EFFECT OF CELLULOSE STRUCTURE ON ENZYMATIC HYDROLYSIS

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Enzymatic hydrolysis of non-dried and dried cellulose samples having various particles size, degree of polymerization, porosity, crystalline polymorph, and content of non-crystalline domains has been studied. Regression analysis was carried out to determine contribution of various structural features of cellulose samples to their hydrolysability. It was found that particle size, degree of polymerization, and crystalline polymorph had a negligible influence on the conversion degree of cellulose into glucose under the effect of the cellulolytic enzyme. Such characteristics as the pores volume had a fair impact on the conversion degree of cellulose. Drying of the wet samples caused decreasing of the hydrolysability of cellulose due to irreversible collapse of the pores volume. The content of non-crystalline domains (A_x) in cellulose had the highest effect on the rate of enzymatic hydrolysis and average conversion degree (α_a) of cellulose into glucose. A linear dependence $\alpha_a = f(A_x)$ was established both for dried and non-dried cellulose samples.

Keywords: Cellulose; Structure; Enzymatic hydrolysis; Correlations

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INTRODUCTION

One of the important branches of biotechnology involves enzymatic hydrolysis of carbohydrates into monosaccharides with subsequent fermentation to produce bio-ethanol. At present, the main source of carbohydrates for the industrial fermentation is juice of sugarcane or sugar beet, starches of corn, wheat, potatoes, and some others agricultural plants. In the USA approximately 85 percent of the total ethanol production, or about 10 billion gallons, relies on the grain of corn. Since carbohydrates of agricultural plants are required by the food industry, their use for biotechnology is limited. Moreover, further expansion of the bio-technologic production to higher volume will cause a deficit of food and feed products and an increase of their prices. It was calculated that the production of sufficient ethanol to fuel an automobile traveling 10,000 miles would need about 1.5 to 2.0 acres of corn. If all US automobiles were fueled with such corn-based ethanol, then the most part of agricultural land area of USA would be needed to grow the corn feedstock to produce biofuel only.

Extraction of fermentable monosaccharides, mainly glucose from non-food cellulose wastes, has been regarded as a promising way to obtain valuable bio-products without competing with food and feed industry. In particular, scraps of pulp, paper, cellulose fabrics and fibers, as well as cotton residues (e.g. linter, fuzz, etc.) and some others cellulose wastes can be used as promising and relative cheap raw materials for

enzymatic hydrolysis for production of the fermentable glucose. Resources of such cellulose wastes accumulating annually in the world reach more than 100 million tons (Asia Paper Markets 2011). Besides, additional huge amounts of cellulose-rich pretreated plant materials (forest and agricultural wastes, bushes, grasses, etc.) can be produced and used for enzymatic hydrolysis to convert cellulose into glucose.

Unfortunately, initial cellulose materials exhibit a high resistance to enzymatic cleavage. Various structural factors have been discussed to explain the limited ability of cellulose to hydrolysis under effect of cellulolytic enzymes: coarse particles, high degree of polymerization, increased crystallinity, low porosity, decreased specific surface, low moisture, presence of residual lignin, etc. (Fan et al.1980, 1981; Hendrikis et al. 2009; Jeoh et al. 2007; Park et al. 2010; Philipp 1983; Wada et al. 2010).

Crystallinity of cellulose is considered to be an important structural parameter that hinders the enzymatic hydrolysis (Fan et al. 1980, 1981; Hall et al. 2010; Park et al. 2010). Amorphization of cellulose by its dissolution followed by regeneration from the solutions leads to extremely rise of hydrolysis rate and degree (Dadi et al. 2006; Hall et al. 2010; Zhang et al. 2006).

Nevertheless, some researchers think that crystallinity of cellulose may not be a key factor determining the enzymatic hydrolysis (Chen et al. 2007; Mansfield et al. 1999; Wang et al. 2006; Zhang et al. 2004). Thompson et al. (1992) concluded that just pore size of substrates in relation to the diameter of enzyme molecules is the main structural factor influencing on enzymatic hydrolysis. Grethlein et al. (1985) showed that initial rate of the hydrolysis by cellulolytic enzyme of *Trichoderma reesei* is correlated with the pore volume of the substrate. Drying of the wet cellulose can cause an irreversible collapse of the pore structure; the result is in a decreased hydrolysability of the dried sample (Philipp 1983). A relationship between accessible surface area of pores and digestibility was also found (Sinitsyn et al. 1991). However, in other investigations the accessible surface area was not considered as a main dependent factor that affects cellulose digestibility (Fan et al. 1980; Millet et al. 1976). Decreasing in degree of polymerization was discussed also as an additional factor promoting the digestibility of cellulose at the enzymatic hydrolysis (Puri 1984).

Several investigations showed that large particles of substrates are slowly hydrolysable; however, reduction of particle size below 0.3-0.5 mm did not enhance the hydrolysis rate (Chang et al. 1997; Draude et al. 2001). To loosen the structure and improve access of enzymes, the cellulose substrates were treated with concentrated alkali solutions, liquid ammonia, amines, and other activating agents. It was found that activation of cellulose structure accelerates the enzymatic hydrolysis (Igarishi et al. 2007; Philipp et al. 1979, 1983; Wada et al. 2010). To describe kinetics of cellulose bio-digestion, the equation of pseudo-first order was proposed (Philipp et al. 1979, 1983; Weimer et al. 1990). However, the used activating agents can cause various changes of cellulose structure; they raise the porosity and specific surface, decrease of crystallinity and size of crystallites, increase volume of crystalline unit cells, and change the crystalline modification of cellulose. Intensive milling of cellulose can lead to simultaneous decreasing of the particle size, degree of polymerization, and crystallinity of cellulose and increasing of the specific surface area (Caulfield et al. 1974). Therefore,

it is impossible to conclude unambiguously, which structural factor is crucial for improving the enzymatic cleavage of cellulose.

The purpose of this paper was to establish quantitative relationships between the structural features of the cellulosic substrates and their hydrolysability under effect of the cellulolytic enzymes.

