# Influence of Homogenization Treatment on Physicochemical Properties and Enzymatic Hydrolysis Rate of Pure Cellulose Fibers

**N. Jacquet · C. Vanderghem · S. Danthine · C. Blecker · M. Paquot**

**Abstract** The aim of this study is to compare the effect of different homogenization treatments on the physicochemical properties and the hydrolysis rate of a pure bleached cellulose. Results obtained show that homogenization treatments improve the enzymatic hydrolysis rate of the cellulose fibers by 25 to 100 %, depending of the homogenization treatment applied. Characterization of the samples showed also that homogenization had an impact on some physicochemical properties of the cellulose. For moderate treatment intensities (pressure below 500 b and degree of homogenization below 25), an increase of water retention values (WRV) that correlated to the increase of the hydrolysis rate was highlighted. Result also showed that the overall crystallinity of the cellulose properties appeared not to be impacted by the homogenization treatment. For higher treatment intensities, homogenized cellulose samples developed a stable tridimentional network that contributes to decrease cellulase mobility and slowdown the hydrolysis process.

**Keywords** Homogenization · Microcrystalline cellulose · Enzymatic hydrolysis · Crystallinity · Water retention

## Introduction

In the recent economic context, there has been increasing concern for the use of cellulose-based products as raw materials to produce second-generation biofuels [1, 2] or high added values products [3, 4]. In this sense, lignocellulosic materials, which are widely composed of polymer materials such as cellulose, hemicelluloses, and lignin appears to be promising [5].

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However, the economic feasibility of using lignocellulosic materials depends on the ability to perform a complete fractionation of the biomass. To achieve this goal, materials must be subjected to a pretreatment [6].

A large number of pretreatment processes for the fractionation of biomass are highlighted in recent review articles. These pretreatment technologies can be classified into biological, physical, chemical, and physicochemical technologies according to the different forces or energy used in the process [7]. Literature established that physicochemical pretreatments offer several attractive features such as lower environmental impact, lower capital investment, more potential for energy efficiency, fewer hazardous process chemicals and conditions, possibility of using a large chip size, etc. when compared to other fractionation technologies. Physicochemical pretreatments include technology such as steam explosion, liquid hot water, ammonia fiber explosion (AFEX), wet oxidation, microwave and ultrasound pretreatments, and $CO_2$ explosion. [8] Steam explosion and $SO_2$ steam explosion are actually the most widely employed pretreatments for lignocellulosic biomass. These technologies allow the breakdown of lignocellulosic structural components by steam heating, shearing forces due to the expansion of moisture and hydrolysis of glycosidic bonds by the acid formed during the process [9, 10]. Liquid hot water is another easy and widely used hydrothermal treatment, with parameters very close to the steam explosion but does not require rapid decompression or any catalyst or chemicals [11]. The third pretreatment widely used is the AFEX process, which pretreats biomass with liquid anhydrous ammonia and high pressure. The rapid release of pressure at the end of the process causes the expansion of ammonia gas that induces swelling and physical disruption of lignocellulosic materials and a partial decrystallization of cellulose [12]. Wet oxidation, $CO_2$ explosion, microwave, and ultrasound pretreatments are seldom used in the industry and remains actually at a pilot scale [13–16]. All these different studies highlight the positive effect induced by the shearing on the structure opening and hydrolysis yields of the biomass. However, none of them mention high shear homogenization technology as a potential method to pretreat biomass.

Actually, high-pressure homogenization technology is used to disperse, mix, emulsify, and process different products in various industrial sectors: chemical, pharmaceutical, specialty foods, and biotechnology [17]. The homogenization technology consists of a positive displacement pump to which is attached a homogenizing valve. The pump forces fluids through the homogenizing valve under pressure and then the liquid flows through the valve at high velocity. [18]. As the velocity increases, the pressure decreases producing an instantaneous pressure drop and inducing simultaneously phenomena of cavitation, shear, turbulence, and temperature increases, which are very difficult to rank in order of importance and to quantify but have sufficient energy to disrupt materials structure [19, 20].

Several studies have already shown the impact of homogenization technology on biomass material and mainly on the cellulose fiber structure. Dinant et al. have shown that homogenization treatment, applied on a cellulosic residue of sugar beet pulp parenchyma cells, induce the breakdown of the macroscopic fiber structure and lead to an aqueous suspension of either individual or bundles of cellulose microfibrils [21] with outstanding rheological properties with solidlike viscoelastic behavior and shear-thinning flow behavior [22].

Similar results were obtained by Bhatnagar (2003), Nakagaito (2004), and Habibi (2008) on cellulosic fraction of various lignocellulosic materials (fibrillated kraft pulp, peel of prickly pear, …) that confirmed that homogenization treatment induces irreversible changes in the cellulose fiber structure and increases its bonding potential by modification of its morphology that could induce a potential increase of the cellulose hydrolysis rate [23–25].

The objective of this study was to evaluate the effect of homogenization treatment on the enzymatic hydrolysis yield of the cellulose fibers. Various treatment intensities were applied

on a pure cellulose fiber in order to identify the impact of the process on the physicochemical properties and hydrolysis rate. Links between cellulose hydrolysis yields, crystallinity rate, and moisture retention were established. In addition, rheological analyses were performed to characterize the viscoelastic properties developed by the homogenized cellulose suspension and their possible influence on the hydrolysis process.

## Material and Methods

Cellulose Material

The sample tested in this study was a microcrystalline cellulose purchased from Mikro-Technik GmbH (Germany) under the trade name of Alba-Fibre C200. This commercial product is bleached cellulose obtained from wood pulp and purified from hemicelluloses and lignin. The average fiber size is around 200 μm in length with a diameter of 30 μm. Its density is between 120 and 150 g/dm$^3$ with a moisture content of at least 8 %.

