# IN-SITU PHYSICAL ANALYSIS OF CELLULOSE FIBRE SUSPENSIONS DURING ENZYMATIC HYDROLYSIS

**NGUYEN Tien-Cuong[a*], ANNE-ARCHARD Dominique[b], COMA Véronique[c], CAMELEYRE Xavier[a], LOMBARD Eric[a], TO Kim Anh [d], FILLAUDEAU Luc[a]**

[a] *Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP), University of Toulouse, CNRS UMR 5504, INRA UMR 792, INSA, 135 avenue de Rangueil 31400 Toulouse, FRANCE*

[b]*Institut de Mécanique des Fluides de Toulouse (IMFT), University of Toulouse, CNRS UMR 5502, Allée du Professeur Camille Soula, 31400 Toulouse, FRANCE.*

[c]*Laboratoire de Chimie des Polymères Organiques (LCPO), UMR 5629 CNRS/Université Bordeaux 1, IPB/ENSCPB, Pessac, FRANCE*

[d]*School of Biotechnology and Food Technology, Hanoi University of Science and Technology, Hanoï, VIET-NAM*

## 1. INTRODUCTION

Lignocellulose biomass is one of the most abundant renewable resources and certainly one of the least expensive. It was considered as a glucose source for obtain energetic or chemical molecules by bioconversion. This enzymatic conversion was so complicate therefor 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, stand out as a major determinants of process efficiency and determine equipment to be used and the strategies applied. Rheological behaviour of fibre suspensions is usually described by an apparent yield stress, a shear viscosity (Hershel-Buckley or Bingham models) and elasticity. During biological hydrolysis, the apparent viscosity of suspensions decreases in parallel with a decrease of particle size (Nguyen et al., 2013). This study focuses on the characterisation of cellulose suspensions (Microcrystalline cellulose, Whatman paper and extruded paper pulp) during enzymatic hydrolysis using in-situ and ex-situ physical analysis. The complex relationships between fibre structure, degradation, chemical composition and rheological behaviour was scrutinised.

## 2. METHODS

### *2.1. Experimental setup*

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). This allows on-line torque measurements. The rotational speed ranged between 0.5 and 800 rpm and torque between 1 and 30mN.m. The bioreactor was a homemade glass tank (diameter: 130mm, Hmax: 244mm, V: 2.0L) fitted with a water jacket. Suspension homogeneity was maintained with a shaft equipped with double impeller. The first impeller consists in a 3 inclined blades (diameter: 73.5mm, angle: 45°, h=38mm) located at 75mm height from the bottom to ensure mixing. The second impeller is a close bottom mixer including 2 large blades (diameter: 120mm, h=22mm) to avoid substrate decantation. Temperature was controlled by circulation (cryostat Haake DC30 and K20) through the water jacket. The viscometer and the cryostat were controlled by software from HaakeRheoWin Job Manager (Thermo Fisher Scientific) which also ensured data recording (temperature, torque, mixing rate). A focused beam reflectance sensor was located in bulk in order to measure the distribution of particle chords.

### *2.2. In-situ and ex-situ rheometry*

Power consumption curve was identified and modelled with the semi-empirical model (Churchill, 1983) including laminar and transition regions. It was considered as the reference curve:

$Np = \left( \left( \frac{Kp}{\mathrm{Re}} \right)^n + (Np_0)^n \right)^{1/n}$ with n=0.834, Kp=98.1, $Np_0$=0.25. The in-situ viscosity of suspension was determined using the torque and mixing rate measurements and considering the Metzner & Otto concept (1957) and Rieger and Novak's approach (1973).
Ex-situ viscous and elastic modules were measured by rheometer (MARS III, Thermo Scientific, $3.10^{-8}$ < C < 0.2 N.m) under oscillation with parallel striated plate (D: 60mm, gap: 1mm, frequency range: 0.05 to 20Hz, 20°C).

*2.3. In-situ and ex-situ granulometry*

In-situ particle size characterization was realised by chord length measurement of particles using a solid-state laser light source (FBRM G400, Mettler-Toledo, range: 0.1 to 1000µm).