EXPERIMENTAL

Materials

Various cellulosic materials were used. Bleached sulfite spruce pulp (SFI) was obtained from Weyerhaeuser Co, WA, USA. Non-dried bleached spruce kraft pulp was delivered from Södra plant, Sweden. Linter of the middle-length cotton "Acala" cultivated in Israel was refined by a soda cooking (Ioelovich et al. 2008).

Herbaceous biomasses of bagasse (from Brazil), switchgrass (from Canada), and rice straw (from China) were used as initial raw materials to isolate cellulose by Kürschner-Hoffer method with subsequent soda cooking (Obolenskaya et al. 1991). Filter paper No 1 of Whatman and microcrystalline cellulose (MCC) Avicel PH-301 also were used.

To achieve partial depolymerization, the refined cotton linter was irradiated by means of Gamma-rays of ^{60}Co up to absorption dose 50 to 300 kGy. Cotton linter was hydrolyzed with boiling 2.5N HCl for 30 min with subsequent washing up to a neutral pH value. Regenerated cellulose (RC) was prepared from the MCC solution in ortho-phosphoric acid (Zhang et al. 2009). Low-crystalline cellulose (LC) was obtained by treatment of SFI pulp with liquid ammonia for 30 min with following drying at 60 ^{o}C up to constant weight. Treatment of the cellulose materials with 5 to 20 wt.% solutions of sodium hydroxide was carried out at room temperature for 1 h with subsequent washing until a neutral pH value was reached. To remove excess water, the non-dried cellulose samples were preliminary squeezed up to solid content about 20 wt. %. Drying of the wet samples was carried out at 105 ^{o}C up to constant weight.

To reduce size of particles, the SFI and filter papers were cut on 2-5 mm pieces and screened through sieve of 5 mesh size in order to select pieces with average size about 3000 μm. These paper samples were also knife-milled by "Waring" blender at 5000 rpm for 1 min and screened through sieves of 15 and 30 mesh to select the fiber fraction with average size about 700 μm. To achieve lower particles size, these papers were hydrolyzed with boiling 2N HCl for 30 min, washed up to neutral pH, dried, knife-milled, and screened through sieves set to select the particles with average size of 50 and 90 μm. Dried regenerated cellulose and alkali-treated filter paper were knife-milled and screened through sieves to select the particles with average size about 500 and 1000 μm. Dried alkali treated MCC after knife-milling and screening through sieves 100 and 200 meshes had average size about 100 μm.

Enzymatic Hydrolysis

Cellulose samples were hydrolyzed with a mixture of commercial cellulolytic enzyme GC-220 (Genencor Inc, Danisco, NY, USA) and β–glucosidase Novozyme-188

(Novozymes A/S, Bagsvaerd, Denmark). The loading of GC-220 was 10 FPU per 1 g of cellulose and of Novozyme-188 was 8 IU per 1 g of cellulose. Hydrolysis of the samples was carried out in 50-mL polypropylene tubes. The 1 g of solid cellulose sample and 10 mL of 50 mM/L acetate buffer having pH=4.8 were put into the tubes. Then, cellulolytic enzyme GC-220 and β–glucosidase Novozyme-188 were added. Further, an additional amount of the buffer was supplemented to obtain total volume of the liquid phase 20 mL and concentration of the cellulose sample 50 g/L. The tubes closed with covers were placed in a shaker incubator at 50 ^{o}C and shaken at 150 rpm during various times. Three samples of the same cellulose type were hydrolyzed simultaneously to obtain an accurate result of conversion degree (α). The standard deviation of determination of the α-value was not greater than ±0.02.

X-Ray Diffraction

X-ray investigations of dried and swollen samples were carried out with a Rigaku-Ultima Plus diffractometer (CuK_α – radiation, λ=0.15418 nm). To hold the swollen structural state, the non-dried cellulose samples were washed with absolute ethanol, then with acetone and pentane and dried at 60 ^{o}C up to constant weight. X-ray diffractograms were recorded in the $\varphi=2\Theta$ angle range from 5 to 80°. Different crystalline polymorphs (CP) of cellulose - CI, CII, CIII, and others, give diffraction peaks at the different 2Θ angles (Bikales et al. 1971). After recording of the diffractograms, the background was separated, and selected X-ray patterns were corrected and normalized. Then diffraction intensities from crystalline and non-crystalline regions were separated by a computerized method (Vonk 1973). The part of non-crystalline amorphous domains (A_x) in a cellulose sample, as determined by the X-ray method, was calculated according to Equation 1 (Ioelovich et al. 2009, 2010),

$$A_x = 1 - (\int J_c \, d\varphi / \int J_o \, d\varphi) \quad (1)$$

where J_c and J_o are the corrected and normalized diffraction intensities for crystalline regions and sample respectively; φ=2Θ diffraction angle.

Three diffractograms were recoded for the each cellulose type to calculate average A_x-value and its standard deviation that was in the range ±0.02.

Solid-state CP-MAS 13C NMR

The samples were packed uniformly in a zirconium oxide rotor. Solid-state CP-MAS 13C NMR measurements are conducted on a Bruker Avance DPX300 NMR spectrometer, operating at 75.46 MHz 13C-resonance frequency. The magic angle speed rate was in the range of 4 to 5 kHz. Measurements involved the following parameters: time of 90-pulse was 4.3 μs, cross-polarization contact time was 1ms, repetition interval was 3 s, and the number of accumulations was about 1200. Glycine was used as an external standard for calibration of the chemical shift scale relative to tetramethylsilane. The amorphous index *(AI)* of cellulose samples was calculated by the following equation,

$$AI = I_a / (I_a + I_c) \quad (2)$$

where I_a - intensity of the C4 "amorphous" peak at 84 ppm; I_c - intensity of the C4 "crystalline" peak at 89 ppm.

Three CP MAS 13C NMR spectra were recoded for the each cellulose type to calculate average *AI*-value and its standard deviation that was in the range ±0.01.

