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Illumination of Cellulose with Linearly Polarized Visible Light

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Summary: Aqueous suspensions of cellulose of long polysaccharide chains, were illuminated with visible polarized light (VPL) for 20 and 50 hrs. Crystal structure, thermal properties with Differential Scanning Calorimetry (DSC) and degree of polymerization (DP) of the samples were determined. Additionally, kinetic of enzymatic as well as acid-catalyzed hydrolysis of cellulose was estimated. Illumination of cellulose with VPL for 50 hrs increased its DP by 15%. X-ray diffraction patterns revealed that the illumination resulted in an increase in the amount of cellulose crystalline phase. The DSC measurements indicated differences in the water molecules distribution depending on the sample treatment confirming an increase in the crystallinity of the illuminated cellulose. After prolonged illumination, cellulose was resistant to oxidation and had lower susceptibility to enzymatic and acid-catalyzed hydrolysis.

Keywords: cellulose; crystal structure; degree of polymerisation; polarized light

Introduction

Cellulose consists of long chains of $(1 \rightarrow 4)$ linked β-D-glucopyranose units, which are interconnected by hydrogen bonds. It forms fibrous structures within crystalline and non-crystalline areas.[1] Water molecules catalyze the formation of the natural cellulose crystals by their involvement in the hydrogen-bond bridging.

Six types of the crystalline cellulose $I_{\alpha+\beta}$, II, III_1, III_2, IV_1, IV_2 with different acing arrangement, size of the unit cell, hydrogen bonding scheme was described in literature [2] with main focus on native cellulose I.[3]

Degradation, acid-catalyzed hydrolysis, enzymatic hydrolysis, oxidative degradation, and alkaline degradation are the most common reactions of cellulose.[4] Hydrolytic cleavage of the glycosidic bonds between two anhydroglucose units is the most important route of the polymer degradation on cellulose processing and analysis. That cleavage of the β-1,4-glycosidic bonds is catalyzed by either protons [4] or by cellulolytic enzymes.[5] On the depolymerisation of cellulose, there is a symbiotic action of catalyzing acid and oxidation.[6,7] Cellulose is also susceptible to degradation by the UV light [8], and solar radiation.[9]

Plants react to the polarized light.[10] Reports on effects of the polarized light upon the activation of enzymes such as α-amylase in the starch α-amylolysis,[11] xylanase in the degradation of xylan,[12] cellulase in the hydrolysis of cellulose,[13] and glucosyltransferase in the production of cyclodextrins [14] recently appeared. Enhancement rate of enzymatic hydrolysis of starch polysaccharides[15] and rearrangement of the molecular structure of starch polysaccharides[16] could be noted. This work provides results of our study on the changes of physicochemical properties of cellulose on illumination with VPL.

Materials and Methods

Materials

Cellulose long fibers from cotton (C6663 Sigma-Aldrich, Poznan, Poland), cellulase

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from *Aspergillus* species of $\geq$1000 U/g activity (Carezyme 1000L®; EC 3.2.1.4) (C2605 Sigma-Aldrich, Poznan, Poland), cupri-ethylene-diamine (CED) for dissolved samples of cellulose (Amara, Kraków, Polska or Merck, Warszawa, Polska).

Methods

Samples of cellulose before and after illumination were conditioned and their DP, water content and distribution, crystalline structure, and susceptibility of to enzymatic and acid-catalyzed hydrolysis were determined.

Conditioning: All samples of cellulose were conditioned prior to experiments. The conditioning was performed at constant partial water vapour pressure over a saturated solution of magnesium nitrate (at $RH \approx 50\%$) for at least 24 hrs at 23 °C.[17]

Illumination: Aqueous suspensions of cellulose (0.1 cellulose/1ml water) were illuminated with VPL for 20 and 50hrs from the 30cm distance with a KB 502 slit illuminator (Kabid, Chorzów, Poland) equipped with 150W xenon arc (XBO 150, Oriel, Maidston, UK). An HN 22 linear polarizing filter (Polaroid, Waltham MA, USA) with glass filter cutting out wavelengths below 500nm was mounted between slit illuminator and the sample. The light source emitted continuous radiation in the visible range. Its energy flux at the position of the sample was 8mW/cm^2 as checked by YSI radiometer (Yellow Spring OH, USA). Control, non-illuminated samples of cellulose were stored in the dark under the same conditions as illuminated samples. After illumination, celluloses were filtered and dried at room temperature.