Ex-situ particle size distribution was determined through laser diffraction analyses (Mastersizer 2000 Hydro, Malvern Instruments, red λ=632.8nm and blue λ=470.0nm lights, range: 0.02 to 2000µm).

*2.3. Enzyme, substrate and operation conditions*

Three cellulose matrices were studied in order to investigate different fibre morphologies and particle size distributions (Table 1): microcrystalline cellulose (ACROS Organics), a dried and milled Whatman paper (Whatman International Ltd., Maidstone, England) and paper-pulp (Tembec Co., Saint-Gaudens, France, type FPP31) after extrusion. 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). An enzyme cocktail (ACCELLERASE® Genecor) containing exoglucanases, endoglucanases (2800 CMC U/g ie. 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 to 65°C) and pH 4.8 (range 4 to 5). A dosage rate of 0.1 - 0.5 mL per gram of cellulose.

Table 1: Substrate properties (MCC: microcrystalline cellulose, WP: Whatman paper and PP: extruded paper pulp)

| | MCC | WP | PP |
|---|---|---|---|
| Dry matter (%) | 99 | 99 | 26 |
| Cellulose (%) | 100 | 90 | 75 |
| D[4,3] (µm) | 70 | 250 | 190 |
| ρ (g/L) | 1623 ± 28 | 1200 ± 2 | 1346 ± 2 |
| Crystallinity (%) | 79.0 | 88.6 | 64.5 |

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, 20µL 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 24h at a mixing rate of 100 rpm and using the selected concentrations: 1%, 3%dm at different enzyme/substrate ratios: 0.1 and 0.5 mL/g cellulose. Samples were taken manually by a 6mm diameter flexible connected to a 50mL syringe. Each sample was about 15mL, sufficient to perform analyses on 8 sub-samples. Glucose concentration was checked in the supernatant along enzymatic hydrolysis (Analyser YSI model 27A; Yellow Springs Instruments, Yellow Springs, Ohio, range 0-2.5g/L ± 2%, sample volume=25µL).

## 3. RESULTS AND DISCUSSIONS

*3.1. In-situ and ex-situ rheometry*

Under the action of enzymes, the cellulose chains were cut giving simple products such as glucose (ultimate monomer). The glucose concentration (i.e bioconversion percentage) was increased with the time of hydrolysis (between 1 and 24 hours) to reach a final value that was very different for the three substrates (Table 2). For the lowest enzyme/substrate ratio, the enzymatic attack strongly depends on fibre structures and substrate compositions; by consequence the bioconversion rate of MCC was higher than PP and WP. On the contrary, this dependence was limited when the ratio enzyme/substrate increased. For MCC and PP, at the ratio 0.5 mL/g cellulose, their bioconversion rates reached 61% and 72% respectively which were superior to the results reported by Dasari et al., 2007; Pereira et al., 2011 and Szijarto et al., 2011 (bioconversion rates between 3.6% and 45%).

Table 2. Bioconversion percentage at 24h of hydrolysis

| Substrate | Concentration (%dm) | Ratio enzyme/substrate | Bioconversion rate (%) |
|---|---|---|---|
| MCC | 1% | 0.1 mL/g cellulose | 34.8 |
| MCC | 1% | 0.5 mL/g cellulose | 61.2 |
| PP | 1% | 0.1 mL/g cellulose | 17.3 |
| PP | 3% | 0.5 mL/g cellulose | 72.1 |
| WP | 1% | 0.1 mL/g cellulose | 12.8 |

The changes in the physical appearance of the slurry are associated to the biochemical changes of fibres. A sharp decrease of viscosity was observed with WP and PP during hydrolysis whereas with MCC its viscosity remain stable having value of water viscosity (≈0.6mPa.s) (Fig 1-A). Under 100 rpm, it was the same observed phenomena for WP, 0.62 to 0.006 Pa.s and PP, 0.574 to 0.007 Pa.s. However, this reduction is faster for WP than for PP. It takes 3h for one reduction of 100 times with WP comparing 10h with PP. This evolution of viscosity is supported by the literature over a wide range of matrices, particle sizes and enzyme/cellulose ratios (Geddes et al., 2010; Nguyen et al., 2013; Pereira et al., 2010; Um. 2007; Wiman et al., 2010). The ex-situ rheometry results showed a typical viscoelastic behaviour being confirmed both in the initial step and during hydrolysis for WP and PP (for MCC, it is impossible to realise this measurement because of substrate decantation). The elastic behaviour was predominately comparing with viscous module; however these two modules decreased regular during hydrolysis (fig 1-B).

