

Modulation of Acid Hydrolysis Reaction Time for the Extraction of Cellulose Nanocrystals from *Posidonia oceanica* Leaves

F. Luzi, E. Fortunati*, D. Puglia, R. Petrucci, J.M. Kenny and L. Torre

University of Perugia, Civil and Environmental Engineering Department, UdR INSTM, Strada di Pentima 4, 05100 Terni, Italy

Received November 20, 2015; Accepted January 21, 2016

ABSTRACT: In this research, the revalorization of *Posidonia oceanica* leaf sea waste was studied and the acid hydrolysis processing times were modulated in order to optimize the extraction of cellulose nanocrystals (CNCs). The obtained CNCs were deeply investigated. A two-step treatment was applied to extract cellulose nanocrystals from *Posidonia oceanica* leaves. First, a chemical treatment leads to the removal of lignin and production of holocellulose, while the second chemical process of acid hydrolysis allows the obtainment of cellulose nanocrystals in aqueous suspension. The unbleached and bleached leaves and cellulose nanocrystals were characterized by using thermogravimetric analysis, infrared spectroscopy and morphological investigation; the birefringence properties were also studied in order to determine the efficiency of the acid hydrolysis treatment. Cellulose nanocrystals were successfully obtained from *Posidonia oceanica* leaves and they showed a monocrystalline rod-shaped acicular structure with a 5–10 nm diameter and 200–450 nm length.

KEYWORDS: Marine waste revalorization, *Posidonia oceanica*, leaves, cellulose nanocrystals, hydrolysis

1 INTRODUCTION

In the last few decades, the revalorization of cellulosic resources, agro-forest and marine wastes as valid substitutes for traditional materials have attracted the interest of academic researchers. *Posidonia oceanica* is a sea grass plant in the Mediterranean Sea composed of balls and leaves; important quantities of this plant are accumulated on the coast, generating sea waste on the touristic coastal beaches, and should be removed every year. For these reasons, the attention of the scientific community is now oriented to the revalorization of marine wastes such as *Cladophorales* algae and *Posidonia oceanica*. Green filamentous of *Cladophorales* algae were studied to prepare high-tech composite materials [1]. The fibers of *Posidonia oceanica* balls have been analyzed as reinforcement in composites based on traditional polymers [2] or based on biodegradable polymers such as gluten protein [3] or as cellulosic nanoreinforcements in poly(lactic acid) (PLA) matrix [4]. Recently, the rheological behavior of cellulose

nanofiber suspensions from *Posidonia oceanica* as a function of the oxidation treatment was also studied [5].

The chemical composition of *Posidonia oceanica* leaves was previously determined according to the standard Tappi methods that attested to a high amount of extractives (19.2%), 31.4% of α -cellulose, 57.1% of holocellulose, 10.5% of ashes and 29.3% of lignin [6]. The amount of cellulose in balls is quite a bit higher (40.0%) [6, 7] with respect to leaves (31.4%), determining a possible different amount in the cellulose nanocrystal extraction yield [6].

Cellulose is the most abundant green polymer on Earth and it has been widely used as a reinforcing element in thermoplastic polymers to modulate some functional properties [8]. It is characterized by high-molecular-weight linear syndiotactic homopolymer constituted of β -d-glucopyranosyl units joined by 1–4 glycosidic linkages in different arrangements [9, 10]. The microfibrils and nanofibrils are characterized by a highly crystalline structure arranged into lattices within the cell wall; moreover, crystalline cellulose materials are resistant to reagents, acid attacks and are insoluble in water [11, 12]. In this context, cellulose nanocrystals (CNCs), extracted from natural cellulosic-based materials, are usually characterized

