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On the supramolecular structure of cellulose allomorphs after enzymatic degradation

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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. 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. The biodegradation of different celluloses by *Trichoderma reesei* was investigated. Changes in supramolecular structure of cellulosic residues resulting from enzymatic hydrolysis were established by X-ray diffraction method. The action of the exocellulosic components is demonstrated by the reduction of the dimensions of crystallites for all the allomorphic forms that were studied. The study of X-ray diffractograms show the fact that after biodegradation, the crystalline structure of allomorphic forms I and II does not suffer significant modifications. For the polymorphic form of cellulose III, a partial return to the crystalline structure of cellulose I was observed.

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1. Introduction

The potential importance of cellulose hydrolysis in the context of conversion of plant biomass to fuel and chemicals is widely recognized and cellulose hydrolysis also represents one of the largest materials flows in the global carbon cycle [1-3].

Many researchers have studied cellulose degradation to glucose, as glucose could easily be converted to a lot of chemicals [4, 5]. Cellulose biodegradation studies have been generally oriented upon physiological characteristics of cellulolytic microorganisms and also on biochemical properties of the enzymes synthesized by them [6-8].

It is well-known the diversity of the structure organization forms of cellulose and their complexity, nevertheless there are only a few studies on the relation between the fine structure of cellulose and its biodegradability [9, 10].

One of the cellulose features which was not yet investigated in detail concerns with the effect of the variety of physical structures adopted by the cellulose molecules in its different crystalline forms, on biodegradation. The allomorphs of cellulose are different from supramolecular structure point of view, like dimensions of monoclinic unit cell, density of intra- and interchain bonds, polarity and the packing degree of macromolecular chains. An important feature of cellulosic materials is their two-phase morphology of crystalline (ordered) and amorphous (disordered) regions, which influence the accessibility and chemical reactivity of fibers. To increase chemical reactivity of cellulose it is important to make more accessible the crystalline regions of cellulose to reactants, by swelling and de-crystallization [11-13].

Because the crystalline domains of native cellulose are formed almost exclusively of cellulose I, the other allomorphs may be considered as analogue substrates and represent useful materials for testing the hydrolysis mechanism of cellulose at molecular level [14].

The degradation rates of the cellulose I, II and of a mixture of the allomorphs II and IV by *Trichoderma viride*, were examined, and was established that the fungi growth on each sample determine the formation of a cellulasic complex with a much bigger activity for a particular cellulosic material [15].

The aim of this paper was to examine the effect of polymorphism on the biodegradability of cellulose using *Trichoderma reesei* enzymes and to analyze the relative importance of different structural parameters on the enzymatic hydrolysis.

2. Materials

Three kinds of cellulose were prepared:

- *Cellulose I (BI)* - Pakistan cotton was extracted in a Soxhlet extractor with ethanol and benzene for 8 hours. It was then boiled in 1% aqueous solution of sodium hydroxide for 6 hours, washed with distilled water, immersed in 1% acetic acid, washed with water, and finally dried in air.

- *Cellulose II (BII)* - Mercerized cellulose with crystalline form of cellulose II was prepared from cotton cellulose by soaking it in 17.5% NaOH for 24 hours at 15^{o} C, followed by washing thoroughly with distilled water and dried in air.

- *Cellulose III (BIII)* - Samples with the crystalline form of cellulose III_I were prepared from cotton cellulose by soaking in organic amine (100% ethylenediamine) for 24 hours at room temperature. The cellulose amine complex was washed with anhydrous methanol and finally cellulose III_I samples were air-dried.

- *Amorphous cellulose* (B amorph) – was obtained by regeneration from cellulose solutions in the solvent system SO_2 – dietilamine -dimethylsulphoxide (SO_2 – DEA - DMSO), in a medium of ethylic alcohol [16].

3. Methods and measurements

Method of hydrolysis – The allomorphs were subjected to enzymatic hydrolyses using 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 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.

Degrees of polymerization of cellulose (DP) were measured by the viscosity method in 0.5 mol Cuen [17].

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-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 [18].

The estimation of the crystallinity index of cellulose samples was established by the ratio from crystallinity area (S_C) and total area (S_T) [19].

2. Results and discussion

In Fig. 1 - 3 are presented the diffractograms of the initial samples and those for the sample treated enzimatically for 6 and 10 hours, respectively.

To observe the structural modifications suffered by cotton cellulose allomorphs during the process of enzymatic degradation, we performed the deconvolution of the peaks presented in the diffractograms, with the help of a soft PeakFit 4.11.

It can be observed that the residues obtaining after enzymatic hydrolysis of cotton cellulose, at different period of time, presents a structure characteristic to allomorph form of cellulose I.

