



## Preliminary Studies on the Production of Nanofibrils of Cellulose from Never Dried Cotton, using Eco-friendly Enzymatic Hydrolysis and High-energy Sonication

E. Bittencourt <sup>a</sup>, M. de Camargo <sup>b</sup>

a. *Universidade Estadual de Campinas, Campinas, SP, e\_bittencourt@uol.com.br*

b. *Universidade Estadual de Campinas, Campinas, SP, marcelo.camargo@intertek.com*

---

### Abstract

An ecologically friendly method, to obtain cellulose nanofibrils, starting from Never Dried Cotton (NDC) is described, where cotton bowls are opened and maintained in water. NDC cotton exhibits a highly accessible structure and porosity, thus allowing a more efficient enzyme action and chemical treatments and derivatization. In this work, the conditions utilized to synthesize nano-fibrils from NDC were also tested on once dried cotton; the latter failed to produce nano-fibrils when submitted to the experimental conditions applied. A first-drying of cotton fibers results in a structure characterized by a collapse of the NDC fiber structure, which change from a circular cross section to its typical “bean-like” cross section, with reduced accessibility and porosity, and lower water sorption capacity. Those changes are of the same nature as the well known *hornification* described in pulp and paper science studies, associated with irreversible reduced accessibility, which affects paper properties, and in general, the utilization of cellulose for utilization as materials or fuel (i.e. alcohol). In this work, enzymatic hydrolysis of the fibers was followed the by high energy sonication for 20 to 50 minutes, resulting in the production of nanofibrils when using NDC. Similar treatment applied to once-dried cotton failed to produce nanofibrils. Although analysis of films made from hydrolyzed and sonicated NDC material, with scanning electronic microscopy, disclosed micro-fibers lengths of approximately 30  $\mu\text{m}$ , and some nano-scale structures, only with Transmission Electron Microscopy was possible to confirm the presence of nanofibrils, Structures with 50 nm in diameter, were present after submitting the NDC to enzymatic hydrolysis, and high-energy sonication. Both processes are considered eco-friendly: enzymatic hydrolysis and, especially, high energy sonication which is gaining impressive industrial utilization in the last decade.

**Keywords:** *Never -Dried Cotton, Cellulose, nanofibrils, ultrasound, hydrolysis*

---

### 1 Introduction

In the excellent paper “Cellulosic Nanocomposites: a review”, Hubbe et al. (2008) point out the increasing interest in the study of nano and micro fibrillar structures that can be produced from different sources of cellulosic materials such as wood; lignocellulosic fibers; cellulose containing residues; and bacterial cellulose, motivated in part by its potential utilization in nanocomposites. Keeping cellulose materials in the never-dried state has important advantages: vapor saturated wood chips, for instance, require half the time of impregnation with sodium hydroxide solution compared to the once dried material as pointed out by Zanuttini et al.

(2005). According to Rajinder (2001), energy is required to dry pulps, but the drying process stiffens fibers, in a way that refining restores only a portion of the fiber original conformability. Therefore, pulps should only be dried if storage, transport, or use of wet pulps is uneconomical or impractical. Drying is a critical process affecting the properties of cellulosic materials, and consequently affects negatively the efficiency of processes intended to modify and utilize lignocellulosic yield of biomass products, due to the reduced accessibility resulting from removal of the water initially present. According to Imai et al. (2004), "The annual net yield of photosynthesis is 1.8 trillion tonnes of biodegradable substances, about 40% of which is estimated to be cellulose". However, only a small fraction of available cellulosic resources is currently utilized in products such as fuel, textiles, paper, and plastics. There is a large potential to increase the efficiency in the utilization of the cellulose containing biomass, an objective which demands increasing our knowledge on the nature of the drying process of the fibrous materials, in order to obtain the desired fibrillar structures, and maximize accessibility, to increase productivity. In order to study never dried cellulosic materials, and evaluate the behavior of the never dried systems, NDC was investigated with respect to its response to a process which combined enzymatic hydrolysis and high energy sonication. As mentioned below, NDC has been studied earlier, but as of the time this work was conducted, never as a material to produce nano-fibrils. NDC has a strong potential to shed light in the drying process of cotton cellulose, and on the drying of more complex cellulosic materials, with regards to composition, containing large amounts of lignin.