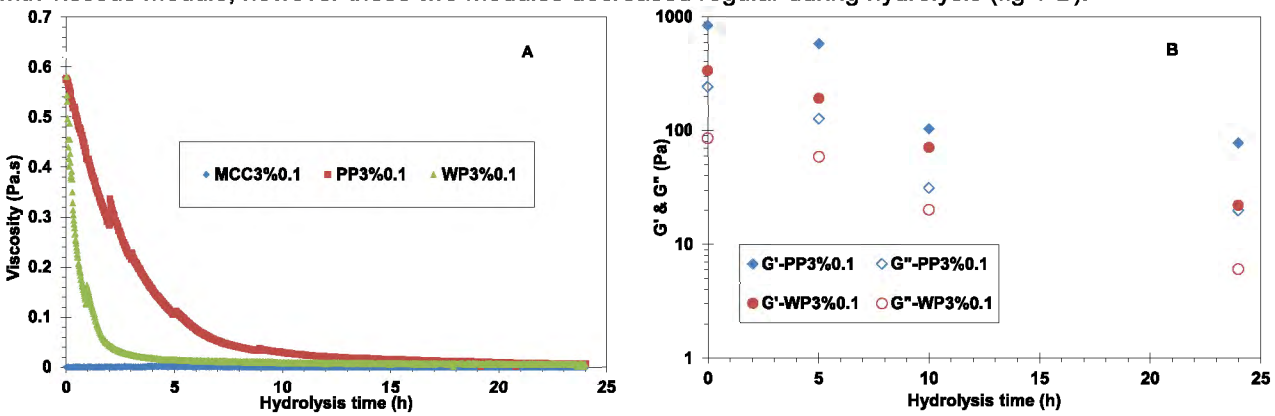


Fig. 1. Evolution of in-situ viscosity (A) and ex-situ viscous and elastic modules (B) during enzymatic hydrolysis (Suspension at 3%dm and 0.1mL enzyme/g cellulose).

*3.2. Evolution of particle size during enzymatic hydrolysis*

During hydrolysis, as the fibres were degraded, their morphology (shape, area, length…) significantly changed (Nguyen et al., 2012). The large particles were hydrolysed; their mean diameter decreased for all substrates (Fig 2-B). This led to the reduction of viscosity suspension. The hydrolysis effect was mainly observed on coarse particles. The fine population increases and translates to a smaller diameter. The same result was reported by Nguyen et al., 2013; Um. 2007; Wiman et al., 2010. However, coherence of in-situ and ex-situ data need to be explored to strengthen our understanding of particle deconstructing.