Chemical and Physicochemical Tests

The content of alpha-cellulose and average degree of polymerization *(DP)* of the cellulose samples were studied by standard TAPPI methods T-203 and T-230. Water retention value (WRV) of the samples characterizing total volume of pores (V_p) in the water medium was tested by the method of Jayme et al. (1960, 1968) using a centrifugal force 3000 G for 15 min (see SCAN-C 62:00 procedure). SD at determination of alpha-cellulose content was at most ± 1%, of DP ± 10, and of WRV ± 0.2 cm^3 /g.

The concentration of the glucose in solutions after enzymatic hydrolysis of the cellulose samples was tested by the conventional *DNS*-assay using glucose for calibration (Miller 1959). Weight loss of the samples after the hydrolysis also was studied. The conversion degree α_g of cellulose into glucose was calculated from results of glucose concentration in the solution. On the other hand, the conversion degree α_w can be determined from weight loss of the cellulose sample during the hydrolysis,

$$\alpha_g = C/C_o \quad (3)$$

$$\alpha_w = 1-(W/W_o) \quad (4)$$

where C is the concentration of glucose in solution (mM/L) after hydrolysis of the sample for the certain time, C_o = 308.6 mM/L is the maximal concentration of glucose in the solution for the fully hydrolyzed cellulose, and $W(g)$ and W_o = 1 g are the weights of the hydrolyzed and initial samples, respectively.

RESULTS AND DISCUSSION

Characteristics of the Initial and Modified Cellulose Materials

Some characteristics of the dried cellulose samples having various crystalline polymorphs - CI, CII and CIII, are shown in Table 1. The samples contained relatively high levels of alpha-cellulose, indicating that they had sufficient purity. Samples of commercial MCC Avicel and also of cotton linter partially hydrolyzed with hydrochloric acid were characterized as having decreased proportions of non-crystalline domains, while a sample of regenerated cellulose (RC) showed highly amorphized structure (Fig. 1). Grass-based celluloses (BAC, SGC, and RSC) were less crystalline than wood pulps and cotton cellulose.

The 13C NMR method can be used directly for structural investigations of the swollen cellulose samples in the presence of water. For example, it can be shown that treatment of the cellulose with alkali solutions increases intensity of the "amorphous" peak at the chemical shift 84 ppm and decreases intensity of the "crystalline" peak at the chemical shift 89 ppm in 13C NMR spectrums (Fig. 2).

Table 1. Characteristics of Dried Cellulose Samples

Samples	*CP*	*Alfa-Cellulose, %*	*DP*	*AI*	A_x
Sulfite pulp (SFI)	CI	95	1100	0.35	0.37
Kraft pulp (KP)	CI	92	960	0.33	0.35
Mercerized Kraft pulp (KPM)	CII	99	910	0.50	0.47
Filter paper (FP)	CI	99	1200	0.30	0.29
Refined cotton linter (CL)	CI	98	1600	0.27	0.31
Acid-hydrolyzed cotton linter (CLH)	CI	86	180	0.25	0.23
Mercerized cotton linter (CLM)	CII	99	1520	0.48	0.45
Avicel MCC (AV)	CI	87	170	0.26	0.25
Mercerized Avicel (AVM)	CII	98	160	0.45	0.42
Bagasse cellulose (BAC)	CI	92	480	0.43	0.44
Switchgrass cellulose (SGC)	CI	90	440	0.47	0.46
Rice straw cellulose (RSC)	CI	89	400	0.52	0.50
Low-crystalline SFI pulp (LC)	CIII	93	1000	0.60	0.62
Regenerated cellulose (RC)	CII	-	150	0.76	0.74

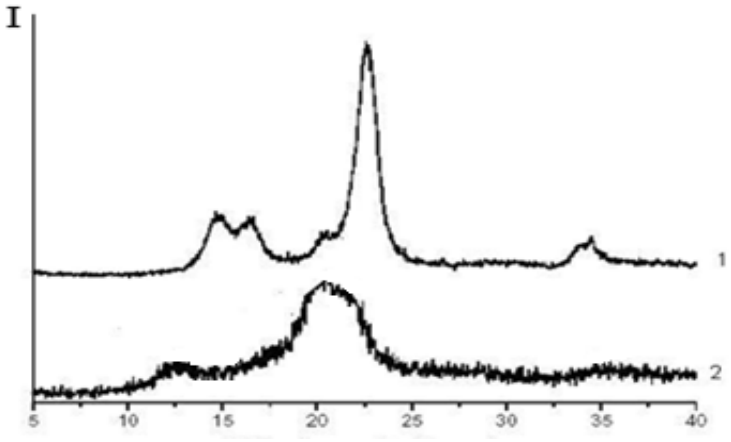

Fig. 1. X-ray diffractograms of acid-hydrolyzed cotton (1) and regenerated cellulose (2)

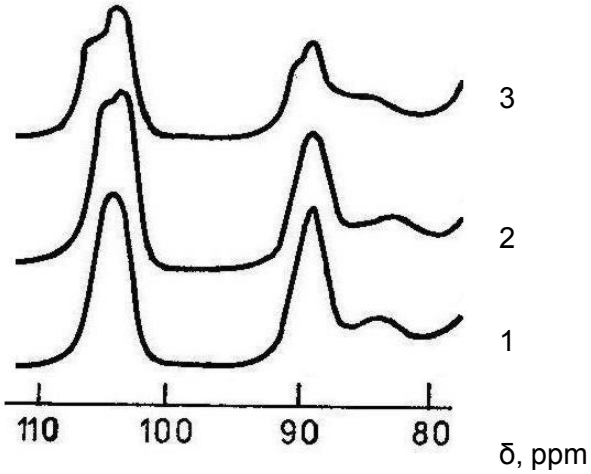

Fig. 2. NMR 13C spectrums of non-dried samples of cotton linter after cooking (1) and alkalization with 12 wt. % (2) and 20 wt. % (3) of NaOH in the range of chemical shift *(δ)* 80-110 ppm

The 13C NMR and XRD investigations also showed that the same swollen or dried samples had similar content of non-crystalline (amorphous) domains (Table 2).