Homogenization Treatment

Ten liters of cellulose fiber suspension, at 2 w/w% concentration in deionized water, was homogenized through a pilot-scale Rannie homogenizer Model LAB 12.51H (APV Inc., Charlotte, NC, USA) in a continuous recycle configuration. Homogenization treatment was performed with a constant flow rate of 2 l/min and a range of homogenizer conditions including various pressures (250 and 500 MPa) and recycle operations times (60, 120, and 240 min). The valve and seat valve configuration were a standard flat system, usually used in a variety of food, dairy, and chemical applications. The high recirculation rate relative to the volume of solution in the feed tank (2 l/min, 10 l solution) provided adequate mixing. Temperature of the product was controlled with an ice cooling system to maintain the product at a constant temperature of 55–60 °C. At the end of the process, one part of the homogenized suspension was recovered in 250-ml Duran bottles for the enzymatic hydrolysis step. The other part was lyophilized to achieve x-ray diffraction analyses and to keep the product for further use.

Enzymatic Hydrolysis, Analysis of Cellulose Hydrolysis Products, and Determination of Hydrolysis Rate

Cellulose homogenized suspension was enzymatically hydrolyzed by a cellulase and cellobiase mixture (*Trichoderma reesei* ATCC 26921 (Celluclast® 1.5 l) and *Aspergillus niger* (Novozym 188) (Novozymes A/S, Denmark)). The method was adapted from a process described by Vanderghem [26]. Both enzyme mixtures were purchased from Sigma-Aldrich (St. Louis, MO, USA). The activities of Celluclast® 1.5 l and Novozym 188 were of 30 FPU/ml and 295 CBU/ml, respectively. Applied enzyme loadings were 7.5 FPU/g (Celluclast® 1.5 l) and 37 CBU/g (Novozym 188).

Hydrolysis took place in 250-ml capped Duran bottles incubated in a water bath equipped with a submersible magnetic stirrer (2mag MIXdrive 6, Germany) at 52 °C for 24 h. The pH was adjusted to 4.8 with tampon citrate buffer and continuous stirring was performed with a stir bar at 400 rpm.

Samples obtained were filtered (0.45 μm). Separation and quantification of hydrolysis products (glucose, cellobiose) were performed with a high-performance anion exchange

chromatography using a CarboPac PA100 column set (guard and analytical) coupled with pulsed amperometric detection (HPAEC-PAD, ICS-3000) (Dionex, Sunnyvale, CA, USA). Solvent A was 100 mM NaOH, solvent B was 600 mM CH3COONa and 100 mM NaOH, solvent C was 500 mM NaOH, and solvent D was distilled water (HPLC-grade solvents). The gradient was as follows: 0–8 min 50 % A and 50 % D, 8–12 min linear gradient up to 100 % A, 12–15 min 100 % A, 15–25 min linear gradient up to 30 % B and 70 % A, 25–28 min 30 % B and 70 % A, and 30–40 min 50 % B and 50 % C (cleaning). The cellulose conversion to glucose and cellobiose was calculated using the following equation (Eq. 1) described in the work of Vanderghem [26]:

$$\text{Hydrolysis rate (\%)} = \left( \frac{\text{Glucose produced} + 1.053 \times \text{cellobiose produced}}{\text{Total amount of glucose in the pretreated biomass}} \right) \times 100. \quad (1)$$

Optical Microscopy

Observations were performed on homogenized suspensions with a Nikon Eclipse E400 microscope (Kanagawa, Japan) equipped with a Basler video camera (Vision Technologies, Inc., Ahrensburg, Germany) and mounted with a 20× objective (Carl Zeiss, Germany). Micrographs were treated with the image processing software LUCIA G 1500 program.

X-ray Diffraction Analyses

The crystalline state of the homogenized samples was determined isothermally using a D8 Advance diffractometer (Bruker, Germany) (λ Cu=1.54178 Å, 40 kV, 30 mA) equipped with a Vantec (Bruker, Germany) detector and a TTK450 low-temperature Chamber and TCU 110 Temperature Control Unit (Anton Paar, Graz, Austria) connected to a circulating water bath (Julabo, Germany). The diffraction intensity was measured between the Bragg angles 15° and 27°. Diffrac Plus Evaluation 14.0.0.0 program (Bruker, Germany) was used to normalize and process the results.

Water Retention Values

Water retention values (WRV) were determined by the method adapted from Ioannis [27]. Homogenized samples were placed in a filter centrifugation tube (pore diameter: 0.2 μm) and centrifuged at 8,000g for 10 min (Beckman Coulter Allegra X-15R Centrifuge). Wet samples obtained were weighed (Ww), dried in an oven for 24 h at 105 °C, and cooled in a desiccator. The weight of the dried samples was recorded (Wd). The WRV was then calculated by using the following equation (Eq. 2):

$$\text{WRV(\%)} = \frac{W_w - W_d}{W_d} \times 100. \quad (2)$$

Rheological Analysis

A Bohlin CVO120 controlled stress rheometer (Malvern, Worcestershire, UK) fitted with an upper rotary cone (diameter: 40 mm, angle: 4 %) and a fixed lower plate (diameter: 60 mm), connected to a circulating water bath (Julabo, Germany) was used for this study. Homogenized samples were loaded on the rheometer plate with care to minimize shearing during

the loading and silicon oil was placed on the probe periphery to prevent side effects. The gap closure was fixed at 150 μm.

