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In situ rheometry of concentrated cellulose fibre suspensions and relationships with enzymatic hydrolysis

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Rheology
Paper pulp
Hydrolyse
Viscosity

A B S T R A C T

This work combines physical and biochemical analyses to scrutinize liquefaction and saccharification of complex lignocellulose materials. A multilevel analysis (macroscopic: rheology, microscopic: particle size and morphology and molecular: sugar product) was conducted at the lab-scale with three matrices: microcrystalline cellulose (MCC), Whatman paper (WP) and extruded paper-pulp (PP). A methodology to determine on-line viscosity is proposed and validated using the concept of Metzner and Otto (1957) and Rieger and Novak’s (1973). The substrate suspensions exhibited a shear-thinning behaviour with respect to the power law. A structured rheological model was established to account for the suspension viscosity as a function of shear rate and substrate concentration. The critical volume fractions indicate the transition between diluted, semi-diluted and concentrated regimes. The enzymatic hydrolysis was performed with various solid contents: MCC 273.6 gdm/L, WP 56.0 gdm/L, PP 35.1 gdm/L. During hydrolysis, the suspension viscosity decreased rapidly. The fibre diameter decreased two fold within 2 h of starting hydrolysis whereas limited bioconversion was obtained (10–15%).

1. Introduction

Lignocellulose biomass is one of the most abundant renewable resources and certainly one of the least expensive. Its conversion into ethanol fuel is eventually expected to provide a significant portion of the world’s energy requirements. The substrates used are varied. They include woody substrates (hardwood and softwood), products from agriculture (straw) or those of lignocellulosic waste industries (food processing, paper).

In order to achieve economic viability, the bio refining of ligno-cellulosic resources must be operated at very high feedstock dry matter content. Paper pulp is quite appropriate for modern bio-refining, because it displays a low lignin content, it is free of inhibitory compounds that can perturb fermentations and devoid of microbial contaminants.

Nevertheless, the enzyme liquefaction and saccharification of paper-like pulps are subject to the same constraints as other pulps obtained via alternative methods such as steam explosion or dilute acid hydrolysis. Therefore, a better scientific understanding and, ultimately, good technical control of these critical biocatalytic
reactions, which involve complex matrices at high solid contents, is currently a major challenge if biorefining operations are to become commonplace.

Amongst the main parameters to be studied, the rheological behaviour of the hydrolysis suspension and the fibre particle size of, stand out as a major determinants of process efficiency and determine equipment to be used and the strategies applied (Wiman et al., 2010). The choice of agitation system, fundamental to heat and/or mass transfer, and to disruption of agglomerated particles, influences the bioconversion of cellulose into simple sugar (Um, 2007). It requires detailed knowledge of the rheological behaviour of the substrate suspensions. However, these suspensions present such complex and unique properties that there are no standard method for studying fibre network deformation and pulp flow behaviour (Blanco et al., 2006).

Fibre suspension flow is a key factor and extensive studies have been reported in the pulp and paper scientific literature. Cellulose fibres in suspension form three-dimensional networks that exhibit viscoelastic properties (Wahren et al., 1964; Kerekes et al., 1985 cited by Antunes, 2009). Measuring the rheological properties of fibre suspensions is complex, owing to multiple factors: (i) fibre physical and mechanical properties and concentration ranges, (ii) fibre contacts and surface forces and (iii) forces on fibres and flocculation. Rheological behaviour of fibre suspensions is usually described by an apparent yield stress, a shear viscosity (Herschel–Buckley or Bingham models) and elasticity. The physical properties of cellulose fibre are considered such as swelling, dissolution, structure and strength of network. The strength of the network of the coarsest fibres determines the rheology of these materials (Wiman et al., 2010). The rheology of lignocellulosic suspensions is of special interest and studies are numerous at different temperatures and concentrations, from dilute solutions 0.2–3.0% (Agoda-Tandjawa et al., 2010; Ferreira et al., 2003) to concentrated solutions 10–20% (Um and Hanley, 2008; Zhang et al., 2009). Both of these studies conclude that a shear-thinning behaviour occurs for any lignocellulosic substrate suspension: microcrystalline cellulose (Agoda-Tandjawa et al., 2010; Chaussy et al., 2011; Tatsumi and Matsumoto, 2007; Um and Hanley, 2008); hardwood paper-pulp (Blanco et al., 2006; Zhang et al., 2009); softwood paper-pulp (Ferreira et al., 2003; Wiman et al., 2010); sugar cane bagasse (Pereira et al., 2011). The viscosity of the suspension depends not only on the temperature and concentration (Ferreira et al., 2003) but also on the average fibre length (Lapiere et al., 2006). A longer fibre has a higher degree of polymerisation and generates a higher viscosity. During biological hydrolysis, the apparent viscosity of suspensions decreases (Pereira et al., 2011; Um, 2007) in parallel with a decrease of particle size (Wiman et al., 2010).