Table 2. Content of Non-crystalline Domains for Some Swollen and Dried Cellulose Samples

Samples	*AI*		*A_x*	
	Swollen and non-dried	*Dried*	*Fixed swollen state*	*Dried*
Kraft pulp (KP)	0.34	0.33	0.36	0.35
Refined cotton linter (CL)	0.27	0.27	0.29	0.31
CL alkalized with 12 wt. % NaOH	0.40	0.40	0.36	0.37
CL alkalized with 20 wt. % NaOH	0.47	0.48	0.43	0.45
Regenerated cellulose (RC)	-	0.76	0.75	0.74

Gamma-irradiation of cellulose leads to its selective depolymerization without changing in crystallinity degree (Ioelovich et al. 1997, 1999). As can see from Fig. 3, as a result of the Gamma-irradiation with dose of 300 kGy the average *DP* of the cotton cellulose decreased to the range 100-120.

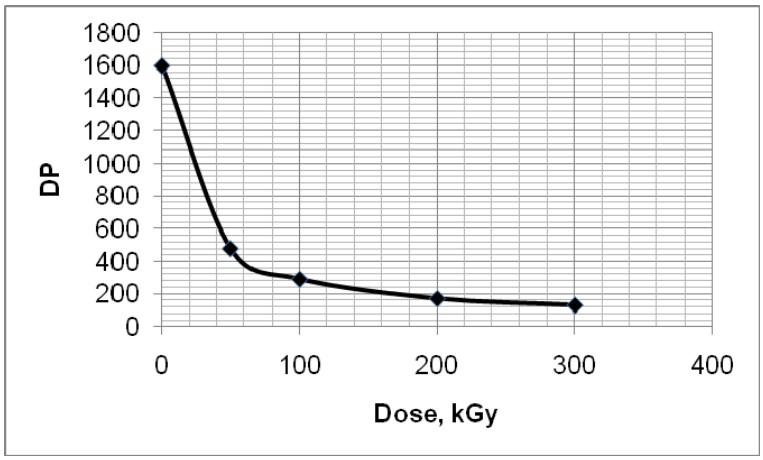

Fig. 3. Dependence of *DP* of refined cotton linter on dose of Gamma-irradiation

Investigations of water retention value *(WRV)* of various cellulose samples were carried out to estimate total volume of pores *(V_p)*. After swelling of cellulose in solutions of sodium hydroxide with concentration up to 10 wt. % and washing, a significant rise of V_p-value had taken place, while further increasing of alkali concentration caused some dropping of the pore volume (see e.g. Fig. 4). The similar dependence of *WRV* on concentration of NaOH-solutions was found also by other researchers (Papkov et al. 1976). Drying of the wet samples led to falling in V_p-value, but it did not violate the amorphous-crystalline relation (Table. 2).

Though high swelling in solution of sodium hydroxide, a crystalline structure of cellulose was not changed, if concentration of the alkali solution was lower than 10 wt. %. Treatment of the cellulose with more concentrated solutions of sodium hydroxide brings the irreversible disruption of its crystalline structure and increasing content of non-crystalline domains (Fig. 5).

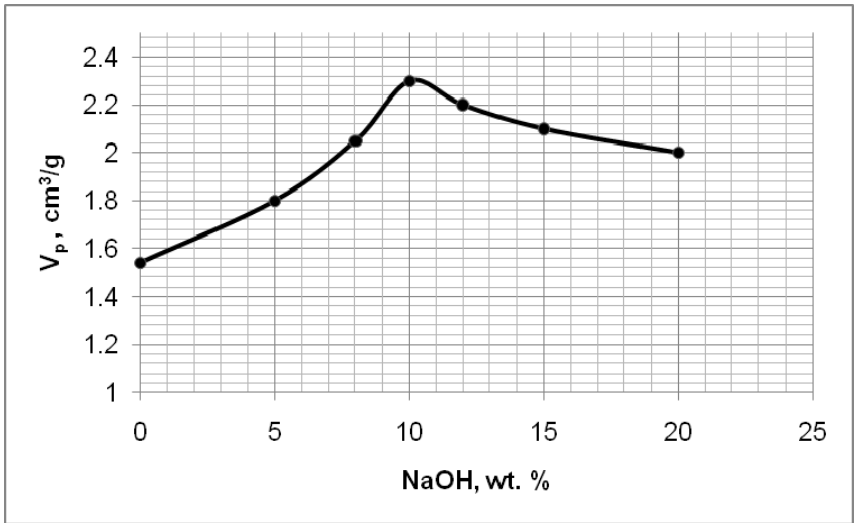

Fig. 4. Dependence of pores volume of refined cotton linter on alkali concentration

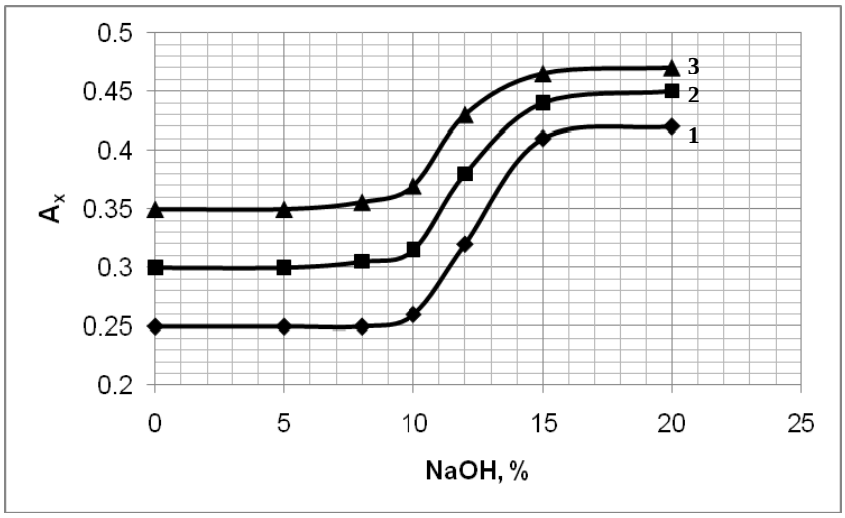

Fig. 5. Dependence of content of non-crystalline domains on the alkali concentration for dried cellulose samples: 1- AV, 2- CL, 3 - KP