Oscillatory stress sweep tests were then conducted over the range from 0.1 to 100 Pa with a frequency of 1 Hz at 20 °C. Frequency sweep tests were realized at 20 °C with a specific shear stress (in the linear viscoelastic region (LVR) at a value of 50 % of the critical stress ($\sigma$)) with a range of frequency of 0.01 to 10 Hz.

## Results and Discussion

Multiple-pass Homogenization and Degree of Homogenization

In most product processing systems, homogenization treatment is generally performed in a single-pass process. In the case of cellulose, several studies have showed that cellulose must be homogenized with additional passes to obtain the individual or bundles of cellulose microfibrils [21, 23–25].

In this study, cellulose was continuously treated, implicating that the product from the homogenizer was recycled during different times. A first theoretical pass number applied in this condition could be easily estimated by the following relation (Eq. 3):

$$T = \frac{V}{D} \times M \tag{3}$$

where:

T    process time (minute)
V    volume of the sample (liter)
D    flow rate (liter per minute)
M    theoretical number of pass.

However, the recycle system configuration is not so easy to analyze because there is no assurance that the entire batch has undergone the theoretical number of passes. A certain fraction of the batch does, but there are some fractions of the batch that have undergone greater or fewer numbers of passes. To take account of this phenomenon, Leviton has developed a mathematical relation (Eq. 4) that allows the calculation of the percentage of the total volume ($f$), which has undergone $P$ homogenization in continuous multipass process for a theoretical number of pass ($M$) [28]:

$$f = \frac{M^{P} e^{-M}}{P!} \tag{4}$$

where:

f    fraction of total volume that has received P passes
M    theoretical number of pass
P    number of passes.

With both these equations, the value of $M$ was easily calculated ($M$=12, 24, and 48), and the following distributions were established (Fig. 1).

From these distributions, it was possible to determine a "degree of homogenization," which corresponds to the minimum number of passes undergone by 99 % of the cellulose suspension. The degrees of homogenization of each homogenization treatment are presented

**Fig. 1** Percentage of the total volume (f), which has undergone P homogenization for a theoretical number of pass of 12, 24, and 48
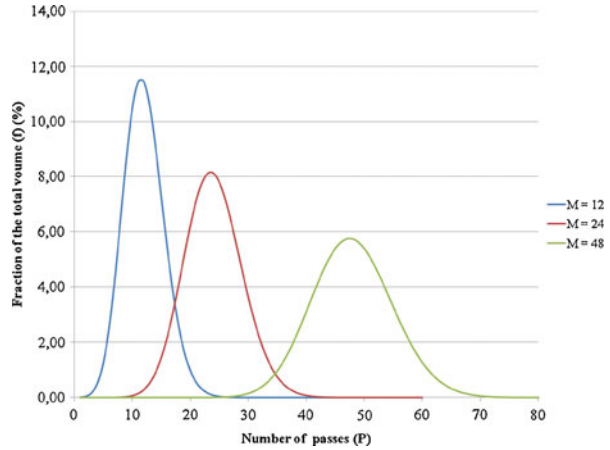


in Table 1 with the corresponding process pressures (megapascal), process time (minute), and theoretical number of passes.

Characterization of Homogenized Cellulose

Cellulose accessibility to enzymatic complex has great influence on hydrolysis yield. Literature shows that cellulose accessibility can be mainly characterized by three physico-chemical properties: particle size, crystallinity, and water retention [29].

Particle size characterizations of homogenized cellulose were performed by microscopic observations. Size characterization with a granulometer was not pertinent because of the important shape factor of cellulose fibers. Microscopic observations of the particle size of homogenized cellulose with the pressure and the degree of homogenization applied are presented in Figs. 2 and 3.

Results show that the shearing treatment has a significant impact on the cellulose fiber structure and was conducted to obtain microfibril suspensions. These results agree with other literature data [21, 23]. Observations noticed that the increase of the pressure and degree of homogenization applied result in a progressive decrease of the fiber size that suggests that a major surface area will be available for enzymatic attack. This increase of the surface area induces an increase of the amount of potential enzymatic binding sites that should lead, under the conditions of this study, to improvement of the cellulose hydrolysis yields.

**Table 1** Pressure, process time, theoretical number of passes, and degree of homogenization for homogenization treatments applied

| Samples name | Pressure (mPa) | Process time (min) | Theoretical number of passes | Degree of homogenization |
|---|---|---|---|---|
| C200 (25/4.2) | 25 | 60 | 12 | 4.2 |
| C200 (25/12.9) | 25 | 120 | 24 | 12.9 |
| C200 (25/32.1) | 25 | 240 | 48 | 32.1 |
| C200 (50/4.2) | 50 | 60 | 12 | 4.2 |
| C200 (50/12.9) | 50 | 120 | 24 | 12.9 |
| C200 (50/32.1) | 50 | 240 | 48 | 32.1 |
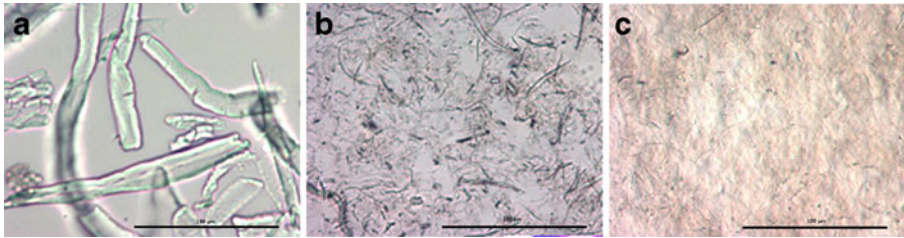
**Fig. 2** Microscopic observations of cellulose particle size with pressure. **a** C200 cellulose, **b** C200 (25/32.1), **c** C200 (50/32.1). *Scale bar* 100 μm

A second factor that may influence the contact between the enzyme and the substrate is cellulose crystallinity. Literature shows that enzymatic hydrolysis of the very compact and resistant crystalline region is much slower than in the amorphous regions. In the crystalline region, hydrolysis is dominated only by exoglucanases activity, whereas less compact amorphous regions are believed to be hydrolyzed by endo- and exoglucanases activities [30]. Therefore, a decrease or an increase of the cellulose crystallinity could lead to modification of the hydrolysis rate of the cellulose.