Traditionally, rotating viscometers have been used (Duffy and Titchener, 1975; Chase et al., 1989; Bennington et al., 1990). However, normal commercial viscometers do not provide enough mixing to maintain uniform fibre distribution, which causes viscosity values close to the viscosity of the pure water (Blanco et al., 1995 cited by Antunes, 2009). Therefore, to study the rheological properties of fibres suspensions there is no standardized method but several measuring devices have been reported in the literature (Cui and Grace, 2007; Blanco et al., 2006; Chaussy et al., 2011; Derakhshandeh et al., 2011). Plate torque-based devices have the highest resolution and can be used to determine the rheological behaviour of pulp suspensions (Blanco et al., 2006). One difficulty remains in the definition of criteria to ascribe a viscosity to a heterogeneous suspension, originally defined for homogenous fluids in laminar flow (Blanco et al., 2006). To attain fluidisation, apparent yield stress must be exceeded throughout the suspension. Although fluidisation generally occurs in a turbulent regime, fluid-like behaviour at the floc level can be attained under non-turbulent conditions. One example is the flow induced in a rotary device at slow rotational speeds just above the apparent yield stress; another example was found in spouted beds (Derakhshandeh et al., 2011).

Then on-line measurement of torque or mixing power in bioreactors may highlight viscosity of concentrated cellulose suspensions and may constitute a way to follow enzymatic hydrolysis reactions. Particle size, rheology, and rate of enzymatic hydrolysis could be correlated to operating conditions for example: mixing rate and impeller speed (Pereira et al., 2011; Samaniuk et al., 2011).

The aim of the present report was to investigate the dynamics of transfer phenomena and limitation of biocatalytic reactions with lignocelluloses resources under high concentration conditions. This study focuses on the characterisation of cellulose suspensions at different concentrations and coupling with the enzymatic kinetics of hydrolysis using on-line viscometry. In the literature, rheometers are used to determine ex situ suspension viscosity. These approaches are limited by the number of samples and the substrate properties, predominately decantation and flocculation of material. To solve these problems, a method allowing the suspension viscosity to be followed is proposed. Firstly, cellulose fibre suspensions at various concentrations are investigated through on-line measurements in purpose-built bioreactor. Three real and model matrices are characterised by fiber morphology, diameter and concentration. Using Metzner and Otto concept (1957), rheograms were determined. Rheological behaviour was then described by structured rheological models. Secondly, the complex relationships between fibre structure, degradation, chemical composition and rheological behaviour was scrutinised. To do so, physical and biochemical on-line and off-line analyses were conducted during the bioreaction. A relationship between viscosity change and biocatalytic degradation of fibre was observed.

2. Methods

2.1. Experimental device

The experimental set-up consists of a tank and an impeller system connected to a viscometer working at imposed speed (Viscotester HAAKEVT550, Thermo Fisher Scientific, Ref: 002-7026) (Fig. 1). This allows on-line torque measurements. The rotational speed ranged between 0.5 and 800 rpm and torque between 1 and 30 mN m. The bioreactor was a homemade glass tank with a flat bottom (diameter: 82 mm, H: 76 mm, V: 0.4 L) fitted with a water jacket. The impeller was a four-pitched blade turbine (IKA A200, stainless steel, d: 50 mm, l: 21 mm, w: 8 mm, 45° angle 25 mm from the bottom of the tank to maintain axial and radial flows. Temperature was controlled by circulation (cryostat HAAKE DC30 and K20) through the water jacket. A bioreactor panel control (B. Braun Biotech International MCU200 + microDCU300) was used for pH control and regulation, dissolved oxygen and temperature measurements. The viscometer and the cryostat were controlled by software from HAAKErheoWin Job Manager (Thermo Fisher Scientific) which also ensured data recording (temperature, torque and mixing rate).

2.2. Substrates and enzymes

Three cellulose matrices were studied in order to investigate different fibre morphologies and particle size distributions (Table 1): microcrystalline cellulose (ACROS Organics, Ref: 382310010), a dried and milled (Bosch MKM6003 mill) Whatman paper (Whatman International Ltd., Maidstone, England, Cat No. 1001 090) and paper-pulp (Tembec Co., Saint-Gaudens, France, type FPP31) after extrusion (7/8 mixing, 1/8 shear stress, Prism
The Tembec paper-pulp was made from coniferous wood and contained 26.1% dry matter (75.1% cellulose, 19.1% hemicellulose, 2.2% Klason lignin and ash). The three substrates are henceforth referred to as MCC, for microcrystalline cellulose, WP for Whatman paper and PP for extruded paper pulp. The density of the three substrates was determined by the volume method (proportion of substrate volume and added water volume in a volumetric flask of 100 mL). This density corresponds to the suspended matrix, including its initial water content. It was used to calculate the volume fraction, even though other definitions can be proposed it characterizes raw matter and emanates directly from the industrial process.