Water content and its distribution: Distribution and water content in cellulose were determined from DSC measurements. DSC experiments were performed in *Mettler-Toledo 821^e* calorimeter equipped with an intracooler *Haake* under constant flow of argon (80ml/min) within temperature range of $(0) \div (+180\,^\circ C)$ and heating rate of $\beta = 5\,^\circ C/min$ for water content determination or within $(+25) \div (-50) \div (+25\,^\circ C)$ and heating/cooling rate of $\beta = 2.5\,^\circ C/min$ for water distribution experiments. The measurements of water distribution in cellulose samples were performed in 40μl lid closed (hermetically) aluminium pans. Water content in cellulose samples was determined by heat effect of water evaporation from sample closed in the 40μl aluminium pans after making a micro-hole ($\varnothing = \sim 0.05$ mm) in the lid. The distribution and water content were determined from heat effects of water evaporation ($\Delta H_{vap} = -2216$ J/g) or freezing ($\Delta H_{fr} = +354.9$ J/g) of pure water.[18]

Degree of Polymerization (DP): The mean *DP* was calculated from the Mark-Houwink equation [19]: $[\eta] = Q \bullet DP^a$, where: $Q = 1.33$ ml/g, $a = 0.905$.[20] The limiting viscosity number $[\eta]$ of cellulose dissolved in the solution of cupri-ethylene diamine (CED) was determined according to standard.[21] All calculations were performed for anhydrous cellulose. Glass viscometers of Werner Glass&Instrument AB, Sweden were applied.

Crystalline structure: The changes in the crystalline structure of cellulose before and after illumination was determined by X-ray powder diffraction technique on PW3710 Philips X'Pert apparatus using Cu $K_{\alpha 1}$ ($\lambda = 0.154178$ nm) radiation with a graphite monochromator.

IR spectra: The illuminated and non-illuminated cellulose samples were submitted to FT-IR analysis by the diffuse reflectance method (DRIFT) using a Mattson 3000 spectrophotometer. All spectra were acquired as 64 signal averaged scans between 400 and 4000 cm^{-1} at a resolution of 2 cm^{-1}.

Acid-catalyzed hydrolysis (Accelerated Aging Tests): Low rate of cellulose acid-catalyzed hydrolysis resulted in a necessity of using artificial ageing tests. Ageing tests are based on accelerating the observed changes of cellulose properties – usually by using high relative humidity and high temperature.[22,23] Sample of cellulose was inserted into glass vessel. This vessel was hanged in the polypropylene container of capacity 250 mL containing 50mL water on the bottom in order to attain the 100%

Table 1.
Polymerization degree (DP), total, freezable, non-freezable water content in cellulose samples.

Sample[a)]	Illumination time[h]	DP	Water content [%]		
			Total	Freezable	Non-freezable
C0	–	880	6.7	0.0	6.7
C1	50	1014	18.7	4.5	14.2
C2	0	870	3.8	0.0	3.8
C3	20	870	4.4	0.0	4.4

[a)] C0 - pure cellulose, C1 - cellulose illuminated for 50 hrs, C2 - non-illuminated cellulose, suspended in water for 50 hrs, C3 – cellulose illuminated for 20 hrs.

relative humidity (*RH*). There was no direct contact of cellulose with liquid water. Nonhermetically closed container was inserted into automatic dryers (90 °C) and kept in darkness. Each container was closed after required temperature and pressure stabilized. The stability of temperature was within ±0.5 °C. Accelerated ageing was controlled for 23 days. After the assumed time of ageing the process had been attained and the containers were cooled and opened.[24] The reactions were run in duplicates.

Enzymatic reaction: Cellulose suspended in phosphate buffer 0.1M pH 7.0 (concentrations 5mg/1mL) was placed in 37 °C water bath and enzyme solutions (1.6mL/1g cellulose; 1.76U/1mg cellulose) were added. After enzyme addition, samples were incubated with mild agitation at 37 °C for up to 21 h. The enzyme was deactivated through addition of CED solution and DP was determined. The procedures were duplicated.

Results and Discussion

Four samples of cellulose have been obtained: C0 - pure cellulose, C1 - illuminated for 50 hrs, C2 – non-illuminated, suspended in water for 50 hrs and C3 - illuminated for 20hrs.

DP of cellulose suspended in water for 50 hrs and illuminated for 20 hrs decreased by about 10 units, that is, by only 1%. After 50 hrs of illumination the DP value increased by 134 units, that is by 15% (Table 1).

The highest total water content was observed for 50 hrs illuminated cellulose. Freezable water was present only in the sample illuminated for 50 hrs, as proven by the different water distribution in illuminated cellulose (Table 1). Performed DSC experiments on the cellulose samples revealed that most of that water was non-freezable – probably due to its involvement in the cellulose fibril bridging.