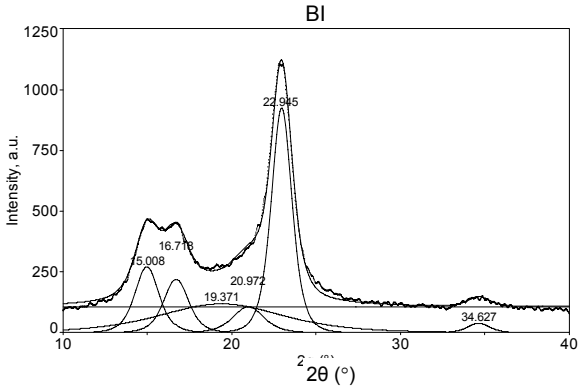

Fig. 1. The X-ray diffractogram of cotton cellulose (BI).

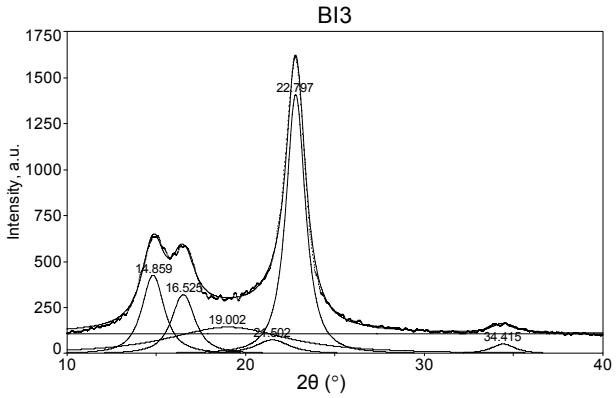

Fig. 2. The X-ray diffractogram of sample BI enzymatically degraded for 6 hours (BI3).

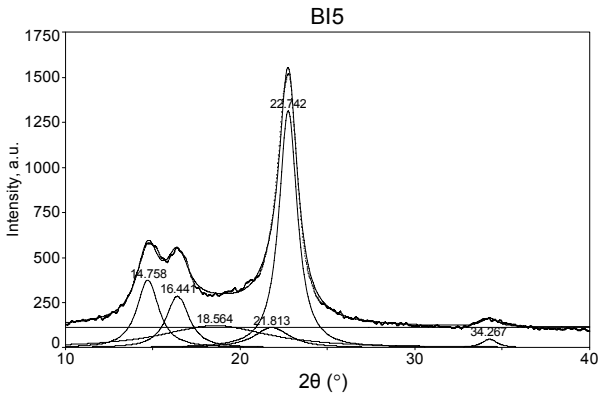

Fig. 3. The X-ray diffractogram of sample BI enzymatically degraded for 10 hours (BI5)

The X-ray diffractograms of cellulosic substrata, by type of cellulose II, on which we performed enzymatic hydrolysis does not present important modifications in their shape compared to the initial samples, before enzymatic treatment (Fig. 4 - 6).

The slight change in the value of the Bragg angle at which we find the reflexes corresponding to the three planes (101), $(10\bar{1})$ and (002) is also confirmed by the study of X-ray diffractograms of the mercerized cotton cellulose sample degraded enzymatically. Thus, for sample BII5 the reflection of plane (101) appears at an angle value of 12.14°, that characteristic to plane $(10\bar{1})$ at 20.06°, and that of plane (002) at 21.98°, while for BII, the reflection values are at 12.34°, 20.38°, and 22.28°, respectively.

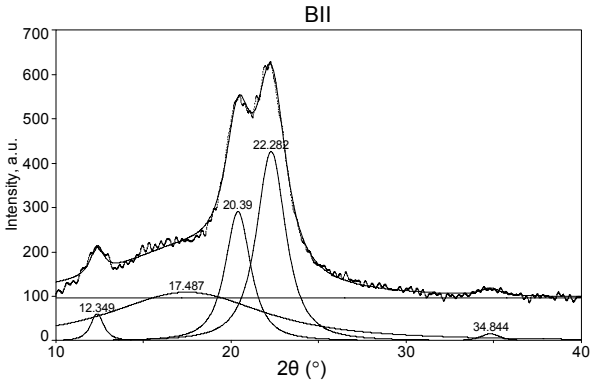

Fig. 4. The X-ray diffractogram of cellulose II obtained from cotton cellulose (BII).