An enzyme cocktail (Enzyme ACCELLERASE® Genecor, Ref. 3015155108) containing exoglucanases, endoglucanases (2800 CMC U/g, i.e. 57 ± 2.8 FPU/mL cited by Alvira et al., 2011), hemicellulases and β-glucosidases (775 pNPG U/g) was used. Its optimal temperature and pH were 50 °C (range 50–65 °C) and pH 4.8 (range 4–5). An ACCELLERASE® 1500 dosage rate of 0.1–0.5 mL per gram of cellulose or roughly 0.05–0.25 mL per gram of biomass (depending on biomass composition) is recommended by the manufacturers.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>MCC</th>
<th>WP</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>99</td>
<td>99</td>
<td>26</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>100</td>
<td>90</td>
<td>75</td>
</tr>
<tr>
<td>Δ[4,3] (µm)</td>
<td>70</td>
<td>250</td>
<td>190</td>
</tr>
<tr>
<td>ρ (g/L)</td>
<td>1623 ± 28</td>
<td>1200 ± 2</td>
<td>1346 ± 2</td>
</tr>
<tr>
<td>Crystallinity (%)</td>
<td>79.0</td>
<td>88.6</td>
<td>64.5</td>
</tr>
</tbody>
</table>

Fig. 1. Experimental set-up.

2.3. Physical and chemical analysis

2.3.1. Laser particle size determination

Particle size distribution was determined through laser diffraction analyses (Mastersizer 2000 Hydro, Malvern Instruments Ltd., SN: 34205-69, range from 0.02 to 2000 µm). A suspension (approximately 5 g/L) was added drop by drop to the circulation loop (150 mL). Analysis are conducted at room temperature (20 °C) with obscuration rates (red λ = 632.8 nm and blue λ = 470.0 nm lights) ranging between 10% and 40%. Particle volume distribution and the associated cumulative curve versus particle diameter were determined. Laser diffraction analysis converts the detected scattered light into a particle size distribution. Successful deconvolution relies on an appropriate description of light behaviour: either Mie theory or the Fraunhofer approximation (of Mie theory). Historically, the use of Mie theory was limited by computing power, which was eliminated in the last decade by dramatic increases in processing power. This method was designed for particles, so relative measurements were made in order take complex particle shape, refractive index and measurement repeatability into consideration.

2.3.2. Morpho-granulometry

Fibre morphology was observed using a mopho-granulometer (Mastersizer G3S, Malvern Instruments Ltd., SN: MAL1033756, software Morphologi v7.21). This optical device includes a lens (magnification: from ×1 to ×50, dimension min/max: 0.5/3000 µm) and a camera (Nikon CF60). Samples were analysed by two methods: “dry” and “wet”. For “dry” analysis, the powders were dispersed using a specific dispersion unit (with air). For “wet” analysis, the suspensions (approximately 5 g/L) were observed between cover glasses and slides. A 1.5 mm × 1.5mm surface was observed under standardized conditions (light intensity:
80 ± 0.2; magnification: ×2.5). The images were filtered and analysed to determine the number of particles and their geometric properties (diameter, aspect ratio, etc.).

2.3.3. Glucose concentration (YSI)

Glucose concentration was checked in the supernatant along with enzymatic hydrolysis (Analyser YSI model 27A; Yellow Springs Instruments, Yellow Springs, Ohio, range 0–2.5 g/L ± 2%, sample volume = 25 μL).