*Corresponding author: elena.fortunati@unipg.it

by rigid rod monocrystalline domains with a large specific surface area, with diameters ranging from 1–100 nm and from 10–500 nm in length [13], while the nanocrystal aspect ratio (diameter/length) can vary from 1:1 to 1:100 [14, 15]. The dimensions of the CNCs depend on the raw material utilized for their extraction [16], agronomic and cultivar factors as plant maturity, fertilized used [17], soil characteristics and the intensity of the chemical process used for their extraction [18]. The CNCs have a crystalline structure [19], a very high elastic modulus of around 150 GPa [20], and a low coefficient of thermal expansion [21]. Cellulose nanocrystals have attracted scientific research due to some specific advantages: they are, in fact, natural renewable resources, very abundant, and have a low density (1.566 g/cm³), and are therefore low cost [22]; moreover, cellulose is considered nontoxic, biocompatible and biodegradable [16]. The nano-dimensions of CNCs have some superior properties compared to cellulose, such as aspect ratio, optical properties and mechanical strength [8, 23]. The effect of the acid hydrolysis conditions was previously investigated by modulating the hydrolysis parameters (temperature: 45 °C and 60 °C; and time of reaction: 30, 60, and 75 min) to extract cellulose nanocrystals from sisal fibers as a strategy to obtain higher crystallinity at the higher temperature for a lower amount of time [24]. Both Dong *et al.* and Teodoro *et al.* [24, 25] observed that the dimensions of CNCs decreased, increasing the time of reaction.

Furthermore, cellulose nanocrystals can be easily chemically grafted by the presence of reactive –OH side groups, thereby obtaining different surface properties [26]; and this aspect encourages their use as reinforcement phase in polymer-based formulation for final practical applications [21, 27–29].

The objective of the present research is focused on the study of the CNC extraction procedure from *Posidonia oceanica* leaves by modulating the hydrolysis parameters (time of reaction). As previously reported in the literature, the dimensions of CNCs decrease, increasing the time of reaction for maintaining a fixed procedure temperature [24, 25], while an increase in the reaction time should lead to improve the quantities of reacted material, also increasing the reaction yield.

For this reason, two different acid hydrolysis procedures were proposed and studied in the present work, with the idea of extracting cellulose nanocrystals from bleached *Posidonia oceanica* leaves, increasing the reaction time and modulating the final properties of the obtained cellulosic materials. A two-step procedure was applied to extract CNCs: an initial chemical treatment led to the removal of lignin and production of holocellulose, while the following acid hydrolysis

allowed the obtainment of cellulose nanocrystals in aqueous suspension. Thermal, chemical and morphological characterizations were applied to characterize both unbleached and bleached leaves and to demonstrate the successful extraction of CNCs.

2 EXPERIMENTAL SECTION

2.1 Materials

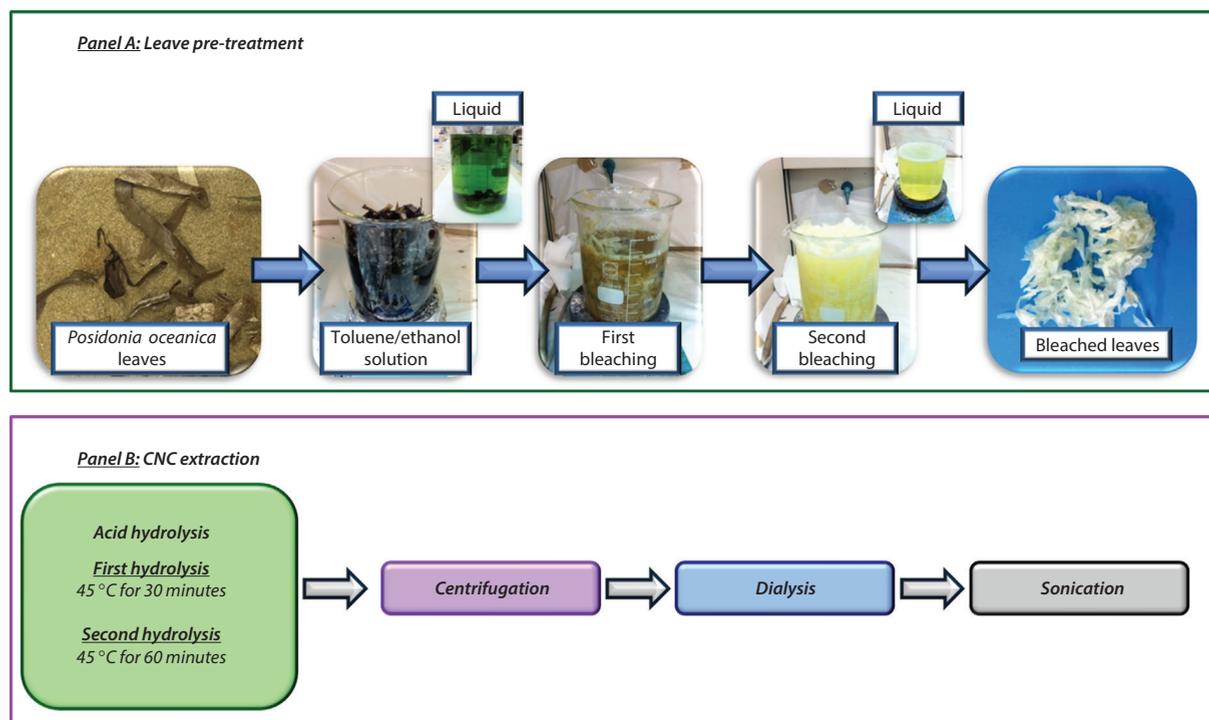
Posidonia oceanica leaves were collected from the Campello Beach in Alicante (Spain) by AITEX (Alcoy, Alicante, Spain).