Figure 4 presents x-ray diffraction pattern of cellulose C200 and homogenized cellulose samples. Analysis of the different patterns shows many similarities in the overall crystallinity and cellulose I/cellulose II content of cellulose C200 and homogenized cellulose samples.
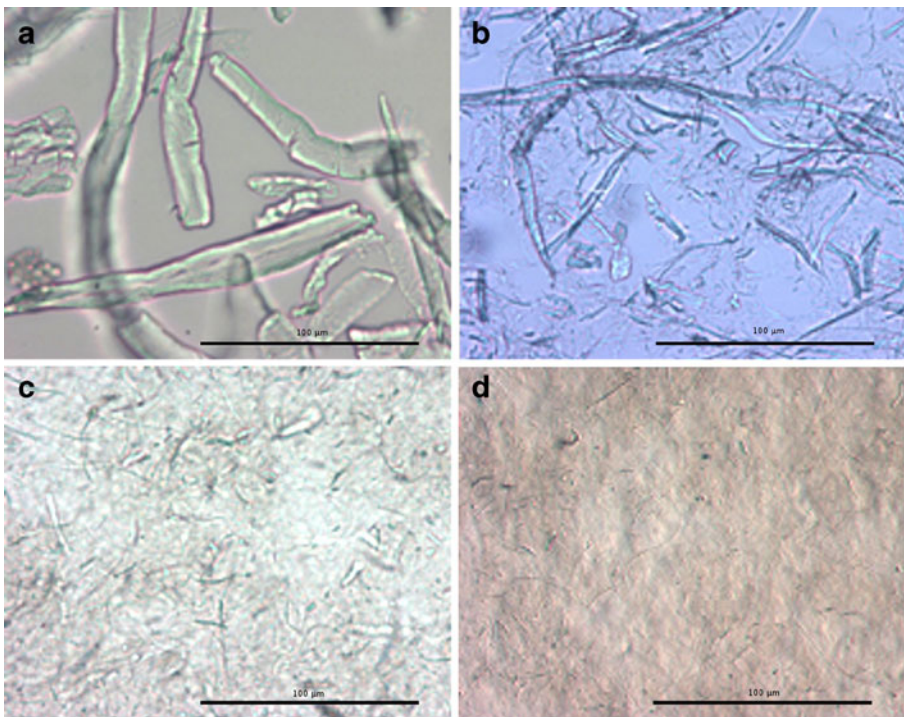


**Fig. 3** Microscopic observations of cellulose particles size with the degree of homogenization, **a** C200 cellulose, **b** C200 (50/4.2), **c** C200 (50/12.9), **d** C200 (50/32.1). *Scale bar* 100 μm
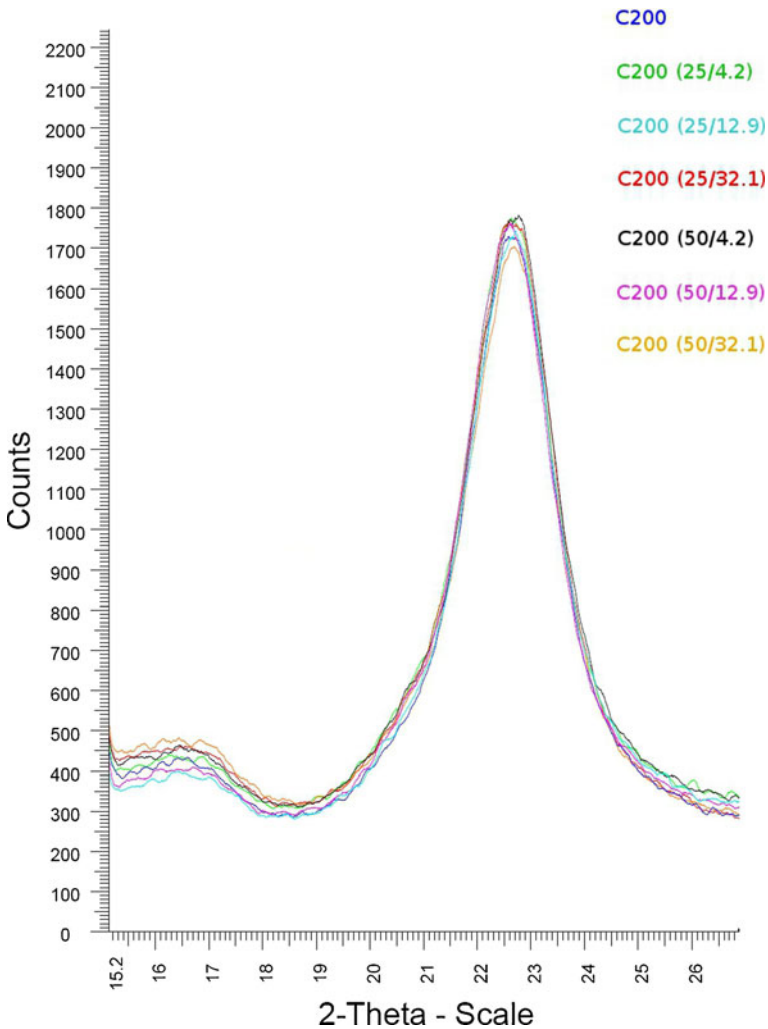
**Fig. 4** X-ray diffraction pattern of cellulose C200 and homogenized cellulose