2.4. Generalised power consumption curve and on-line viscometry

Power consumption is described by the dimensionless power number $N_p$ versus the mixing Reynolds number, $Re$, and was established for Newtonian fluids, with:

$$N_p = \frac{P}{d^5 \cdot \rho \cdot N \cdot d^2} \cdot \frac{\rho}{\mu}$$

$$P = 2\pi \cdot N \cdot C.$$  

This single master curve depends only on impeller/reactor shape and geometry. In the laminar regime ($Re < 10–100$), the product $N_p \cdot Re$ is a constant, named $K_p$, which is then defined as follows:

$$N_p \cdot Re = K_p$$

$K_p$ is a function of impeller shape and geometry for any Newtonian fluid. A deviation from Eq. (2) indicates the end of laminar regime. In fully turbulent flow ($Re > 10^5–10^6$) and for Newtonian fluids, the dimensionless power number $N_p$ is assumed to be independent of mixing Reynolds number and equal to a constant, $N_{p0}$. In this case, three Newtonian liquids (distilled water, Marcoul 52 oil and glycerol) were used to cover a large range of mixing Reynolds numbers. Viscosity for these calibration fluids (also non-Newtonian liquids below) was measured with a cone and plate system (60 mm diameter, angle 2°, Mars III rheometer, Thermo Scientific) and for shear rate varying from 10 to 100 (s$^{-1}$) at two different temperatures: 20 and 40 °C. The density of the fluids was also determined by a densimeter (Metttler Toledo DE40, 0–3 g/cm$^3$, ±0.0001 g/cm$^3$). The torque and mixing rate (ascent/descent cycles, 0.5/800/0.5 rpm) were measured for each fluid at 20 and 40 °C. Calculating $B$ and $Re$, the power consumption curve was then established.

The $K_p$ value obtained was 68.8 which is comparable to values from the literature (Rushston et al., 1950: for propeller $K_p = 40–50$, for flat-blade turbine $K_p = 66–76$). Experimental results confirm that the laminar regime prevails up to $Re = 20$ (Fig. 2).

A semi-empirical model including laminar and transition regions were considered for the reference curve with a one-to-one relationship between $N_p$ and $Re$:

$$N_p = \left(\frac{K_p}{Re_{eq}}\right)^n + \left(c \cdot Re_{eq}^\beta\right)^n$$

The parameters $n$, $c$, and $\beta$ stand for the transition regime and adjustments to the experimental results lead to: $n = 2; c = 3.22; \beta = -0.208$.

In the non-Newtonian case, a generalised mixing Reynolds number has to be defined as the viscosity is not a constant. The well-known Metzner and Otto concept (1957) was used: a viscosity $\mu$ is defined as the Newtonian viscosity leading to the same power number. Metzner and Otto (1957) showed that the equivalent shear rate $\dot{\gamma_{eq}}$ associated to this viscosity (through the rheological behaviour of the fluid) is proportional to the rotation frequency, then introducing the Metzner–Otto parameter $K_s$:

$$\dot{\gamma_{eq}} = K_s \cdot N$$

This leads to the generalized Reynolds number:

$$Re_e = \frac{\rho \cdot N^2 \cdot d^2}{k \cdot K_s^{-1}}.$$  

$K_s$ is a constant depending only on the geometry of the stirring system. Eq. (5) can be extended to the transition region using a power equation (Jahangiri et al., 2001). Xanthan solutions (0.04%; 0.1%; 0.4%) in glucose solution (650 g/L) and in sucrose solution (943 g/L) were used to determine the proportionality constant $K_p$. Using the power consumption curve established with Newtonian fluids, the apparent viscosity $\mu$ was calculated from torque and mixing rate measurements. The corresponding value of the shear rate, $\dot{\gamma_{eq}}$, was extracted from the rheograms of the Xanthan solutions. Rieger and Novak’s approach (1973) was used to determine the value of $K_s$: Eq. (1) with the generalized Reynolds number $Re_k$ is written in a similar form:

$$N_p \cdot Re = K_p(n)$$

With $Re_k = \frac{\rho \cdot N^2 \cdot d^2}{k}$ and $K_p(n) = C_{p0} K_s n^{-1}$.

The value of $K_s$ is directly deduced from the curve $K_p(n) = f(n)$ using the previously determined $K_p$ value. This leads to $K_s \approx 28 ± 4$. In the case studied, the extension to the transition region using a power equation (Jahangiri et al., 2001) is not relevant. Once the experimental set-up was characterized by its power consumption curve $N_p(Re)$ and the $K_p$ value, on-line viscometry of the suspension was performed before and along the biocatalytic reaction.

2.5. Methodology

2.5.1. Mixing substrate

The first step consisted in suspending the substrates in 300 mL of water. Each cycle of suspension is composed of (i) a homogenization phase (500 rpm for 300 s) with substrate addition and (ii) torque measurement based on 100 s phase with increasing and decreasing mixing rates (10, 50, 100, 155, 200, 300, 500, 650 and 800 rpm) within viscometer capacity ($N_{max} \approx 800$ rpm, $C_{max} \approx 30$ mN m). The concentration chosen for a given experiment was reached by successive additions of substrate: 8 × 20 g for MCC, 7 × 3 g for WP and 11 × 3 g for PP.