Enzymatic Hydrolysis of Cellulose Materials having Various Structural Characteristics

Comparison of conversion degrees *(α)* of cellulose samples at the enzymatic hydrolysis obtaining from determination of glucose concentration by *DNS*-assay and from testing of the sample weight loss gives the near α-values (Fig. 6). Low difference between α_g and α_w values permits use an average value of the conversion degree:

$$\alpha_a = (\alpha_g + \alpha_w)/2 \quad (5)$$

Since various factors can influence the enzymatic hydrolysis, a detailed study was performed to found dependence of the cellulose hydrolysability on such structural features as particles size, degree of polymerization, pores volume, type of crystalline polymorph and content of non-crystalline domains.

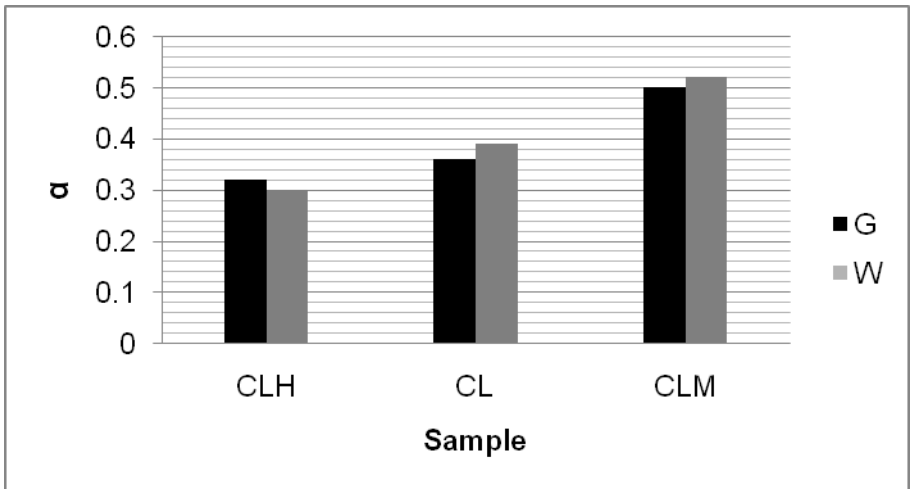

Fig. 6. Conversion degree *(α)* of the dried cellulose samples after enzymatic hydrolysis for 24 h determined by testing concentration of glucose (G) and weight loss (W)

Analysis of the enzymatic hydrolysis of various samples showed a poor correlation between the average size of particles and conversion degree of cellulose (Fig. 7).

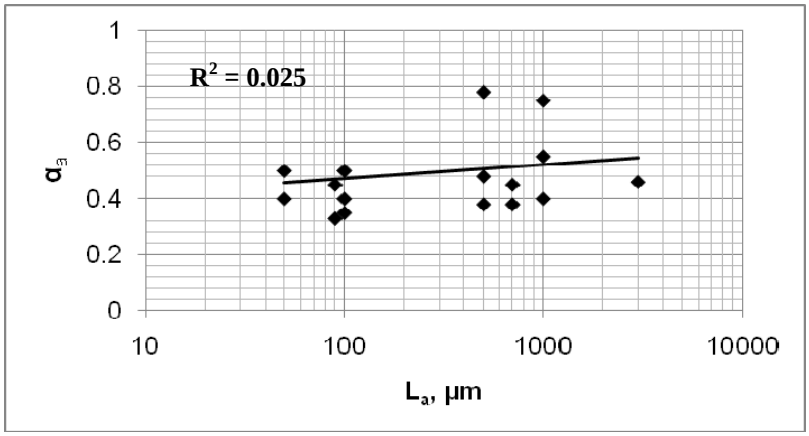

Fig. 7. Correlation between average particles size *(L_a)* and average conversion degree *(α_a)* of the dried cellulose samples after enzymatic hydrolysis for 24 h

Differences in the degree of polymerization *(DP)* of samples had a negligible effect on the hydrolysis rate and conversion degree of cellulose at the enzymatic

hydrolysis (Fig. 8). This finding has been confirmed also by other investigations (Sinitsyn et al. 1991).

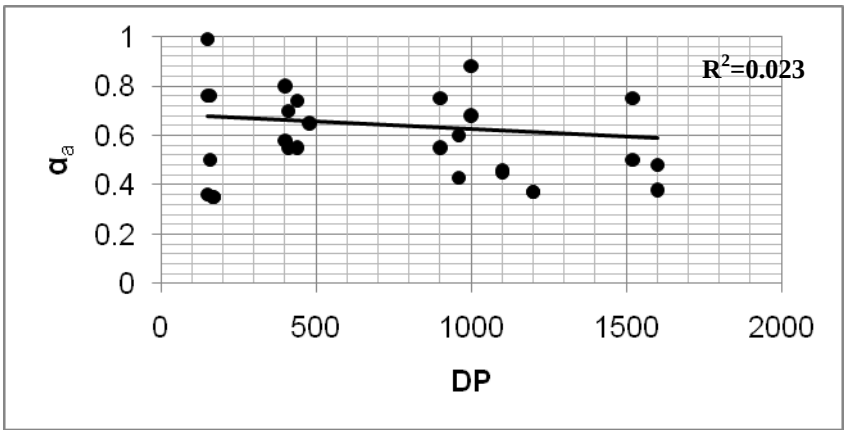

Fig. 8. Correlation between *DP* and average conversion degree *(α_a)* of cellulose after enzymatic hydrolysis of the non-dried and dried samples for 24 h

The influence of pores volume on the conversion degree of cellulose at enzymatic hydrolysis is complicated and ambiguous. Swelling in 5 to 10 wt. % solutions of sodium hydroxide with the subsequent washing of the samples increased pores volume *(V_p)* and promoted enzymatic hydrolysis, while drying of the samples resulted in some decreasing of the conversion degree due to irreversible reducing in pores volume. On the other hand, if cellulose samples were treated with more concentrated alkali solutions, the opposite tendency was observed. Namely, the volume of pores decreased, while the conversion degree increased (see e.g. Table 3).