All samples exhibit high intensities at a scattering angle of 22.7° indicating the presence of cellulose I, which corresponds to the crystalline part of the cellulose fiber [31]. The degree of crystallinity of the samples, estimated from the peak height at 22.7° and 18.5° [32, 33], was included in a narrow range from 70 to 74 %. Evolution of the ratio of scattering intensities at 22.7° and 20.4° (correlated to the cellulose I/cellulose II content) of homogenized samples was also very close to the nontreated cellulose, indicating a high constant in the crystallinity of the samples [34]. So, these observations suggest that cellulose crystallinity was not affected by the homogenization treatment and should not influence the cellulose hydrolysis rate.

Water retention is a further important factor affecting cellulose hydrolysis. This property is an element that contributes toward control over the accessibility of the enzymes since, to be attacked by the enzymatic way, cellulose chains must be hydrated. As the fiber swells, intermolecular bonds are broken as a result of the internal stresses produced by swelling. The

degree of order within the fiber is reduced and there is an increase in the specific surface available for enzymatic hydrolysis [35].

WRV of raw C200 cellulose and homogenized cellulose are presented in Fig. 5. WRV increased in an appreciable way with the pressure and degree of homogenization applied. Results suggest also that high treatment intensities (pressure: 500 b and degree of homogenization higher than 12.9) have a more significant impact on the increase of WRV (275 % for the C200 (50/12.9) and 330 % for the C200 (50/32.1)50). These increases of WRV indicate, in accord with the microscopic observations realized, an increase of specific surface coupled to a swelling by hydration of the cellulose [30, 36].

Rheological Characterization of Homogenized Cellulose

A last factor that may influence the contact between the enzyme and the substrate is linked to rheological properties of cellulose-homogenized samples. Literature showed that homogenized cellulose suspensions developed, at the lowest concentration (0.25 % w/w), a shear-thinning flow behavior and could be considered as classical pseudoplastic material [20, 37].

Oscillatory stress sweep analysis was performed to characterize the rheological properties of each homogenized suspensions. Analyses show a similar profile for all the samples. Figure 6 shows the effect of oscillatory shear stress on storage modulus ($G'$), loss modulus ($G''$), and strain ($\delta$) of a homogenized cellulose sample (C200 50/32.1). The storage modulus profile ($G'$) is constituted of a range of stress where $G'$ is independent of the stress applied. This part of the profile is called the LVR. In this region, the stress applied is in phase with the resulting strain that means that the tridimentional structure present in the sample is not broken [38]. At the end of the LVR, the storage modulus is equal to the loss modulus and the stress applied, which is not in phase with the resulting strain ($\delta > 0$), which indicates that the tridimentional structure is disturbed. The stress values corresponding to this point are called the critical stress ($\sigma$) [38, 39].
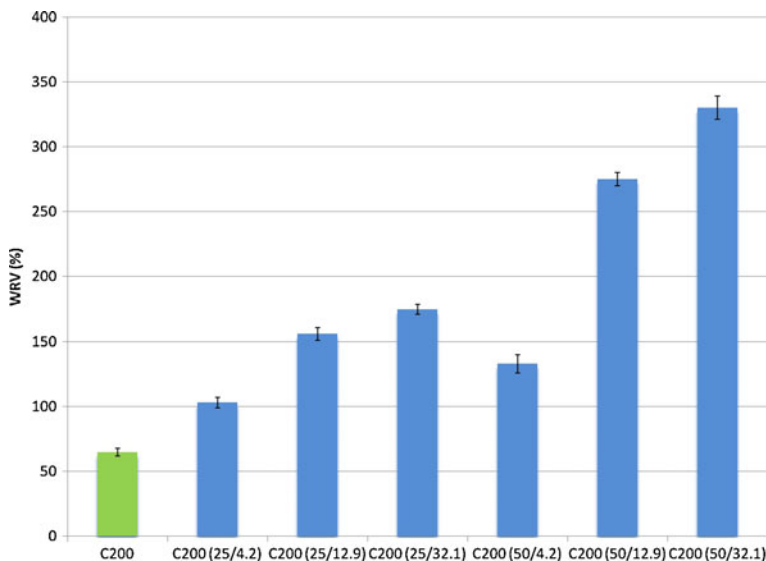


Fig. 5 Water retention values of cellulose C200 and homogenized cellulose (WRV)
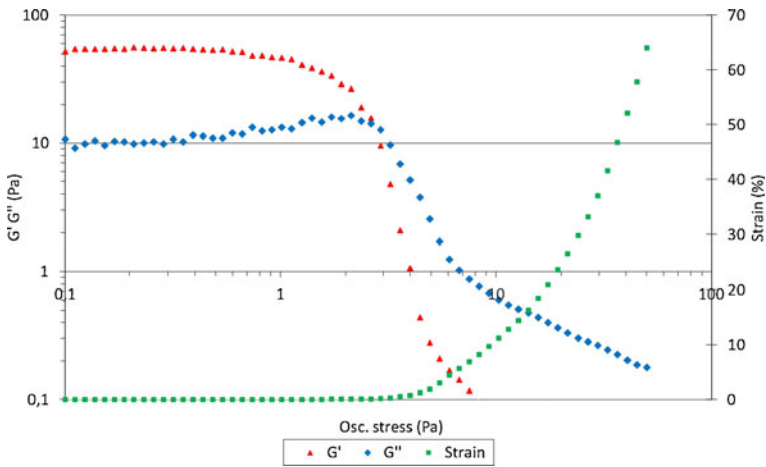
**Fig. 6** Storage modulus ($G'$), loss modulus ($G''$), and strain ($\delta$) measurement in oscillation stress sweep test (C200 50/32.1)