2.5.2. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out at 40 °C due to energy saving and the microbiological step during the fermentation process considering a simultaneous saccharification and fermentation (SSF) operation. The pH of the medium was adjusted to 4.8 using a solution of 85% orthophosphoric acid. To avoid contamination, 5 μL of a solution of chloramphenicol (5 g/L) was added. Then enzymes were added when the suspension reached homogeneity and the torque values were stable.

Hydrolysis was investigated over 25 h at a mixing rate of 450 rpm and using the selected concentrations: 273.8 gdm/L for MCC; 56.0 gdm/L for WP and 35.1 gdm/L for PP. These concentrations were established to obtain a significant initial torque ($C \geqslant 1.7$ mN m) and to ensure accurate monitoring of its derivation during hydrolysis. These concentrations ensure initial laminar regimes for WP and PP and transitional regime for MCC (Table 2). The quantities of enzyme used were in agreement with supplier’s recommendations.

Decantation affects the suspension homogeneity and can lead to deposition under low mixing rates. This problem is exacerbated with MCC due to its higher density and higher compactness. So during the reaction, periods of higher mixing rates (500/650 rpm
for 300–600 s, every 1–3 h) were imposed in order to keep the suspensions uniform.

Samples were taken manually by a 6 mm diameter flexible connected to a 20 mL syringe. Each sample was about 6 mL, sufficient to perform analyses on 5/7 sub-samples. The total volume of samples removed ranged from 30 to 42 mL (10–14% of initial volume). This order of decrease of suspension volume causes negligible impact on the suspension viscosity (at the end, a difference of 1–7% may be observed). The samples were used for rheological, granulometric and biochemical analysis during enzyme degradation.

3. Results and discussions

3.1. Viscosimetry of substrate suspensions

The rheological behaviour of suspensions is complex and is affected by multiple parameters such as concentration, shape, density and surface properties. The viscosity of the suspension was quantified as a function of the type of substrate, its concentration and the mixing conditions. Using the power consumption curve and the associated Churchill model, the on-line viscosity was estimated at 40°C as a function of substrate concentration and mixing rate (Fig. 3). These raw data covered laminar and transition regimes.

For a given mixing rate and substrate concentration, the viscosity of the WP suspension was higher than that of the PP suspension, and the viscosity of MCC was the lowest. As an example, for 155 rpm and a substrate concentration close to 64 g/L, the viscosities observed were $\mu_{WP} = 4560$ mPa s, $\mu_{PP} = 100$ mPa s, and $\mu_{MCC} = 2$ mPa s with a decreasing volume fractions, $\Phi_{WP} = 0.055$ (64.8 gdm/L), $\Phi_{PP} = 0.047$ (16.5 gdm/L) and $\Phi_{MCC} = 0.039$ (64.0 gdm/L) respectively. For identical mixing rates and a substrate concentration close to 16 gdm/L, interpolation of the previous results gives an estimate of $\mu_{WP} = 194$ mPa s, $\mu_{PP} = 90$ mPa s, and $\mu_{MCC} = 8$ mPa s with decreasing volume fractions of $\Phi_{PP} = 0.047$ (64 g/L), $\Phi_{WP} = 0.016$ (19.7 g/L) and $\Phi_{MCC} = 0.01$ (16.5 g/L). For MCC, the results are in agreement with reported data with average fibre length and diameter equal to 1.7 and 0.077 µm, respectively exhibiting $0.01 < \mu < 10$ Pa s for 0.5 < %dm < 5% (Tatsumi et al., 1999). For all the studied concentration of the three suspensions, the viscosity decreased as the mixing rate increased. All the suspensions were found to act as shear-thinning fluids.

The on-line measurements were firstly used to establish rheograms (considering only results in laminar regime) and to determine the rheological behaviour of the suspensions. In a second step the impact of particle volume fraction on relative viscosity was investigated. This approach contributed to establish a structured rheological model including several factors such as shear-rate, volume fraction and particle dimension.

3.1.1. Rheogram

Based on the Metzner and Otto concept, rheograms are identified under the laminar flow regime ($Re \geq 30$). Data obtained with the microcrystalline cellulose suspension were outside the laminar regime, so rheograms were only obtained for WP and PP.

As the suspensions exhibited a shear-thinning behaviour, several approximations, such as power-law, Sisko, Cross, Powell–Eyring, Carreau and “local” power-law models can be used. In the investigated conditions, a power-law model was retained. It is written:

$$\mu = k \cdot \gamma^n \cdot \mu_0$$  \hspace{1cm} (7)

For substrates and WP and PP, the rheological behaviour was described as a function of concentration and modelled by linear and exponential relationships (Table 3). The patterns observed are similar to those reported by Bayod et al. (2005) and Luukkonen et al. (2001). In the concentration range studied, power-law indexes
ranged between 0.28 and 0.50 for WP and between 0.57 and 0.68 for PP. Consistencies ranged between 88.8 and 6.2 Pa s for WP and between 18.0 and 3.5 Pa s for PP.

