT 2500 apparatus, equipped with a transmission type goniometer using nickel

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filtered, CuK_α radiation at 40kV. The goniometer was scanned stepwise every 0.10° from 10 to 40° in the 2θ range. The resulting diffraction patterns exhibited peaks which were deconvoluted from a background scattering by using Lorenzian functions, while the diffraction pattern of an artificially amorphicized sample was approximated by a Gaussian functions curve fitting analysis.[6] The estimation of the crystallinity index of cellulose samples was established by the ratio from crystallinity area (S_C) and total area (S_T).[7]

RESULTS AND DISCUSSION

It can be observed in Fig. 1 the clear differences between the reaction rates of enzymatic hydrolysis in the case of the three polymorphic forms of microcrystalline cellulose. The values of the concentration of reducing sugar, obtained after 10 hours of hydrolysis, are presented in Table 1.

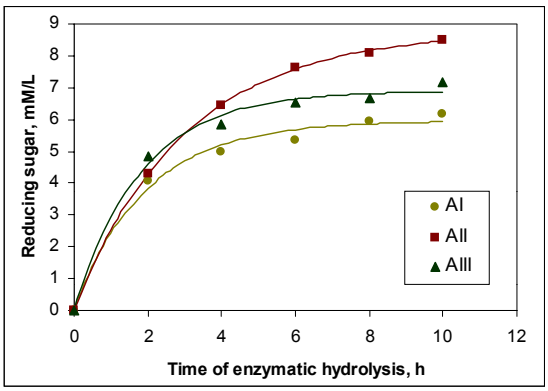

Fig. 1 – The enzymatic degradation of allomorphic forms of microcrystalline cellulose.

Table 1

The concentration of reducing sugar obtained through the enzymatic hydrolysis of the studied cellulose samples

Type of allomorph	The concentration of reducing sugar, g/L
Cellulose I	1.114
Cellulose II	1.532
Cellulose III	1.295

The study concerned with establishing the optimal conditions for the production of enzymatic hydrolysis and with evaluating the influence of the substratum concentration upon the reaction rate, respectively, proved that, in the case of microcrystalline cellulose, the Michaelis-Menten type of kinetics was followed.[8] To evaluate the influence of the crystalline type of organization and the allomorph type of cellulose, respectively, on the process of the enzymatic hydrolysis reaction, we proceeded to determine the kinetic parameters Km and Vmax, based on the integral equation obtained through integrating the Michaelis-Menten equation and determining the integration constant:[9]

$$V = V_{max}\left(\frac{[S]}{[S] + Km}\right) \quad (1)$$

where: V – the rate of product formation; Vmax – the maximum hydrolytic rate; [S] – substrate concentration; Km – the Michaelis constant,

$$[P] = V_{max} \cdot t + K_m \cdot \ln\left(\frac{[S]_0 - [P]}{[S]_0}\right) \quad (2)$$

where: [P] – product concentration; Vmax – the maximum hydrolytic rate; t – time; Km – the Michaelis constant; $[S]_0$ – initial substrate concentration.

The values obtained for the kinetic parameters Km and Vmax are presented in Table 2.

Table 2

The kinetic parameters characteristic to the polymorphic forms of cellulose enzymatic hydrolysed

Sample	Michaels' constant, Km		The initial maximum rate, mM/h
	% (weight/vol.)	mM	
AI	3.05 ± 0.01	188.50 ± 0.02	2.41 ± 0.02
AII	4.88 ± 0.12	302.28 ± 0.93	5.25 ± 0.27
AIII	2.13 ± 0.12	131.76 ± 0.88	3.98 ± 0.08

As is generally known, Km is a parameter that is independent of the concentration of the enzyme and of the substratum, representing after all the affinity of the enzyme with the substratum. A great value of this parameter indicates a weak bond between the enzyme and the substratum and a small stability of the ES complex, while a small value indicates, on the contrary, a great affinity.

It can be observed in Table 2 that the allomorphic forms of cellulose III present compared to the allomorphic forms I and II, much small er values, thus a greater affinity of the enzyme. The hierarchy that we can establish through this parameter regarding the affinity of the enzymatic complex employed toward allomorphic forms decreases in this order:

Cellulose III > Cellulose I > Cellulose II.