The values of $G'$ in LRV and $\sigma$ obtained for different homogenized suspensions are summarized in Fig. 7. These results showed that high intensity treatment was required to develop a tridimentional microfibril network. Samples obtained below these conditions had an extremely weak storage modulus ($G'<20$ Pa) and very low stability ($\sigma<1$ Pa) indicating that shearing treatments were not strong enough to produce a sufficient amount of microfibrils to develop a tridimentional network [40]. For higher intensities, increases in the values of storage modulus at LVR and critical stress were observed. This increase is in agreement with the development of a stable network in the homogenized suspension. These results could be explained by an increase of the amount of microfibrils with the shearing intensity, which allowed the formation of the tridimentional network, with, as consequence, strength stability reinforcement of the hydrogel that could affect the hydrolysis step [41, 42].
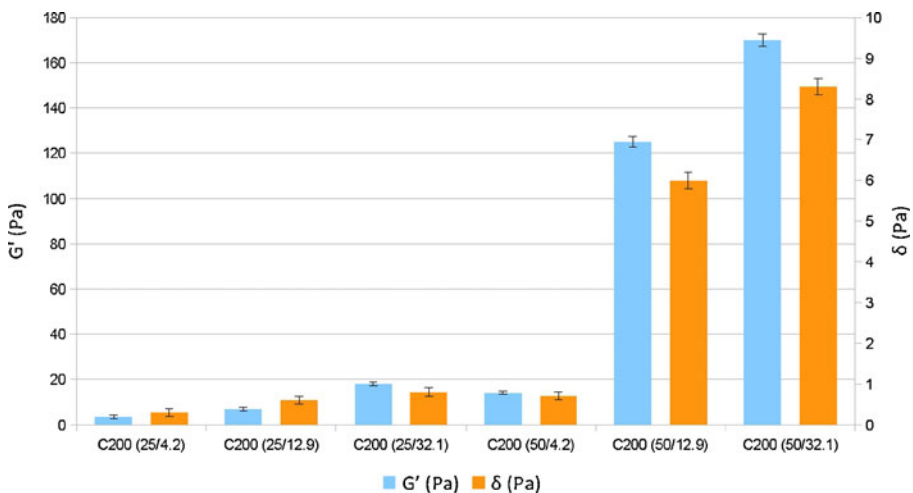


**Fig. 7** $G'$ in LRV and $\sigma$ values of cellulose homogenized suspensions

The results of frequency sweep tests confirm this trend. Profiles obtained for high intensity treatment showed a small gradual increase of $G'$ and $G''$ with the frequency (Fig. 8). This characteristic indicated a large elastic behavior with a comparatively small dissipation of energy that confirms the presence of a tridimentional network stabilized by hydrogen bonds established between the surface hydroxyl groups of the cellulose micro-fibrils [41, 42]. In comparison, profiles of the storage modulus of homogenized cellulose obtained at a pressure below 500 b with a degree of homogenization lower than 12.9 showed a quick increase of $G'$ and $G''$ with the frequency (results not shown) indicating an important viscous character of the homogenized suspensions that results mainly from the encumbered steric cellulose structure in the sample [41, 42].

Effect of Homogenization Treatment on Hydrolysis Rate of Pure Cellulose Fiber

Hydrolysis rates of the different cellulose fractions obtained for each homogenization treatment were evaluated by high-performance anion exchange chromatography. Figure 9 presents the evolution of the hydrolysis rate obtained after 24 h for different treatment intensities.

Results obtained show that homogenization treatment influences the cellulose hydrolysis rate. The increase of the hydrolysis rate comprised between 25 (C200(25/4.2) and 100 % (C200(50/32.1) in comparison to the cellulose C200 and depended on the homogenization treatment applied. Results showed also that increase of the cellulose hydrolysis rate is corralled to the shearing (correlate to the pressure) and the degree of homogenization applied. Comparison of these results with a similar previous study performed on the steam explosion technology, which is commonly used to pretreat the biomass, confirmed the potential of the homogenization. Steam explosion treatment, applied on the same material with different intensities, does not appear improve the hydrolysis rate of pure cellulose fiber [43]. In contrast, the important increase of the hydrolysis rate obtained after applying homogenization treatment reflects that accessibility of the cellulose fiber after homogenization is more important compared with those obtained via steam explosion technology and highlights the strong potential of homogenization in the pretreatment of the biomass. Further, hydrolysis rate values obtained for each homogenized samples could be linked to the physicochemical properties described above.
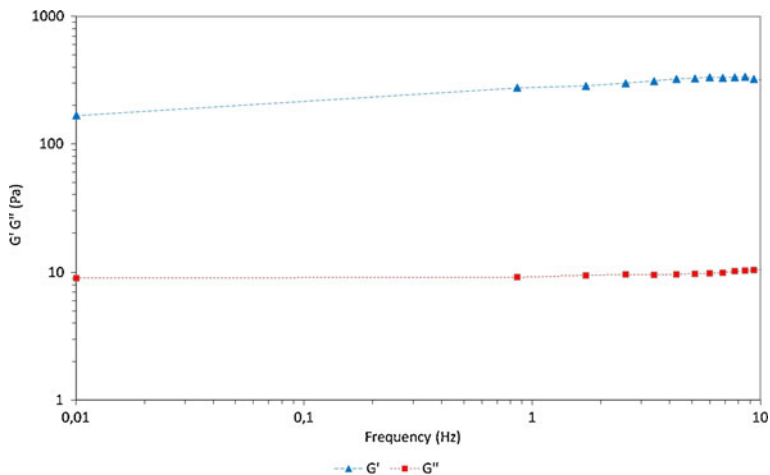