Toluene, ethanol, sodium hydroxide (NaOH, reagent grade ≥ 98%), sodium chlorite (NaClO₂, puriss p.a. 80%), acetic acid (CH₃COOH) and sodium bisulfate (NaHSO₄, purum, anhydrous, ~95.0%) were supplied by Sigma-Aldrich and used for the chemical pretreatment, as specified in Section 2.2.

Sulphuric acid (reagent 95–98%), supplied by Sigma-Aldrich, was used as reagent during the hydrolysis treatment, as specified in Section 2.3.

2.2 Chemical Pretreatment of *Posidonia Oceanica* Leaves

Posidonia oceanica leaves were washed and rinsed several times in distilled water in order to eliminate sand and other soil contaminants; after that they were dried in an oven at 80 °C for 24 h. Scheme 1, Panel A, shows a scheme of the pretreatment procedure for *Posidonia oceanica* leaves. The pristine leaves (Scheme 1, Panel A) were chopped into 5–10 mm fragments and a de-waxing step in a toluene/ethanol mixture (2:1 volume/volume) for 6 h was carried out, followed by filtration and washing with ethanol for 30 min. The green color of the solution was due to the fact that ethanol acted as a detergent/solvent, breaking down the phospholipid bilayer and opening holes in the membrane, making it permeable and promoting the elimination of the chloroplasts [30]. Two bleaching treatments were applied for cellulose extraction. The fibers were first treated with a 0.7% (wt/v) of sodium chlorite (NaClO₂) solution, then were boiled for 2 h (fiber/liquor ratio 1:50). Thereafter, the pH of the solution was lowered to ca. 4 by adding acetic acid (CH₃COOH). As it is possible to observe in Scheme 1, the final obtained solution at the end of the first bleaching treatment appeared completely saturated by the released lignin. For this reason, a second bleaching treatment was applied. The second bleaching was necessary to obtain a complete whitening of the leaves. Then, a 5% (wt/v) of sodium bisulphate (NaHSO₄) solution treatment was carried out [22, 31]



Scheme 1 (Panel A) Scheme of *Posidonia oceanica* leaves pretreatment. (Panel B) CNC step extraction.

and, finally, holocellulose (α -cellulose + hemicellulose) was obtained. The holocellulose was then treated with a 17.5% (wt/v) of sodium hydroxide (NaOH) solution. After filtration and washing, the obtained material was dried at 60 °C in an air-circulating oven.

2.3 Extraction of Cellulose Nanocrystals from *Posidonia Oceanica* Leaves

Cellulose nanocrystals (CNCs) in aqueous suspension were prepared from pretreated *Posidonia oceanica* leaves by sulphuric acid hydrolysis. Scheme 1, Panel B, shows a schematic representation of the procedure used to extract cellulose nanocrystals. Two different acid hydrolysis procedures were carried out using 64 wt/wt % sulphuric acid at 45 °C for 30 min (first hydrolysis approach) [18] and at 45 °C for 60 min (second hydrolysis approach) under vigorous stirring, using for both of the two hydrolyses a cellulose/acid ratio of 0.22 g mL⁻¹ (wt/v). The second acid hydrolysis parameters (45 °C for 60 min) were set after the morphological study of cellulose materials obtained by the first applied procedure (45 °C for 30 min).

After the hydrolysis, the obtained suspensions were diluted 20 fold with deionized water. The aqueous suspensions were centrifuged to remove the acid and dialyzed with cellulose dialysis membranes for 5 days in deionized water until neutral pH was obtained. An

ion exchange resin (Dowex® Marathon™ MR-3 hydrogen and hydroxide form) was added to the cellulose suspensions for 24 h and then removed by filtration. Finally, the cellulosic suspensions were sonicated in an ice bath with ultrasonic treatment (Vibracell 75043, 750W, Bioblock Scientific) to create cellulose nanocrystals of colloidal dimensions. The final obtained solution was neutralized by the addition of 1.0% (wt/wt) of 0.25 mol L⁻¹ NaOH.