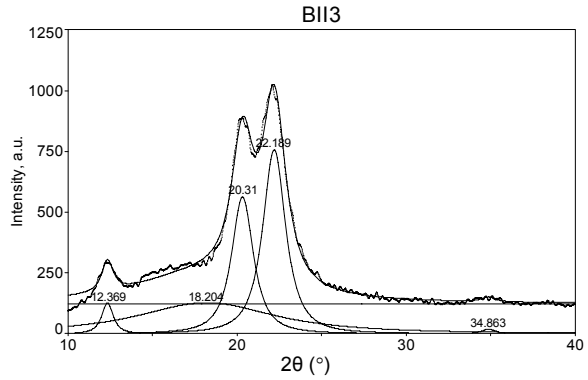

Fig. 5. The X-ray diffractogram of sample BII, enzymatically degraded for 6 hours (BII3).

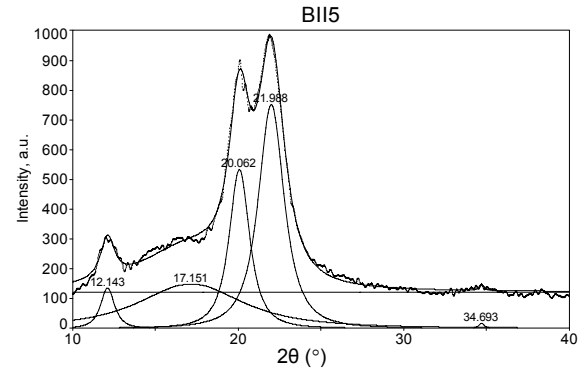

Fig. 6. The X-ray diffractogram of sample BII enzymatically degraded for 10 hours (BII5).

These modifications that appeared in the diffractograms of cellulose II samples degraded enzymatically are not significant and confirm the preservation during the enzymatic hydrolysis reaction of the crystalline structure of cellulose II.

The diffractograms of cellulose III samples that went through the process of enzymatic hydrolysis are presented in Fig. 7 - 9.

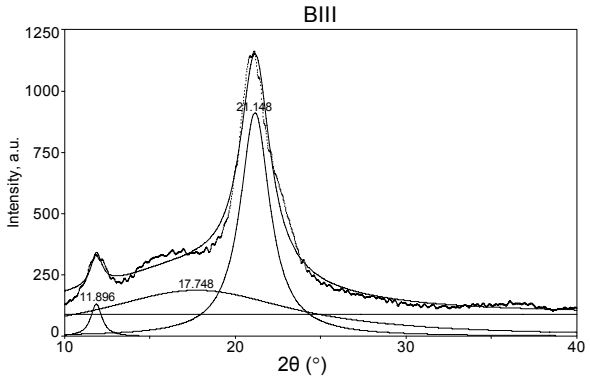

Fig. 7. The X-ray diffractogram of cellulose III obtained from cotton cellulose (BIII).

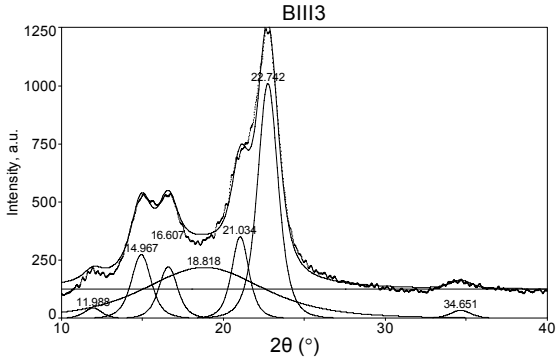

Fig. 8. The X-ray diffractogram of the BIII sample enzymatically degraded for 6 hours (BIII3).

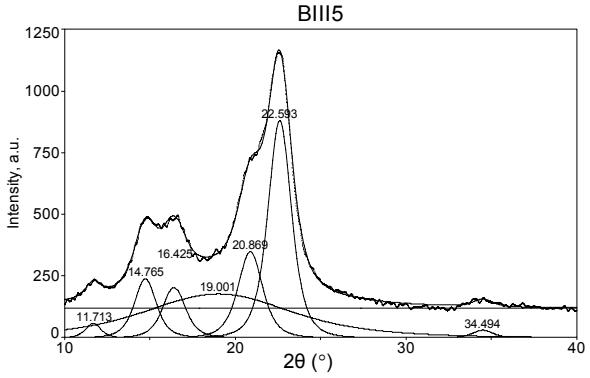

Fig. 9. The X-ray diffractogram of the BIII sample enzymatically degraded for 10 hours (BIII5).

If, in the case of allomorphic forms of cellulose I and II, the X-ray diffractograms of non-degraded materials preserves its shape during the enzymatic reactions, in the case of cellulose III it can be note the partial reversibility to the cellulose I form, demonstrated by the appearance of intensity peaks (101) and ($10\bar{1}$) to the values of the 2θ angles characteristic to cellulose I, namely of 14° and 16°, respectively.