Mild enzymatic hydrolysis, an ecologically friendly process, combined with mechanical shearing, utilizing cellulose I from never dried wood chips, has been used to produce microfibrilated cellulose (MFC), in a process described by Pääkö et al (2007). Alternatives to produce microfibrils or microfibrils aggregates are, as mentioned by Pääkö et al (2007), dissolution in solvents, regeneration, and even potentially, electrospinning. Ultrasonic pretreatment and ultrasonically assisted hydrolysis of biomass has also been used to convert lignocellulosic materials to ethanol by Toma et al. (2006), who observed that saccharification of cellulose was enhanced by ultrasonic pretreatment.

NDC was studied in the 70's, with respect to water content, morphology and transport properties, internal mobility, mechanical properties, pore volume, and fixation of the never-dried state (Peterlin and Ingram. 1970; Ingram et al 1974; Williams et al 1974; Fahmy and Mobarak 1971, a and b; Fahmy and Mobarak 1972; Fahmy and Mobarak, 1976) To the best of our knowledge, NDC was never studied as a source of nanofibrils. It should be pointed out that this work is intended to contribute to the general effort of the utilization of cellulosic biomass, considering the potential elucidation potential of the drying mechanism of a fiber with very high cellulose content, being easily collected and kept in the wet state, in order to avoid the irreversible changes which occur during the first drying. Never drying permits the treatment of the cellulosic material in a more open and accessible state. Quite importantly, it is very relevant to point out the utilization in this work of eco-friendly processes such as enzymatic hydrolysis, and high energy sonication. This latter technology has gained a significant advance as far as its use in industrial processes which include sectors like the food industry, pharmaceuticals, biodiesel production, and other manufacturing areas, such as textiles, paint manufacture. Several examples of industrial scale applications are pointed out by Gallego-Juarez (2010). Large scale environmental applications of high-power sonication are also described by Collins et al. (2010).

## 2 Experimental

A detailed description of the experimental procedures utilized in this work can be found in De Camargo (2010). The cotton bowls were collected approximately a week before expected opening, and the fibers were manually removed from seeds inside water. An alkaline and bleaching step was applied to the fibers to remove lignin, hemicelluloses, pectin, and wax. This treatment consisted of 10 ml/l of sodium hydroxide solution, 38° Bé, 2 g/l of a nonionic detergent (Kieralon, produced by BASF), 8 ml/l hydrogen peroxide 200%, and 0.5 g/l of sodium silicate. NDC cotton, once dried cotton, and viscose were separately immersed in the solutions (20 ml of solution per gram of fiber), and this system was taken to boiling for 15 min. The fibers were then washed with distilled water, neutralized with acetic acid, and washed again until achieving neutrality. This treatment was followed by the enzymatic hydrolysis step, utilizing 30 g/l of a cellulose enzyme, Cellusoft L (produced by Novozymes), with the pH kept between 5.0 and 5.5, with time of exposure of 48 hours, at 55° C. Once-dried cotton and viscose fibers were submitted to the same treatments as NDC for comparison. The once dried cotton fibers were not affected by this enzymatic treatment to the point of producing nano-fibers. After the enzymatic treatment, the fibers were taken to the sonication procedure. The sonication equipment utilized were ultrasonic model UP 400S and UIP 1000, made kindly available by Hielscher Ultrasonics (Brazil Office), respectively, with potencies of 400W and 1000W, and with a frequency of 20 KHz. 60 g of fibers (NDC, was in wet state), were dispersed in 500 ml of water. This dispersions were sonicated both in the 400W and the 1000W equipment. The material for characterization was collected from the supernatant layer after the sonication step. The effective effect of the enzymatic hydrolysis followed by sonication in producing nanofibrils was ultimately verified by transmission electron microscopy (TEM), kindly conducted by Professor Juan P Hinestroza in Cornell University

### 3 Results

Figure 1 and Figure 2 below, were obtained with transmission electron microscopy, and illustrate the successful synthesis of the nanofibrils (in the form of whiskers), resulting from enzymatic hydrolysis followed by high energy sonication of the NDC samples.