The FT-IR spectra of the illuminated and non-illuminated celluloses revealed the same set of absorption bands, typical for cellulose.[25] An insight in the positions and intensity of the groups of bands [26] might suggest, that the oxidation of cellulose during illumination did not occur.

The native cellulose presented the crystalline, type-I form.[26] After 50 hrs illumination, intensity of peaks for cellulose type-I ($2\theta = 14.7, 16.4, 22.5, 34.2$) and type-II ($2\theta = 20.2$) increased (Table 2).

Table 2.
X-ray diffraction data for cellulose illuminated for 50hrs (C1), non-illuminated, suspended in water for 50 hrs (C2) and illuminated for 20 hrs (C3)

Sample	Peak intensity at 2θ cm^{-1}			Full width of half maximum of $2\theta = 22.6$ cm^{-1}
	14.7	16.4	22.6	
C0	104	102	508	0.4
C1	218	173	811	0.7
C2	176	155	806	0.7
C3	147	137	718	0.6

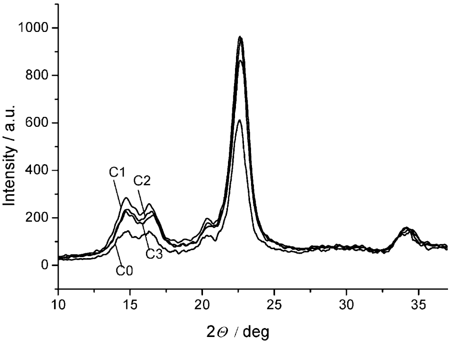

Figure 1.
X-ray diffraction for cellulose illuminated with VPL for 50 hrs (C1), non-illuminated, suspended in water for 50 hrs (C2) and illuminated for 20 hrs (C3).

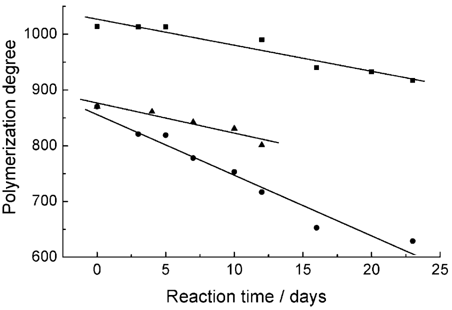

Figure 2.
Acid-catalyzed hydrolysis: ■: with cellulose illuminated with VPL for 50 hrs ($k = 4.7$ day^{-1}); ▲: with cellulose illuminated with VPL for 20 hrs ($k = 5.4$ day^{-1}); ●: with non-illuminated cellulose ($k = 10.9$ day^{-1}).

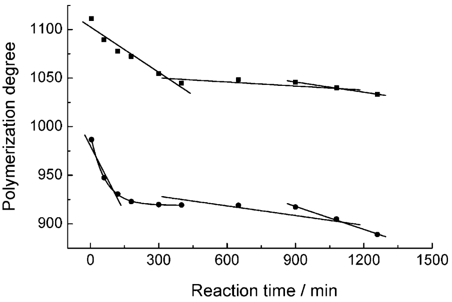

Figure 3.
Enzymatic hydrolysis ■: with cellulose stimulated with VPL for 50 hrs ($k_1 = 0.15$ min^{-1}, $k_2 = 0.013$ min^{-1}, $k_3 = 0.035$ min^{-1}); ●: with non-stimulated cellulose ($k_1 = 0.48$ min^{-1}, $k_2 = 0.033$ min^{-1}, $k_3 = 0.078$ min^{-1}).

Dependence of DP of cellulose on time of the acid-catalyzed hydrolysis was determined (Figure 2). DP was a linear function of time ($R = -0.99$). The acid-catalyzed degradation of samples of cellulose was a zero-order reaction. Degradation of illuminated cellulose was slower than the degradation of non-illuminated cellulose.

In the enzymatic hydrolysis, three stages of the reaction could be distinguished (Figure 3). Enzymatic hydrolysis of illuminated cellulose was slower than that of non-illuminated cellulose.

Conclusion

Illumination of cellulose with linearly polarized light for 50 hrs increased the degree of polymerization by 15%. Such effect was not observed for cellulose illuminated for 20 hrs as well as for non-illuminated cellulose.

Different water content was found for the samples of illuminated and non-illuminated cellulose prepared under identical conditions (water bath, drying and conditioning). Illuminated cellulose (50 hrs) incorporated the highest amount, about 18% water. Only for this sample the heat effect related to water freezing was observed.The cellulose suspended in water was resistant to oxidation.

Long time illumination (50 hrs) increased the quantity of cellulose crystal phase. This effect was not observed for cellulose illuminated at short time (20 hrs).

Illuminated cellulose revealed lower susceptibility to enzymatic and acid hydrolysis.

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