**Fig. 8** Storage modulus ($G'$) and loss modulus ($G''$) in frequency sweep test (C200 50/32.1)
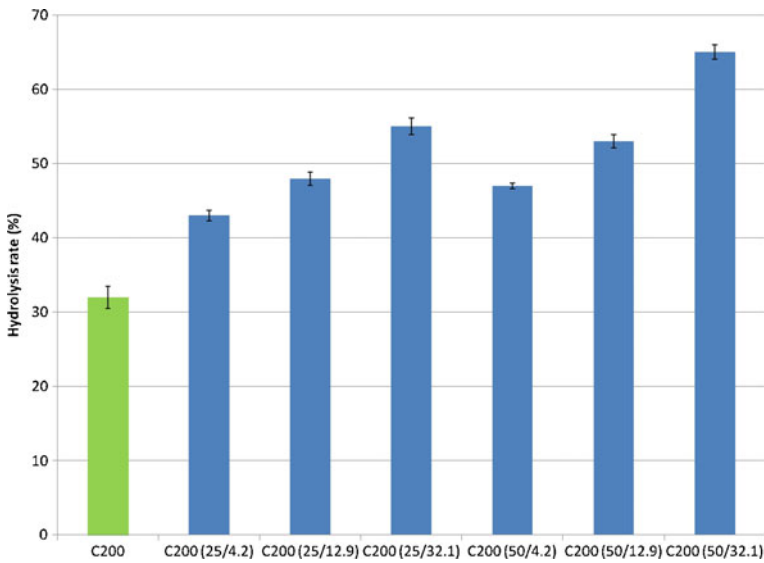
**Fig. 9** Hydrolysis rate (24 h) of cellulose C200 and homogenized cellulose samples

For homogenized samples obtained with moderate treatment intensities (C200(25/4.2), C200(25/12.9), C200(25/32.1), and C200(50/4.2)), the increase of hydrolysis rate are mainly correlated to the evolution of the WRV. WRV could be influenced by the particle size and the pore size, which determinates a specific surface area that will be accessible to the water but also by the dimensions of the crystallites and the degree of crystallinity of the substrate [29]. In this case, it was shown that homogenization did not affect the cellulose crystallinity with the consequence that water retention depended mainly on the specific surface area developed by the homogenized samples. Moreover, it was shown that homogenization treatments applied with moderate intensities were not strong enough to develop a tridimential network that should not influence the accessibility to enzymes.

For higher treatment intensities, the result showed that the important increase of the WRV observed (274 % for C200(50/12.9) and 330 % for (C200(50/32.1))) was not traduced by a very important increase of the hydrolysis rate. The result show even a decrease of the hydrolysis (C200(50/12.9)) with regard to the samples obtained at lower intensities with a lower WRV (C200(25/32.1)) that suggest that the tridimensional network obtained at higher intensities affected the hydrolysis process. The stable network developed in cellulose induces a strength reinforcement of the sample structure that leads to a decrease in mobility of the cellulase molecules [41, 42]. The physical contact between cellulose and cellulase molecules is a prerequisite for hydrolytic reaction. The decrease of mobility induces a decrease of the interaction between cellulases and cellulose that results to a slowdown of the hydrolysis process [44, 45].

## Conclusion

This study investigated the influence of different homogenization treatments on physico-chemical properties and hydrolysis rate of pure cellulose fiber. The main conclusion drawn from this study was that homogenization treatments, applied on a pure cellulose fiber, under

the conditions of this study, appear to improve the enzymatic hydrolysis rate and highlight the potential of homogenization as a pretreatment technology.

Further, this study showed that homogenization had an impact on the physicochemical properties of the cellulose fiber. For moderate treatment, an increase of the WRV of the cellulose, correlated to the hydrolysis rate, was observed. Result also showed that crystalline properties remain similar for all the samples.

For higher treatment intensities, the formation of a stable network in the homogenized samples was highlighted. This network appears to induce a reinforcement of the sample structure that leads to a decrease of the mobility of cellulases and slowdown of the hydrolysis process.