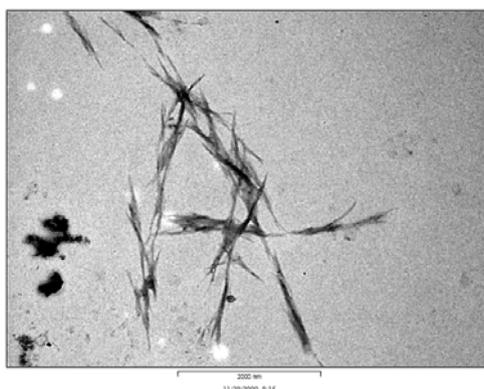


Figure1 – Transmission electron microscopy view of NDC submitted to enzymatic hydrolysis, sonicated for 20 minutes, potency 400 W

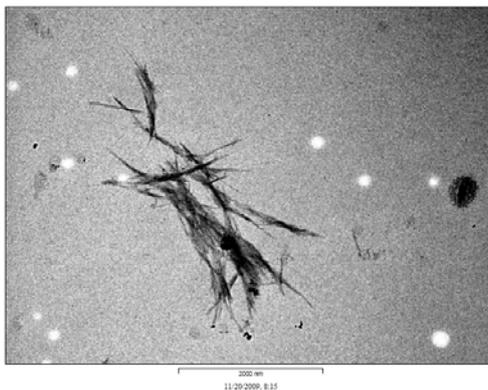


Figure 2 – Transmission electron microscopy view of NDC submitted to enzymatic hydrolysis, sonicated for 50 minutes, potency 400W

Figure 3 below, was obtained with SEM examination of a film of viscose, submitted to the enzymatic hydrolysis, followed by sonication.

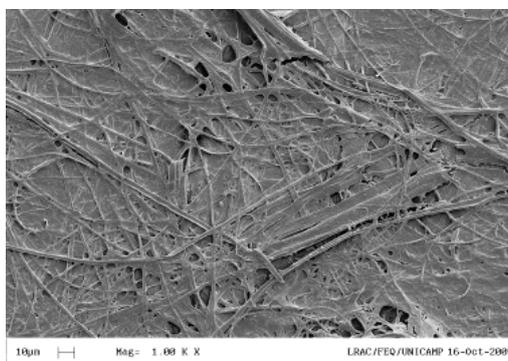


Figure 3. Scanning electron microscopy of viscose films after enzymatic hydrolysis sonicated for 20 minutes, potency 400W

It was observed that 20 minutes sonication, with the 400 W sonication equipment after enzymatic hydrolysis, was enough to obtain MFC with the typical cellulose whisker-like MFC resulting from other investigations, as recently reviewed (Samir et al, 2005). Once dried cotton submitted to the same treatment did not produce the whiskerlike nanofibrils. Viscose fibers films examined by SEM proved that viscose fibers respond to the treatments. Viscose fibers, essentially Cellulose II, are more accessible than once-dried cotton. Viscose fibers remain to be investigated in future work. Future work is also intended, to study other fibrous materials, including the ones originated from the Amazon region, as a result of our ongoing collaboration with the Universidade Federal do Amazonas, centered at discovering, investigating, and utilizing new and structurally unknown, or not well characterized fibrous materials from the Amazon region.

#### 4 Conclusions

As pointed out by Kim and Dale (2004), Brazil and the US are responsible for 62% of the production of ethanol in the world, with sugar cane as the major feedstock in Brazil and corn the major feedstock in the US, authors point out that this technology is still under development, "Another potential resource for ethanol is lignocellulosic biomass, which includes materials such as agricultural residues (i.e., corn stover, crop straw, sugar cane bagasse), herbaceous crops (i.e., alfalfa, switchgrass), forestry wastes, wastepaper, and other wastes". Furthermore, Kim and Dale (2004) add that "Wasted sugar cane and sugar cane bagasse could

produce globally about 53 GL of bioethanol, replacing 38 GL of gasoline in an E85 midsize passenger vehicle, or about 3.4% of the global gasoline consumption. Asia can produce about 22 GL of bioethanol." Utilization of wasted or inadequate used biomass for fuel or materials, especially those chosen among those not competing with food crops, motivates an intense effort to develop scientific and technology knowledge and expertise on efficient utilization of lignocellulosic sources from the biomass. Hydrolysis, acid and enzymatic (eco-friendly), sonication, high energy homogenizers, can be combined to improve processes destined to utilize lignocellulosic materials, in more efficient processes. An innovative treatment, vaccination of biological cellulose fibers with glucose (Fahmy and Mobarak, 2008) has been recently developed to maintain the porous structure of never dried cellulose, keeping its super-absorbent properties and high accessibility of cellulose fibers, as suggested by an early work of Higgins and McKenzie (1963) this vaccination has high potential as an important technique to preserve the accessibility of lignocellulosic materials to chemical and enzymatic modification, as necessary for efficient utilization, either as a source of energy or material. NDC, as mentioned before in this paper, has been studied since the early 70's. In this work we have synthesized nanofibril structures starting with NDC, under conditions which do not produce the nanofibrils if applied to once dried cotton. Although much work remains to be done, to the best of our knowledge, this is the first time NDC has been studied with respect to the synthesis of nanofibrils. Furthermore, the combination of enzymatic hydrolysis and high-energy sonication, consist of two eco-friendly processes that have gained increasing studies and utilization, including in the more efficient, and cleaner utilization of the biomass.