Their rheological behaviour generally exhibited viscoelastic properties (Agoda-Tandjawa et al., 2010; Tatsumi et al., 2001; Paakko et al., 2007). At a concentration of 10% dm and shear rates ranging from 1 to 100 s\(^{-1}\), the viscosity of corn stover (maize thresh and residue) and pre-treated softwood suspensions, decreased from 1.87 to 0.03 and 9 to 0.20 Pa s, respectively (Pimenova and Hanley, 2004; Wiman et al., 2010) (Table 4). Considering dimension criteria, these values are much higher than those for MCC found in the present work.

Surprisingly, the viscosity appears to have the same order of magnitude for dilute and concentrated MCC suspensions (Bayod et al., 2005; Luukkonen et al., 2001) (Table 4). For an MCC concentration of 40% dm and for shear rates ranging from 1 to 100 s\(^{-1}\), the viscosity of the suspension decreased from 8.0 to 0.3 Pa s (Luukkonen et al., 2001). This is similar to the values measured.

3.1.2. Relative viscosity of suspensions

In dilute suspensions, the particles are hydrodynamically independent and a linear relationship between viscosity and volume fraction is observed. The relative viscosity can be modelled by the Einstein equation:

\[
\frac{\mu}{\mu_0} = 1 + k_1 \cdot \Phi = 1 + [k] \cdot Cm
\]

For semi-dilute suspensions, the interactions between the particles begin to interfere and can at first be taken into account by introducing a quadratic term.

Table 3

<table>
<thead>
<tr>
<th>Substrate</th>
<th>(n)</th>
<th>(k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP: 28.7–64.8 gdm/L</td>
<td>(n = -0.006 Cm + 0.701)</td>
<td>(k = 0.724e^{0.075Cm})</td>
</tr>
<tr>
<td>PP: 27.9–42.0 gdm/L</td>
<td>(n = -0.008 Cm + 0.895)</td>
<td>(k = 0.138e^{0.116Cm})</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Author</th>
<th>Substrate</th>
<th>(D(4,3)) (µm)</th>
<th>(Cm) (%)</th>
<th>(n)</th>
<th>(k) (Pa.s(^n))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pimenova and Hanley (2004)</td>
<td>Corn stover</td>
<td>120</td>
<td>5–30</td>
<td>0.05–0.9</td>
<td>0.05–1684</td>
</tr>
<tr>
<td>Wiman et al. (2010)</td>
<td>Dilute acid pre-treated softwood</td>
<td>109</td>
<td>4–12</td>
<td>0.15–0.4</td>
<td>1–16</td>
</tr>
<tr>
<td>Bayod et al. (2005)</td>
<td>MCC</td>
<td>33</td>
<td>0–7</td>
<td>0.8–0.9</td>
<td>0.8–2.5</td>
</tr>
<tr>
<td>Luukkonen et al. (2001)</td>
<td>MCC</td>
<td>60</td>
<td>40–55</td>
<td>0.14–0.29</td>
<td>8–177</td>
</tr>
</tbody>
</table>
\[
\frac{\mu}{\mu_0} = 1 + \alpha \cdot \Phi + \beta \cdot \Phi^2
\]  

(9)

The third regime corresponds to concentrated suspensions with a lot of contacts between the particles. The viscosity of the suspension increases rapidly with volume fraction. When \( \Phi \) reaches a critical value, each particle is confined in a cage formed by its nearest neighbours. For volume fractions above this value, only a vibration of the particles inside the cage remains possible, and disappears completely when \( \Phi \) reaches the value of dense packing.

Covering all concentration ranges, the most commonly used relationship between relative viscosity and volume fraction was proposed by Quemada (2006). Eq. (10) is used for a Newtonian regime.

\[
\frac{\mu}{\mu_0} = \left(1 - \frac{\Phi}{\Phi_{\text{max}}}\right)^n \quad 1 \leq n \leq 2
\]  

(10)

The relative viscosity \( \mu/\mu_{\text{water}} \) is plotted versus the volume fraction at the same mixing rate for three suspensions (Fig. 4). In the plot for PP and WP, two regions are observed corresponding to two concentrations: (i) a dilute/semi-dilute concentration range exhibiting a low relative apparent viscosity (\( \mu/\mu_0 \leq 100 \) under 300 rpm) and a quasi-Newtonian behaviour (low viscosity variations with the rotation frequency) with a linear variation of viscosity versus volume fraction and (ii) a concentrated regime indicating higher relative viscosity (\( \mu/\mu_0 > 100 \)), a shear-thinning behaviour (displayed by the decreasing values of the relative viscosity when the mixing rate increases) and a strong increase with volume fraction. A critical volume fraction, \( \Phi_c \), may be assumed at the transition between two concentration regimes for all suspensions.