As for the maximum initial rate, given that $V_{max} = k_3[E]_0$, in the conditions of comparing the results obtained at the same enzyme concentration, the maximal rate can be considered proportional to the reactivity of the substratum. The generally order in relation to the reactivity of allomorphic forms is:

Cellulose II > Cellulose III > Cellulose I.

This fact can be explained through the modifications that take place in the supramolecular structure of the allomorphs of cellulose.[10,11] Evidently, the cellulose with the less ordered structure is the most susceptible to the topochemical reaction of hydrolysis.

However, during the process of the degradation reaction, a partial reversibility of the structure of the allomorphic form cellulose III takes place, because of the presence of the water-based medium and of the high temperature of the reaction (50°C). Thus, through the partial return of cellulose III to cellulose I, even though its structure presents similarities in the parallel type of bonding of chains, with the structure of cellulose I, it still retains a certain disorganized state at the level of supramolecular structure, which makes it more accessible to the enzymatic attack.

The studies of X-ray diffraction of sample AI, have led to the observation that after biodegradation, the residues resulting from enzymatic hydrolysis reaction, performed with different reaction times, presents diffractograms that do not modify their shape characteristic to the crystalline form of cellulose I. In Figs. 2 and 3 are presented the diffractograms of the initial samples and that for the sample treated enzimatically for 10 hours, respectively.

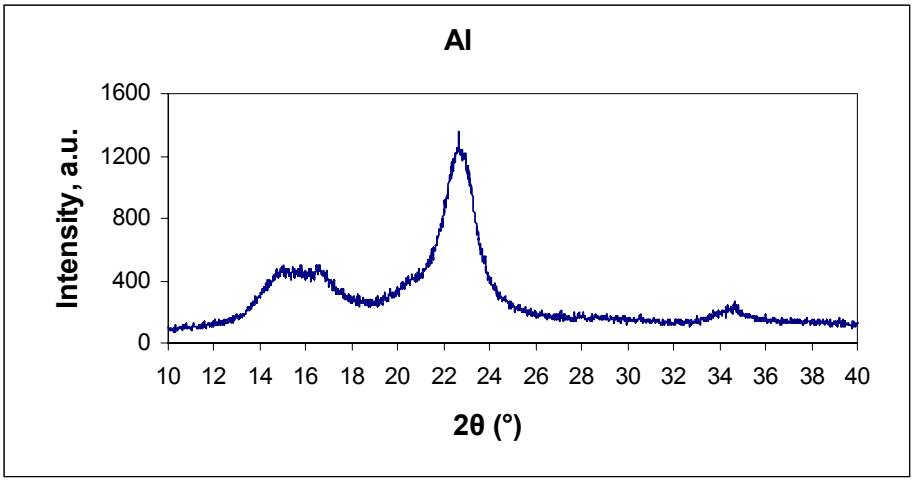

Fig. 2 – The X-ray diffractogram of microcrystalline cellulose (AI).

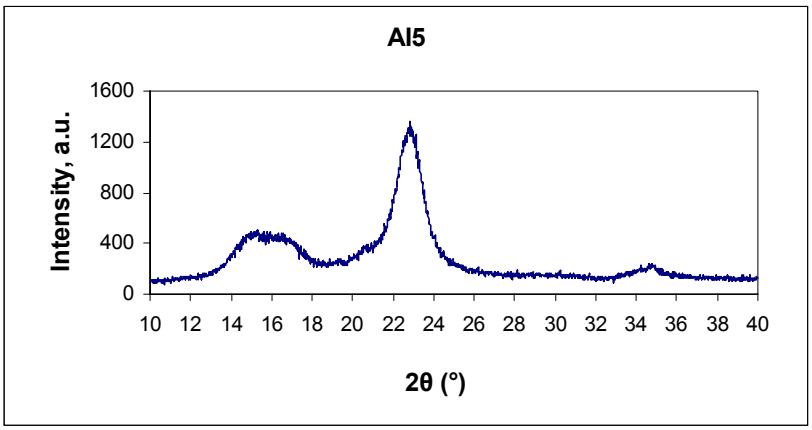

Fig. 3 – The X-ray diffractogram of sample AI enzymatically degraded for 10 hours.