Table 3. Effect of Structural Characteristics of Alkali-treated and Non-dried Samples of Cotton Linter on Conversion Degree at the Enzymatic Hydrolysis for 24 h

NaOH, wt. %	A_x	V_p , cm^3 /g	α_a
5	0.30	1.8	0.46
10	0.30	2.3	0.50
15	0.44	2.1	0.75
20	0.45	2.0	0.76

Therefore, the results are inconsistent with the interpretation that just an accessibility of pore structure only is responsible for degradability of cellulose at the enzymatic hydrolysis. The main cause for improving hydrolysability of cellulose can be the disruption of crystalline structure (Fan et al. 1980, 1981; Hall et al. 2010; Zhang et al. 2006). Nevertheless, when comparing various non-dried and dried samples, the contribution of porosity to cellulose hydrolysability also can be found (Fig. 9).

As is known, the swollen cellulose materials can contain inter-fibrillar pores and capillaries with radius >10 nm (Hubbe 2006). Therefore, it is not a problem for penetration of the cellulases having average radius 4 to 5 nm (Davies et al. 1993; Receveur et al. 2002) into developed pore structure of such substrates. Drying of the wet

cellulose causing irreversible collapse its pores delays access of the enzyme and decreases the conversion degree. Low porosity of cellulose generally doesn't limit the enzyme hydrolysis, because the high active cellulolytic enzyme itself is able to generate the nano-holes (new meso-pores) mainly in non-crystalline amorphous domains of cellulose and provides access of the enzyme molecules inside the cellulose structure.

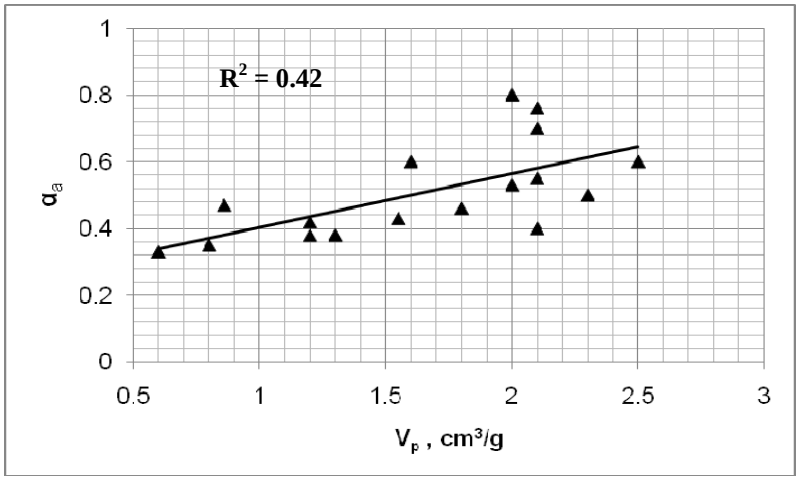

Fig. 9. Correlation between volume of pores *(V_p)* and average conversion degree *(α_a)* of cellulose after enzymatic hydrolysis of the non-dried and dried samples for 24 h

The kinetics of the conversion degree of studied cellulose samples at enzymatic hydrolysis was characterized by decreasing in hydrolysis rate over time up to final stage (time > 20 h) having the slow rate (Fig. 10). Drying of the non-dried samples caused a drop in the conversion degree, probably due to decreasing porosity. The initial hydrolysis rate and total conversion degree were higher for the cellulose samples having more decrystallized and more porous structure.

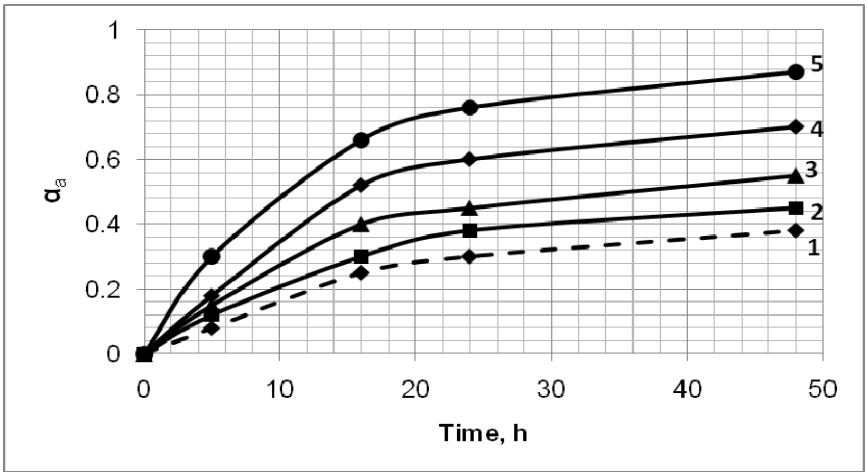

Fig. 10. Kinetics of the enzymatic hydrolysis of dried cellulose samples
1- CLH, 2-CL, 3-KP, 4-KPM, 5-RC

Kinetics of the enzymatic hydrolysis at times < 20 h can be linearized by the equation of pseudo-first order (see e.g. Fig. 11),

$$F = -ln(1 - \alpha_a) = Kt \quad (6)$$

where K is the rate constant for the initial stage of the hydrolysis and t is time.