## References

1. Heungjo, A., Wilbert, E. W., & Stephen, W. S. (2011). *Biomass and Bioenergy, 35*, 3763–3774.
2. Sarkar, N., Ghosh, S. K., Bannerjee, S., & Aikat, K. (2012). *Renewable Energy, 37*, 19–27.
3. Franklin, K.R., Hopkinson, A., Webb, N., White, M.S. (2002). Patent WO/2002/032914. 280.
4. Han, S., Baigude, H., Hattori, K., Yoshida, T., & Uryu, T. (2007). *Carbohydrate Polymers, 68*(1), 26–34.
5. Wang, K., Jiang, J. X., Xu, F., & Sun, R. C. (2009). *Polymer Degradation and Stability, 94*, 1379–1388.
6. Sassner, P., Galbe, M., & Zacchi, G. (2008). *Biomass and Bioenergy, 32*, 422–430.
7. Alvira, P., Tomás-Pejó, E., Ballesteros, M., & Negro, M. J. (2010). *Bioresource Technology, 101*, 4851–4861.
8. Galbe, M., & Zacchi, G. (2007). *Advances in Biochemical Engineering/Biotechnology, 108*, 41–65.
9. Avellar, B. K., & Glasser, W. G. (1998). *Biomass and Bioenergy, 14*, 205–218.
10. Jacquet, N., Vanderghem, C., Blecker, C., & Paquot, M. (2010). *BASE, 14*(S2), 561–566.
11. Pérez, J. A., Ballesteros, I., Ballesteros, M., Sáez, F., Negro, M. J., & Manzanares, P. (2008). *Fuel, 87*, 3640–3647.
12. Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., & Lee, Y. Y. (2005). *Bioresource Technology, 96*, 1959–1966.
13. Palonen, H., Thomsen, A. B., Tenkanen, M., Schmidt, A. S., & Viikari, L. (2004). *Applied Biochemistry and Biotechnology, 117*, 1–17.
14. Schacht, C., Zetzl, C., & Brunner, G. (2008). *Journal of Supercritical Fluids, 46*, 299–321.
15. Zhu, S., Wu, Y., Yu, Z., Wang, C., Yu, F., Jin, S., et al. (2006). *Biosystems Engineering, 93*, 279–283.
16. Sun, R. C., & Tomkinson, R. C. (2002). *Carbohydrate Polymers, 50*(3), 263–271.
17. Floury, J., Desrumaux, A., Axelos, M., & Legrand, J. (2002). *Food Hydrocolloids, 16*(1), 47–53.
18. Pandolfe, W. D. (1999). *Wiley encyclopedia of food science and technology* (2nd ed.). New York: Wiley.
19. Walstra, P. (1983). In P. Becher (Ed.), *Encyclopedia of emulsion technology, vol. 1: Formation of emulsion* (pp. 57–127). New York: Marcel Dekker.
20. Siro, I., & Plackett, D. (2012). *Cellulose, 17*(3), 459–494.
21. Dinand, E., Chanzy, H., & Vignon, R. M. (1999). *Food Hydrocolloids, 13*(3), 275–283.
22. Agoda-Tandjawa, G., Durand, S., Berot, S., Blassel, C., Gaillard, C., Garnier, C., et al. (2010). *Carbohydrate Polymers, 80*, 677–686.
23. Bhatnagar, A., & Sain, M.M. (2003). Canadian Intellectual Property Office No. 2437616.
24. Nakagaito, A. N., & Yano, H. (2004). *Applied Physics A-Materials Science Processing, 78*, 547–552.
25. Habibi, Y., Mahrouz, M., & Vignon, M. R. (2008). *Food Chemistry, 115*(2), 423–429.
26. Vanderghem, C., Boquel, P., Blecker, C., & Paquot, M. (2010). *Applied Biochemistry and Biotechnology, 160*(8), 2300–2307.
27. Ioannis, N., Konstantina, T., & Stavros, M. (2006). *European Journal of Pharmaceutics and Biopharmaceutics, 63*, 278–287.
28. Leviton, A., & Pallansch, M. J. (1959). *Journal of Dairy Science, 42*(1), 20–27.
29. Vanderghem, C., Jacquet, N., Danthine, S., Blecker, C., & Paquot, M. (2012). *Applied Biochemistry and Biotechnology, 166*(6), 1423–1432.

30. Yeh, A. I., Huang, Y. C., & Chen, S. H. (2010). *Carbohydrate Polymers, 79*, 192–199.
31. Nelson, M. L., & O'Connor, R. T. (1964). *Journal of Applied Polymer Science, 8*, 1325–1341.
32. Segal, L., Creely, J. J., Martin, A. E., & Conrad, C. M. (1962). *Texas Research Journal, 29*, 786–794.
33. Thygesen, A., Oddershede, J., Lilholt, H., Thomsen, A. B., & Ståhl, K. (2005). *Cellulose, 12*, 563–576.
34. Bibin, M. C., Alcides Lopes, L., Ferreira de Souza, S., Sabu, T., Pothan, L. A., & Kottaisamy, M. (2010). *Carbohydrate Polymers, 81*, 720–725.
35. Mantanis, G. I., Young, R. A., & Rowell, R. M. (1995). *Cellulose, 2*, 1–22.
36. Paquot, M., Thonart, P., Jacquemin, P., & Rassel, A. (1981). *Holzforschung, 35*, 87–93.
37. Azizi Samir, M. A. S., Alloin, F., & Dufresne, A. (2005). *Biomacromolecules, 6*, 612–626.
38. Tabilo-Munizaga, G., & Barbosa-Canovas, G. V. (2005). *Journal of Food Engineering, 67*, 147–156.
39. Guo, Q., Cui, S. W., Wang, Q., Douglas Goff, H., & Smith, A. (2008). *Food Hydrocolloids, 23*, 1542–1547.
40. Eichhorna, S. J., Young, R. J., Davies, R. J., & Riekel, C. (2003). *Polymer, 44*, 5901–5908.
41. Eriksson, R., Pajari, H., & Rosenholm, J. B. (2009). *Journal of Colloid and Interface Science, 332*, 104–112.
42. Rudraraju, V. S., & Wyandt, C. M. (2004). *International Journal of Pharmaceutics, 292*, 53–61.
43. Jacquet, N., Vanderghem, C., Danthine, S., Quiévy, N., Blecker, C., Devaux, J., Paquot, M. (2012). Bioresour Technol., In press.10.1016/j.biortech.2012.06.073
44. Lee, S. B., Shin, H. S., Ryu, D. D. Y., & Mandels, M. (1982). *Biotechnology and Bioengineering, 24*, 2137–2153.
45. Stone, J. E., Scallan, A. M., Donefer, E., & Ahlgren, E. (1969). *Advances in Chemistry Series, 95*, 219–307.