2.4 Characterization of Bleached Fibers and Cellulose Nanocrystals

The morphology of the unbleached and bleached *Posidonia oceanica* leaves was investigated by means of field emission scanning electron microscopy (FESEM, Supra 25-Zeiss). The leaves, before and after the bleaching, were gold sputtered and analyzed.

A morphological study of the obtained CNC-based solution was also conducted by FESEM. A few drops of CNC suspension, obtained both after the first and second hydrolysis procedures, were cast onto silicon substrate, vacuum dried and gold sputtered before the analysis. The FESEM micrographs of untreated leaves and CNC were analyzed with NIS-Elements BR (Nikon) software and sixty measurements were taken in order to obtain the values of the wall thickness, the lumen width of the cell element and the diameter and the length of cellulosic nanostructures.

Moreover, the birefringence property of cellulose nanocrystal suspension at 0.4% (wt/wt) extracted after the second hydrolysis procedure was detected in a dark box between crossed polars. The pictures were taken using a Nikon D 7000 digital camera.

The reaction yield and dry content, after the hydrolysis process, were calculated as % (of initial weight) of used pretreated leaves and percentage of obtained CNCs with respect to the water. Specifically, the solutions obtained after the extraction procedures (2 mL, five measurements for each solution) were maintained at 105 °C for 2 h, leading to the evaporation of liquid phase. Residues were weighed with an analytical balance (Sartorius ATILON) with ± 0.01 mg precision and the dry content, expressed as percentage with respect to the liquid phase. The yield (%) was calculated as $\text{CNC}_{\text{weight}} / \text{Pre-treated leaves}_{\text{weight}}$.

Thermogravimetric analysis (TGA) measurements of unbleached and bleached *Posidonia oceanica* leaves and CNCs were performed by a Seiko Exstar 6300 TG analyzer with heating scans from 30 to 600 °C at 10 °C min⁻¹ in a nitrogen atmosphere (250 mL min⁻¹).

Fourier infrared (FT-IR) spectra of unbleached, bleached leaves and CNCs were recorded by a Jasco FT-IR 615 instrument in the 400–4000 cm⁻¹ range, in attenuated reflection mode (ATR).

3 RESULTS AND DISCUSSION

3.1 Morphological Investigation of Unbleached and Bleached Leaves

The morphological characteristics of unbleached and bleached leaves were investigated by FESEM and are shown in Figure 1 at different magnifications.

It can be seen in Figure 1a,b that the internal structure of unbleached leaves is composed of vascular tissue, epidermis and parenchyma (cell element). The different elements of the internal structure are determinant in many functions of the plant growth such as photosynthesis, tissue regeneration, synthesis and processing of substances. The cell elements of untreated structures have wall thickness ranging between 1–4 μm , depending on the quantity of lignin, ash and hemicelluloses, while the lumen width ranges between 8–20 μm [32, 33]. The inset in Figure 1b shows the presence of calcium carbonate on the surface of untreated leaf, as previously observed by other authors [34]. Figure 1c,d shows the morphological aspect of bleached leaves. The dimensions of the cell wall element and the lumen width decrease as a consequence of the chemical pretreatment being able to remove the lignin, hemicelluloses and ashes. After the chemical pretreatment, the cell elements are no longer observable and the structure appears collapsed in on itself.