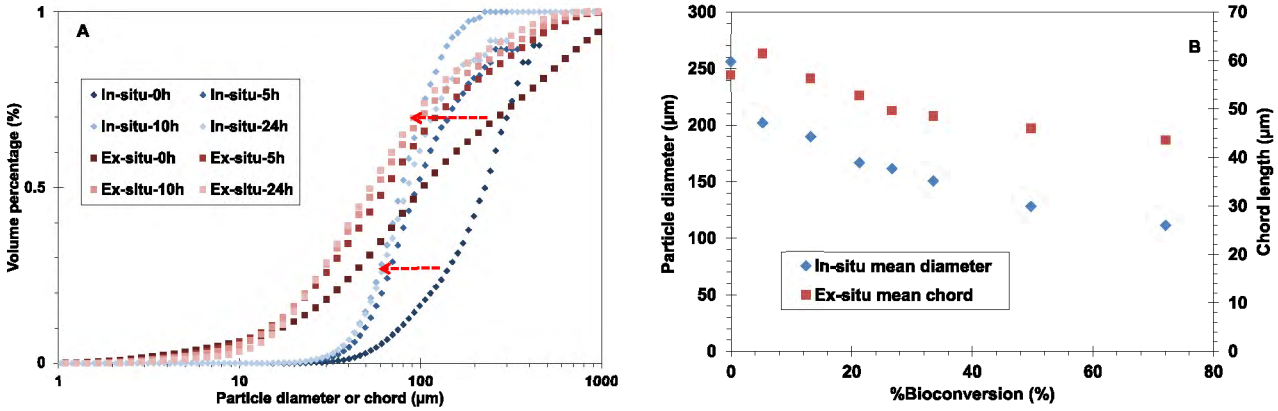


Fig. 2. Evolution of volume cumulative distribution as a function of particle diameter or chord (A) and mean chord (number weighted) and mean diameter (volume weighted) as a function of bioconversion rate (B) (Suspension of PP 3%dm, 0.5mL enzyme/g cellulose)

## 4. CONCLUSION

This study focussing on the rheometry of lignocellulosic suspensions explored enzymatic hydrolysis based on physical parameters. A method for following viscosity and particle size on-line was proposed and used to characterise the cellulose suspensions. 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.

## REFERENCES


Alvira, P., Negro, M.J., Ballesteros, M. 2011. Effect of endoxylanase and alpha-L-arabinofuranosidase supplementation on the enzymatic hydrolysis of steam exploded wheat straw. Bioresource Technology, 102(6), 4552-4558.

Dasari, R.K., Berson, R.E. 2007. The effect of particle size on hydrolysis reaction rates and rheological properties in cellulosic slurries. Applied Biochemistry and Biotechnology, 137, 289-299.

Fillaudeau, L., Babau, M., Cameleyre, X., Lombard, E., Anne-Archard, D. 2011. Libération de substrats fermentescibles à partir de matrices lignocellulosiques issues de l'industrie papetière. Récents Progrès en Génie des Procédés, 101.

Geddes, C.C., Peterson, J.J., Mullinnix, M.T., Svoronos, S.A., Shanmugam, K.T., Ingram, L.O. 2010. Optimizing cellulase usage for improved mixing and rheological properties of acid-pretreated sugarcane bagasse. Bioresource Technology, 101(23), 9128-9136.

Nguyen, T.-C., Anne-Archard, D., Coma, V., Cameleyre, X., Lombard, E., Binet, C., Nouhen, A., To, K.A., Fillaudeau, L. 2013. In situ rheometry of concentrated cellulose fibre suspensions and relationships with enzymatic hydrolysis. Bioresource Technology, 133(0), 563-572.

Nguyen, T.C., Anne-Archard, D., Cameleyre, X., Lombard, E., Binet, C., Fillaudeau, L. 2012. Production of fermentescible sugar from paper-pulp: Looking for a dynamique and multiscale integrated models based on physical parameters. in: 8th International Conference on Renewable Resources and Biorefineries Toulouse, France, pp. P62.

Pereira, L.T.C., Pereira, L.T.C., Teixeira, R.S.S., Bon, E.P.D., Freitas, S.P. 2011. Sugarcane bagasse enzymatic hydrolysis: rheological data as criteria for impeller selection. Journal Of Industrial Microbiology & Biotechnology, 38(8), 901-907.

Szijarto, N., Siika-aho, M., Sontag-Strohm, T., Viikari, L. 2011. Liquefaction of hydrothermally pretreated wheat straw at high-solids content by purified Trichoderma enzymes. Bioresource Technology, 102(2), 1968-1974.

Um, B.-H. 2007. Optimization of Ethanol Production from Concentrated Substrate, Auburn University, pp. 268.

Wiman, M., Palmqvist, B., Tornberg, E., Liden, G. 2010. Rheological Characterization of Dilute Acid Pretreated Softwood. Biotechnology And Bioengineering, 108(5), 1031-1041.