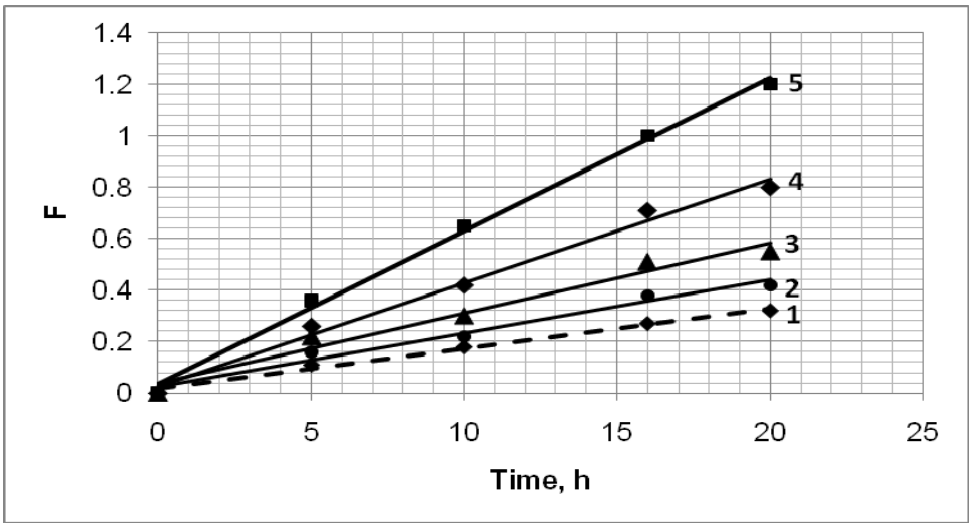

Fig. 11. Linearized kinetics of the enzymatic hydrolysis of dried cellulose samples
1- CLH, 2-CL, 3-KP, 4-KPM, 5-RC

The calculated constants of the hydrolysis rate for some cellulose samples are shown in Table 4.

Table 4. Constants of the Hydrolysis Rate for Dried (K_d) and Non-dried (K_{nd}) Cellulose Samples

Samples	A_x	K_d , h^{-1}	K_{nd} , h^{-1}
CLH	0.23	0.015	0.023
CL	0.29	0.020	0.034
KP	0.36	0.025	0.042
CLM	0.45	0.033	0.065
RC	0.75	0.060	0.121

Use of the constants permits calculation the kinetics of the enzymatic hydrolysis. The obtained results showed that experimental and calculated graphs are near if hydrolysis time < 20 h, but at the final stage of the hydrolysis a divergence between experimental and calculated graphs is taken place (Fig. 12, 13). Moreover, such divergence for the high-crystalline MCC-sample was significant higher than for the amorphized RC-sample.

Hence, the final stage of the hydrolysis is connected first of all with the increased resistance of crystalline regions of cellulose to enzymatic attack. Additional non-structural factors can be responsible for the slow conversion of cellulose on the final

stage of the process, such as: inhibition of enzymes by accumulated end products of the hydrolysis (e.g. glucose), thermal inactivation of enzymes, etc. (Converse et al. 1988; Eriksson et al. 2002; Holtzapple et al. 1990; Kristensen et al. 2009; Yang et al. 2006).

As follows from the experiments (Table 4), increase of content of non-crystalline domains positively affected the hydrolysis rate constant. Besides, a linear correlation was observed between the content of non-crystalline domains and the conversion degree of cellulose into glucose for a certain hydrolysis time, e.g. 24 h (Fig. 14). The rate constant and conversion degree for the non-dried sample having more developed porosity was higher than for the dried cellulose sample.

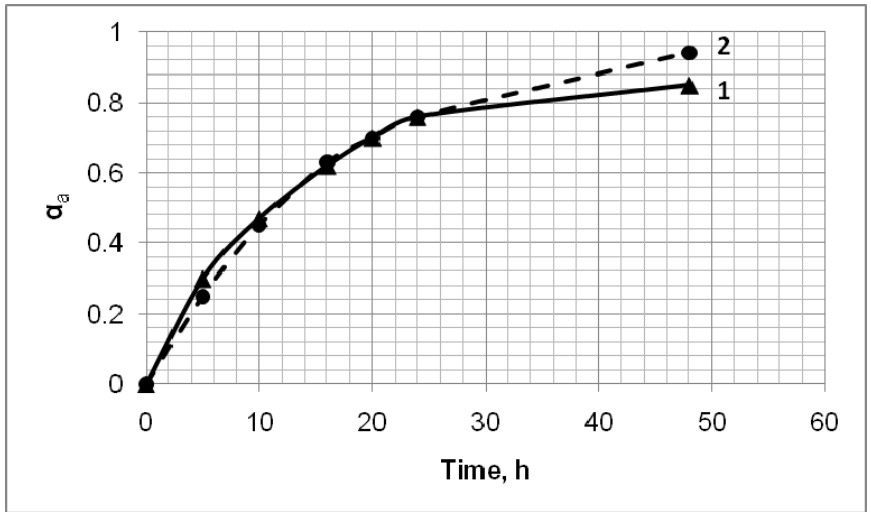

Fig. 12. Experimental (1) and calculated (2) kinetic graphs of the enzymatic hydrolysis for the dried sample of regenerated cellulose (RC) having amorphized structure