Maintaining the crystalline form of organization is also confirmed in the case of the X-ray diffractograms of residues resulting from enzymatic hydrolysis of cellulose II, performed at different reaction times (Figs. 4 and 5).

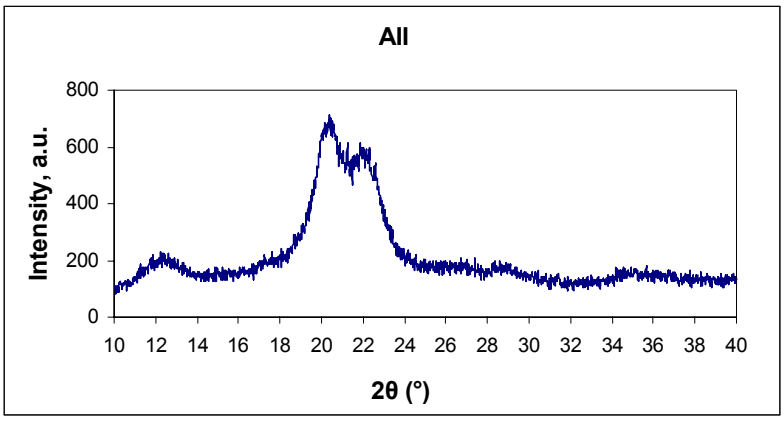

Fig. 4 – The X-ray diffractogram of cellulose II obtained from microcrystalline cellulose (AII).

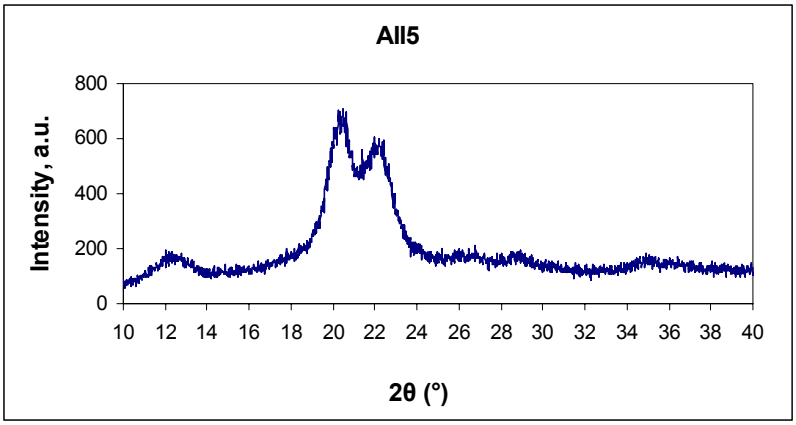

Fig. 5 – The X-ray diffractogram of sample AII, enzymatically degraded for 10 hours.

In the case of cellulose III it can be observed the partial reversibility to the cellulose I form, demonstrated by the appearance of intensity peaks (101) and (10-1) to the values of the 2θ angles characteristic to cellulose I, namely of 14° and 16°, respectively. The diffractograms of cellulose III samples that went through the process of enzymatic hydrolysis are presented in Fig. 6 and Fig. 7.