With an identical substrate volume fraction and mixing rate, the relative viscosity decreased from WP, PP to MCC. This may be explained by the differences in particle size and morphology. The particle diameter of the WP fibre is the largest so the relative viscosity of this suspension is greater than that of PP and MCC (e.g. for \( \Phi_c = 0.05, \mu_{\text{MCC}} = 2 \text{ mPa s}, \mu_{\text{PP}} = 100 \text{ mPa s} \) and \( \mu_{\text{WP}} = 4000 \text{ mPa s} \)). For all suspensions, a transition from semi-dilute to concentrated regime is observed. A linear variation was shown for MCC in dilute regime. For an identical mixing rate, one critical volume fraction was identified for each suspension \( \Phi_c = 0.03; 0.09 \) and >0.24 for WP, PP and MCC, respectively (Table 5). Luukkanen et al. (2001) proposed a critical volume fraction \( \Phi_c = 0.3 \) (equivalent to 47%dm) for MCC.

These results show that the viscosity of suspensions is strongly dependent on physical fibre properties among which size and shape as appear to make the major contributions (Horvath and Lindstrom, 2007; Lapierre et al., 2006; Wiman et al., 2010).

### 3.2. Enzymatic hydrolysis: impact on viscosity and particle size distribution

**3.2.1. On-line viscosity**

The changes in the physical appearance of the slurry are associated to the biochemical changes occurring in the fibres. Under the action of enzymes, the cellulose chains are cut giving simple products such as glucose (ultimate monomer). The glucose concentration increased with the time of hydrolysis (between 1 and 25 h) to reach a final value that was very different for the three substrates: roughly 42 g/L for MCC (i.e. 13% bioconversion), 7 g/L for WP (i.e. 12% bioconversion) and 3 g/L for PP (i.e. 10% bioconversion). If amorphous cellulose is taken as reference, the bioconversions attain 66.4%, 100%, 30.8% for MCC, WP and PP respectively. Amorphous cellulose was totally or almost totally hydrolysed indicating the efficiency of enzymatic attack. The bioconversion into glucose of the matrices studied was comparable to the results reported in the literature which vary between 3.6% and 45% (Dasari and Berson, 2007; Pereira et al., 2011; Szijarto et al., 2011).

Considering the conditions investigated (substrate, concentration, and mixing rate) the initial viscosities were coherent with values observed during suspension viscometry.

Firstly, a sharp decrease of viscosity was observed with WP and PP during hydrolysis whereas with MCC the reduction was only

![Fig. 4. Evolution of the relative viscosity (MCC: microcrystalline cellulose, WP: Whatman paper and PP: extruded paper pulp) versus substrate volume fraction at mixing rate of 300 rpm.](image-url)
moderate (Fig. 5A). Under 450 rpm, it was greater for WP, 0.976–0.001 Pa s and PP, 0.656–0.002 Pa s than for MCC, 0.104–0.029 Pa s. Viscosity decreased 100 times after 5 h hydrolysis for WP and PP with final values almost reaching that of water. Surprisingly, viscosities of WP and PP fell lower than that of MCC.

With WP and PP, the viscosity fell during the first 5 h to reach similar levels. These results are supported by the literature over a wide range of matrices, particle sizes and enzyme/cellulose ratios.

For acid-pretreated sugarcane bagasse, viscosity was reduced by 77% to 95% after 6 h (Geddes et al., 2010) and by 75% to 82% within 10 h (Pereira et al., 2011). This decrease and final plateau depended on the enzyme loading (Geddes et al., 2010).

For spruce pulp (diameter initial: 91 µm), initial and final viscosities ($\mu_{\text{initial}}/\mu_{\text{final}}$) were 0.24/0.028, 0.4/0.058 and 0.84/0.087 µm for concentrations of 10, 15 and 20% (w/w), respectively. These data were correlated to mean diameters: 44, 53 and 57.5 µm and conversion yields: 40%, 32% and 25%, respectively (Um, 2007).