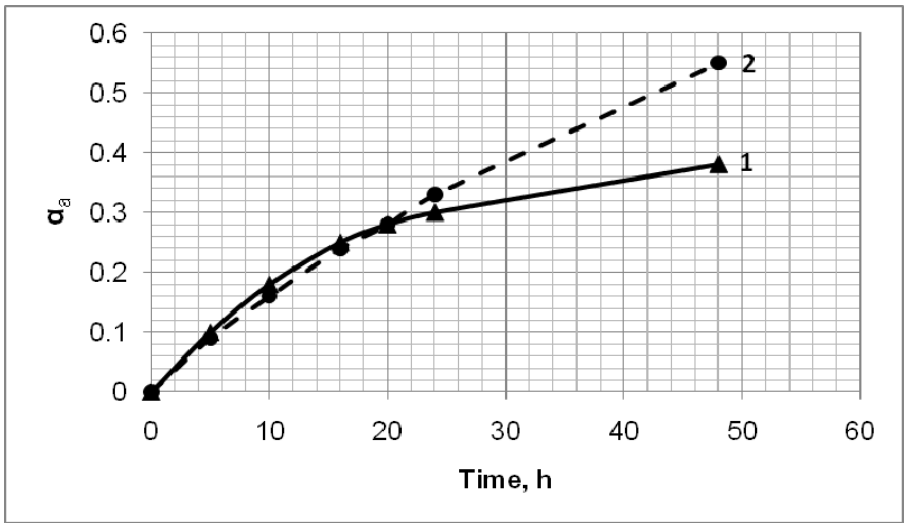

Fig. 13. Experimental (1) and calculated (2) kinetic graphs of the enzymatic hydrolysis for the dried sample of MCC Avicel (AV) having a high-crystalline structure

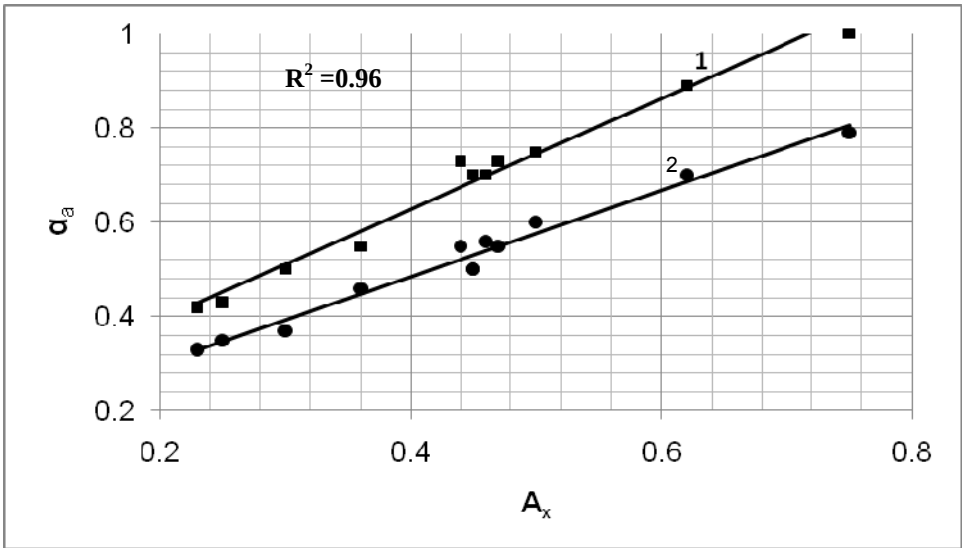

Fig. 14. Dependence of the cellulose conversion degree at enzymatic hydrolysis for 24 h on the content of non-crystalline domains for non-dried (1) and dried (2) cellulose samples listed in Table1

To compare the effects of various structural characteristics of cellulose samples on their hydrolysability, correlation coefficients (*R*) were calculated (Table 5).

Table 5. Correlation Coefficients *(R)* between Structural Parameters and Conversion Degree of various Cellulose Samples at the Enzymatic Hydrolysis for 24 h

Structural parameters	***R***
Crystalline polymorph	0.10
Degree of polymerization	0.15
Average size of particles, μm	0.16
Volume of pores, cm^3/g	0.65
Content of non-crystalline domains	0.98

The calculations showed that the contributions of crystalline polymorph, degree of polymerization and average size of particles to conversion degree of cellulose is low (*R*=0.10-0.16). The volume of pores had a fair effect on cellulose hydrolysability (*R*=0.65). The most powerful structural factor influencing on the conversion degree of cellulose samples at enzymatic hydrolysis was the content of non-crystalline domains (*R*=0.98).

Results of Fig. 14, graph 2, for the dried cellulose samples at the used conditions of the enzymatic hydrolysis can be described in term of a linear two-parametric regression equation $\alpha_a = f(A_x)$:

$$\alpha_a = 0.92\ A_x + 0.1 \quad (7)$$

To describe hydrolysability of the non-dried cellulose samples, a thee-parametric regression should be used that includes the conversion degree (α_a) of the sample for 24h, the part of non-crystalline domains (A_x) and the volume of pores (V_p) in the wet sample:

$$\alpha_a = 0.92\ A_x + 0.1\ (V_p + 1) \quad (8)$$

Joint impact both of increased content of non-crystalline domains and increased pores volume gives the highest effect on the conversion degree of cellulose at enzymatic hydrolysis, e.g. in the case of the swollen amorphized cellulose.

Introducing the dependence $V_p = f(A_x)$ into Eq. (8) makes it possible to derive the simple two-parametric regression equation (9) $\alpha_a = f(A_x)$ for description the enzymatic hydrolysability of the non-dried samples (see Fig. 14, graph 1):

$$\alpha_a = 1.17\ A_x + 0.16 \quad (9)$$

As follows from this equation, the water-swollen amorphized cellulose samples having A_x >0.7 can be hydrolyzed completely for 24 h at the given conditions of the enzymatic treatment.

CONCLUSIONS

A study of enzymatic hydrolysis of non-dried and dried cellulose samples having various structural characteristics showed that such structural characteristics cellulose as type of crystalline polymorph, degree of polymerization, and particles size have a negligible influence on the conversion degree of cellulose into glucose.

The pore volume of samples has a fair impact on the conversion degree of celluloses at the enzymatic hydrolysis. Drying of the non-dried samples decreases cellulose hydrolysability probably due to irreversible collapse of the pores volume.

The content of non-crystalline domains (A_x) has the highest effect on the average conversion degree (α_a) of celluloses at the enzymatic hydrolysis. The linear dependence $\alpha_a = f(A_x)$ was established both for dried and non-dried cellulose samples. Joint impact both of increased content of non-crystalline domains and increased pore volume gives the highest effect on the conversion degree of cellulose at the enzymatic hydrolysis.

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