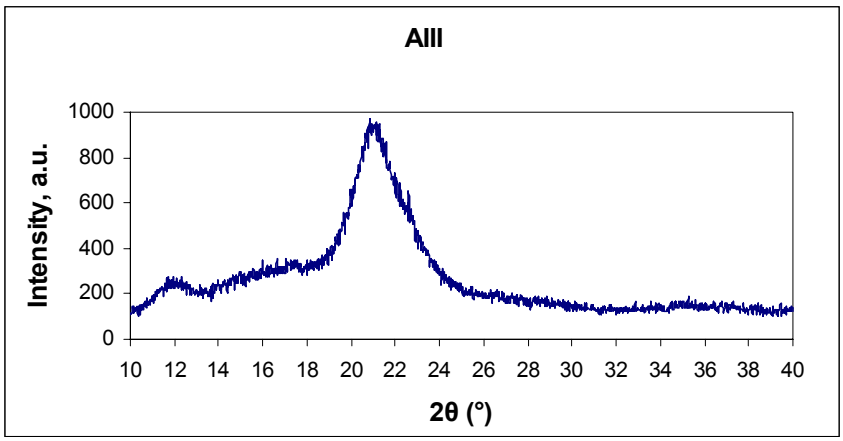

Fig. 6 – The X-ray diffractogram of cellulose III obtained from microcrystalline cellulose (AIII).

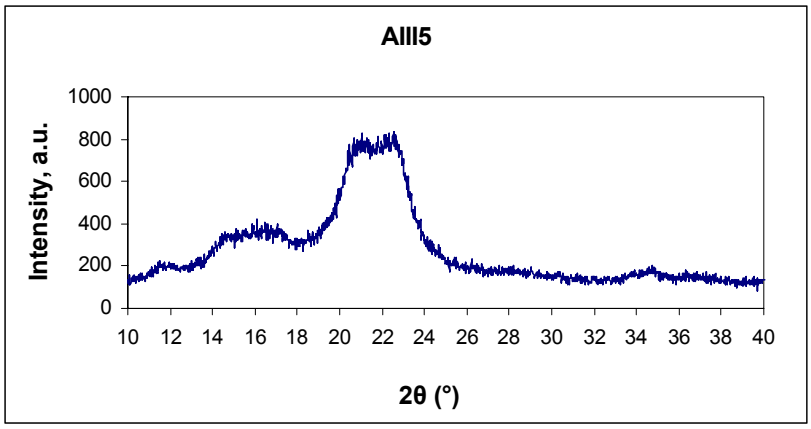

Fig. 7 – The X-ray diffractogram of the AIII sample enzymatically degraded for 10 hours.

The values of the crystallinity index, determined from the X-ray diffractograms for the initial cellulose samples and for those enzymatically biodegraded for 10 hours, are presented in Table 3. It was observed a slight increase of the degree of crystallinity of the cellulosic material during the process of the enzymatic hydrolysis reaction.

Table 3

Crystallinity indexes of the cellulosic samples degraded enzymatically.

Sample	Crystallinity index, %	
	blank	After 10h of enzymatic hydrolysis
AI	83.83	90.38
AII	77.35	84.35
AIII	60.13	63.63

This evolution is explained through the attack on the macromolecular chains placed in accessible, less ordered regions. The scission of chains leads to obtaining a structure that is less dense and consequently less susceptible to the enzymatic attack.

The development of the enzymatic hydrolysis reaction of cellulose can be characterized also by the modification of the degree of polymerization of non-degraded residue obtained after reaction (Table 4).

From the values of the degree of polymerization obtained for the enzymatic hydrolysis of cellulosic substrata, it was observed that an important modification of the DP takes place in the first two hours of hydrolysis, after which the drop takes place more slowly.

Table 4

The DP of the allomorphs of cellulose after enzymatic hydrolysis.

Sample	Degree of polymerization			
	blank	Time of enzymatic hydrolysis, h		
		2	6	10
AI	183	157	153	151
AII	154	128	124	122
AIII	169	155	150	146

CONCLUSIONS

It was demonstrated the fact that both, the morphological structure and the crystalline structure of cellulosic materials are very important in the process of enzymatic hydrolysis. The study of the heterogeneous reaction of cellulose degradation proves to be also adequate for investigating the influence of the modifications induced by chemical treatments of activation of cellulose upon the development of the enzymatic hydrolysis process. The study of X-ray diffractograms of residues resulting from enzymatic hydrolysis show the fact that after biodegradation, the crystalline structure of allomorphic forms I and II does not suffer significant modifications, while for the polymorphic form of cellulose III, a partial return to the crystalline structure of cellulose I was observed. The values of the degrees of crystallinity for all the allomorphic forms enzymatically degradated, at different periods of time, indicate a slight increase during the process of the reaction.

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