Thus, it was demonstrated the hypothesis that compared to the initial sample (enzymatically untreated), in the X-ray diffractograms of the samples enzymatically hydrolyzed appear the reflections of planes (101), ($10\bar{1}$), and (021) to values of the Bragg angle characteristic of cellulose I.

It is well know that in the case of allomorph type of cellulose III, the peaks ($10\bar{1}$) and (002) are superpose at the same Bragg angle, between 20 - 21° [20]. In the case of residues obtained after enzymatic hydrolysis of cellulose II, the reflection of plane (101) of cellulose III appears at approximately the same value for the untreated samples, as well as those treated enzymatically. Instead, the reflection of plane (002) appears at much higher values of the Bragg angle, in the case of the enzymatically hydrolyzed samples, values close to those at which we find the peak (002) in the diffractograms characteristic of cellulose I.

Table 1 illustrates the intensity maximum position of the characteristic peaks of allomorph forms of cotton cellulose.

Table 1. Structural characteristics of cellulose substrates from the analysis of their equatorial X-ray wide angle diffraction.

Structure parameter	Maximum position, 2θ (°)										
	BI	BI3	BI5	BII	BII3	BII5	BIII	BIII3		BIII5	
								III	I	III	I
(101)	15.00	14.85	14.75	12.34	12.36	12.14	11.89	11.98	14.96	11.71	14.76
($10\bar{1}$)	16.71	16.52	16.44	20.38	20.30	20.06	21.14	21.03	16.60	20.86	16.42
(021)	20.97	21.50	21.81	-	-	-	-	-	21.03	-	20.86
(002)	22.94	22.79	22.74	22.28	22.18	21.98	21.14	-	22.74	-	22.59

These values confirm the partial reversion of cellulose III to cellulose I and leads us to assume that there is a juxtaposition of the reflection of the (002) plane of cellulose III with that of cellulose I. An explanation for this reversibility is the presence of the water-based medium and of the high temperature of the reaction (50°C).

Thus, after enzymatically degradation of cellulose III, cellulose with a structure with parallel type of bonding of chains, characteristic to cellulose I, was obtained. This still retains a certain disorganized state at the level of supramolecular structure, which makes it more accessible to the enzymatic attack.

The values of the crystallinity index, determined from the X-ray diffractograms for the initial cellulose samples and for those enzymatically biodegraded for 6 and 10 hours, are presented in Table 1.

It was observed a slight increase of the degree of crystallinity of the cellulosic material during the process of the enzymatic hydrolysis reaction.

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.

Table 1. Crystallinity indices of the cellulosic samples enzymatically degraded.

Sample	Crystallinity index, %		
	blank	Time of enzymatic hydrolysis, h	
		6	10
BI	71.11	76.66	78.75
BII	60.57	68.55	68.87
BIII	53.23	60.32	62.66

The action of the exocellulosic components is also demonstrated by the X-ray diffraction studies by the reduction of the crystallite dimensions for all the allomorphic forms that were studied.

It was find that during the reaction, the size of crystallites in the (101) and ($10\bar{1}$) direction remains approximately around the same value, while in the (002) direction the decrease is much more significant, compared to the initial sample (Table 2).

Table 2. Crystallinity indexes of the cellulosic samples degraded enzymatically.

Sample	Crystallite dimension, Å		
	D (101)	D (10-1)	D (002)
BI	35.97	48.51	30.64
BI3	35.85	48.07	26.03
BI5	30.57	46.98	23.39
BII	46.71	46.92	38.58
BII3	46.59	44.36	31.32
BII5	44.55	44.97	30.46
BIII	58.78	-	41.75
BII3	57.43	-	40.14
BIII5	51.93	-	39.09

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 3).

Table 3. The DP of the allomorphs of cellulose after enzymatic hydrolysis.

Sample	Degree of polymerization			
	blank	Time of enzymatic hydrolysis, h		
		2	6	10
BI	3078	2143	2138	2029
BII	2048	1709	1448	1405
BIII	2829	2138	2082	2045

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.

5. Conclusions

1. We demonstrated the fact that both the morphologic structure and the crystalline structure of cellulosic materials are very important in the process of enzymatic hydrolysis.

2. During the process of enzymatic degradation there are scissions of the macromolecular chains, modifications that are evidenced by the decrease in the degree of polymerization.

3. The study of X-ray diffractograms of residues resulting from hydrolysis show the fact that after biodegradation, the crystalline structure of allomorphic forms I and II does not suffer significant modifications.

4. The polymorphic form of cellulose III suffers a partial return to the crystalline structure of cellulose I.

5. The process of enzymatic degradation produces a decrease, in time, of crystallite dimensions.

6. The values of the degrees of crystallinity for all the allomorphic forms degraded enzymatically, at different points in time, indicate their slight increase during the process of the reaction.

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