## 5 References

- Collins, A. F., Gwan, P. B., Sosa-Pintos, A. P., 2010, Large scale environmental applications of high power ultrasound, *Ultrasonics Sonochemistry* 17, 1049–1053
- De Camargo, M., 2010, Desenvolvimento de processo de produção de nano fibrilas de celulose a partir do algodão nunca seco, Tese de mestrado, Departamento de Tecnologia de Polímeros, Faculdade de Engenharia Química, Universidade Estadual de Campinas.
- Fahmy Y., Mobarak F., 1971a, On fine structure of cellulose fibers. *Svensk Papperstidn*, 74(I), 2–9.
- Fahmy Y., Mobarak F., 1971b, Fine structure and reactivity of cellulose. *J Polym. Sci., Part B, Lett.* 9(10), 767–769.
- Fahmy Y., Mobarak F., 1972, Reactivity of biological cellulose and properties of some of its derivatives. *Cellulose Chem Technol* 6:61–65.
- Fahmy Y., Mobarak F., 1976, On the properties of never-dried and nature-dried cotton, *Cellulose Chem Tech Technol*, 10, 261–264.
- Fahmy T. Y. A., Mobarak F., 2008, Vaccination of biological cellulose fibers with glucose: A gateway to novel nanocomposites. *International Journal of Biological Macromolecules*, 42, 52-54.
- Gallego-Juarez, J. A., 2010, High-power ultrasonic processing: recent developments and prospective advances, *International Congress on Ultrasonics*, Santiago de Chile, January 2009, *Physics Procedia*, 35-47.
- Higgins H. G., McKenzie H. W., 1963, The structure and properties of paper. XIV. Effect of drying on cellulose fibers and the problem of maintaining pulp strength, *Appita*, 16 (6), 145-164.

Hubbe, M. A., Rojas, O. J., Lucia L. A., 2008, Sain M., Cellulosic nanocomposites: A review, 2008, *Bioresources*, 3(3), 929-980.

Imai M., Ikari K., Suzuki I, 2004, High-performance hydrolysis of cellulose using mixed cellulase species and ultrasonication pretreatment, *Biochemical Engineering Journal* 17 , 79–83.

Ingram P., Woods D. K, Peterlin A., Williams J. L., 1974, Never-Dried Cotton Fibers: Part I : Morphology and Transport Properties, *Textile Research Journal* ; 44; 370.

Kim S., Dale B. E., 2004, Global potential bioethanol production from wasted crops and crop residues, *Biomass and Bioenergy*, 26, 361– 375.

Pääkkö M., Ankeforts M., Kosonen H., Nikänem A., Ahola S., Österbertg M., Ruokolainen J., Laine J., Larson P. T., Ikkala O ., Lindström T., 2007, Enzymatic hydrolysis Combined with mechanical shearing and high pressure homogenization for Nanoscale and strong gels, *Biomacromolecules*, 8, 1934-1941.

Peterlin A., Ingram P., 1970, *Morphology of Secondary Wall Fibrils in Cotton*, *Textile Research Journal*, 1970; 40; 345.

Rajinder S. S., 2001, The difference between never-dried and dried chemical pulps. *Solutions! Pulp Properties*, September, Vol. 1 (1).

Said M.A., Samir A., Alloin F., Dufresne A., 2005, Review of recent research into cellulosic whiskers, their properties and theirs application in nanocomposite field, *Biomacromolecules*, 6, 612-626.

Williams J. L., Ingram P., Peterlin A., Woods D. K., 1974, Never-dried cotton fibers. Part II: fixation of the never dried state , *Textile Research Journal* , 44, 370.

Zanuttini M., Citroni M., Marzocci V., Inalbon C., 2005, Alkali impregnation of hardwood chips , *TAPPI Journal*, February, 28-30