As mentioned, the decreasing viscosity during enzymatic hydrolysis is reported in literature. In terms of kinetics and propensity this mechanism could be explained by several assumptions: (i) the initial biochemical structure and composition of matrices, (ii) the ability to dissolve lignocellulosic material, (iii) the reduction

A typical pseudo-plastic behaviour was confirmed both in the initial step and during hydrolysis (Pereira et al., 2011; Wiman et al., 2010).

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![Fig. 5. Online viscosity of suspension versus hydrolysis time (A) and mean diameter (B) (MCC: microcrystalline cellulose, WP: Whatman paper and PP: extruded paper pulp).](image-url)
of particles size and, (iv) the efficiency of the enzyme cocktail (activity, concentration).

3.2.2. Distribution of particle size

The physical properties of each matrix were very different, considering their dimension, shape and compactness. The dimension and shape depend on the morphometry and particle size distribution; they are subject to wide dispersion as illustrated in Table 6.

MCC fibres were dense crystalline particles (1620 kg/m^3) with an angular shape (rectangle, square) resembling crystals. WP occurred as dissociated long curved fibres. PP suspension included long fibres with ramification. Aspect ratios were 0.605 ± 0.027, 0.448 ± 0.026 and 0.598 ± 0.024 for MCC, WP and PP respectively. Initial mean volume diameters and diameters at 10% and 90% of distribution are given in Table 6. Diameter distributions indicate bimodal populations. Equivalent diameters for fine and coarse fractions (maxima) were 30 and 120 µm, 80 and 480 µm, 80 and 350 µm for MCC, WP and PP respectively. The ratio between fine and coarse populations is determined by considering the minima of the distribution curves. Initially, with WP and PP the major population was the fine population, 73.9% ± 1.9 and 70.0% ± 7.0, respectively, while for MCC, the fine population (<60 µm) represented only 34.1% ± 6.6. Specific surface area exhibited wide heterogeneity of mean diameter and associated dispersion.

During hydrolysis, as the fibres were degraded, their length and shape changed significantly (Nguyen et al., 2012). The large particles were hydrolysed; their mean diameter decreased for all substrates (Table 6) suspension heterogeneity is confirmed by D(4,3) variability. The mean diameters were approximately halved within 2 h of hydrolysis, 110.8 to 49.4 µm, 241.6 to 139.2 µm and 276.0 to 167.2 µm for MCC, WP, and PP respectively. This led to the reduction of suspension viscosity (Fig. 5B). However, this effect was observed only for WP and PP for which D(4,3) > 100 µm while for MCC [D(4,3) < 100 µm], the viscosity was not significantly dependent on fibre mean diameter.

The fine populations increased to reach 84%, 94% and 74% for MCC, WP and PP respectively. With MCC, the halving of the mean diameter of Solkafloc within 25 h has already been reported (Um, 2007). For the hydrolysis of dilute acid pre-treated softwood (D(4,3) = 109 µm, concentration: 10%w/w): the coarse population (>100 µm) decreased from 44.2% to 19.7% after 24 h (Wiman et al., 2010). These tendencies are observed for all substrates no matter the mixing rate is. The mean diameter decrease in this present work occurred faster than for Wiman et al., 2010 reporting that the fibre diameter was stable for 10 h and then reduced by 20% at 24 h.

For MCC, the hydrolysis effect was mainly observed on coarse particles (Table 6). The initial population tended towards a log-normal distribution (D(4,3) = 49 µm) after 2 h. For WP, coarse and fine populations were degraded giving four populations whose average diameters were 3, 20, 75 and 350 µm after 25 h which indicates a macroscopic cutting effect on fibres. For PP, several mechanisms seem occur. In the first step (Table 6, t = 0.25 h), the split between coarse and fine is strengthened. The fine population increases and translates to a smaller diameter. The reduction process was observed later for the coarse particles (Table 6, t = 1 h). Around 25 h, a smoothing between coarse and fine particles arose. D(4,3) increased at 25 h of hydrolysis (from 167.2 to 177.5 µm) as a result of swelling and unwinding of macro-fibres during the 100 h hydrolysis (Fillodau et al., 2011). These results are correlated to the decrease of viscosity within 5 h of hydrolysis (Fig. 5A).

4. Conclusion

This study focussing on the rheometry of lignocellulosic suspensions explored enzymatic hydrolysis based on physical parameters. The rheometry was dependent on the substrate concentration, the mixing rate imposed (related to shear rate) and the fibre particle size/shape. A method for following viscosity on-line was proposed and used to characterise the rheological behaviour of suspensions as a function of concentration. During enzymatic hydrolysis, the change in viscosity was found due to enzymatic actions and modifications of fibre properties. The decrease of fibre mean diameter could lead to the decrease of suspension viscosity and the effect of enzymatic attack.

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References