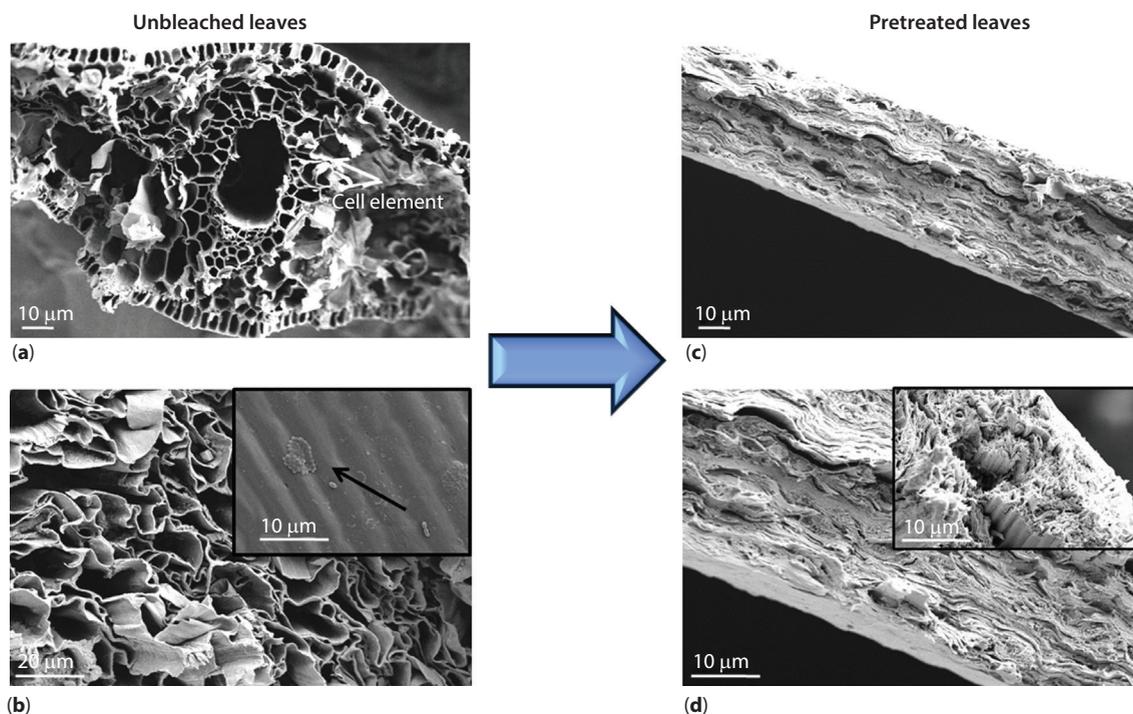


Figure 1 Morphological characterization of fractured unbleached (a, b) (FESEM investigation of leaf surface in inset b) and bleached leaves (c, d) at two different magnifications.

3.2 Acid Hydrolysis Procedures: Morphological and Optical Investigations

Figure 2 shows the morphological investigation of CNC obtained after the first (45 °C, 30 min) and second (45 °C, 60 min) hydrolysis procedures. The choice of the hydrolysis parameters is considered very important in order to modulate the dimensions of CNCs extracted from different natural sources [24, 25].

The FESEM images (Figure 2a,b) at two different magnifications of the first applied hydrolysis procedure show the presence of some unreacted leaf residues, characterized by different shapes and aggregation levels, as indicated by the arrows. The presence of these residues underlined the inefficacy of the applied first hydrolysis that also showed a very low reaction yield (around 8%). However, the higher magnifications revealed the presence of some individualized cellulose nanocrystals that presented an acicular structure characterized by a 15–20 nm diameter and 500–650 nm length.

On the basis of the results obtained for the first hydrolysis procedure, a second treatment with a longer reaction time was applied and this selection was done in order to guarantee the complete reaction process and to obtain cellulosic materials at a

well-defined nanoscale level [25]. Figure 2c,d show the results after the second hydrolysis. The FESEM images underlined the reduced presence of unreacted material, underlining the effectiveness of the new hydrolysis procedure (increased yield, about 15%). The second applied hydrolysis procedure (45 °C and 60 min) guarantees a complete reaction of the material with a consequent higher yield. Morphological investigations confirm that aqueous suspension contains individual cellulose nanocrystals with an acicular structure of 5–10 nm diameter and 200–450 nm length with a consequent aspect/ratio (length/diameter) of 40–45. In a previous work, we proved that the aspect ratio of CNCs extracted from *Posidonia oceanica* balls is 36.7, (180 ± 28) nm in length and diameter of (4.9 ± 1.3) nm [4]. The dimensions obtained for CNCs extracted from leaves are higher with respect to CNCs extracted from balls, as previously reported by other authors [6]; moreover, the higher aspect ratio of CNCs obtained with the second hydrolysis procedure suggests a possible promising interaction between nanofiller and polymer used in nanocomposite applications.

Finally, the aqueous suspension derived from the second hydrolysis procedure has been shown to exhibit the typical shear-induced birefringence of CNCs (Figure 2d – inset), highlighting their ability to form a chiral nematic liquid crystalline phase in equilibrium

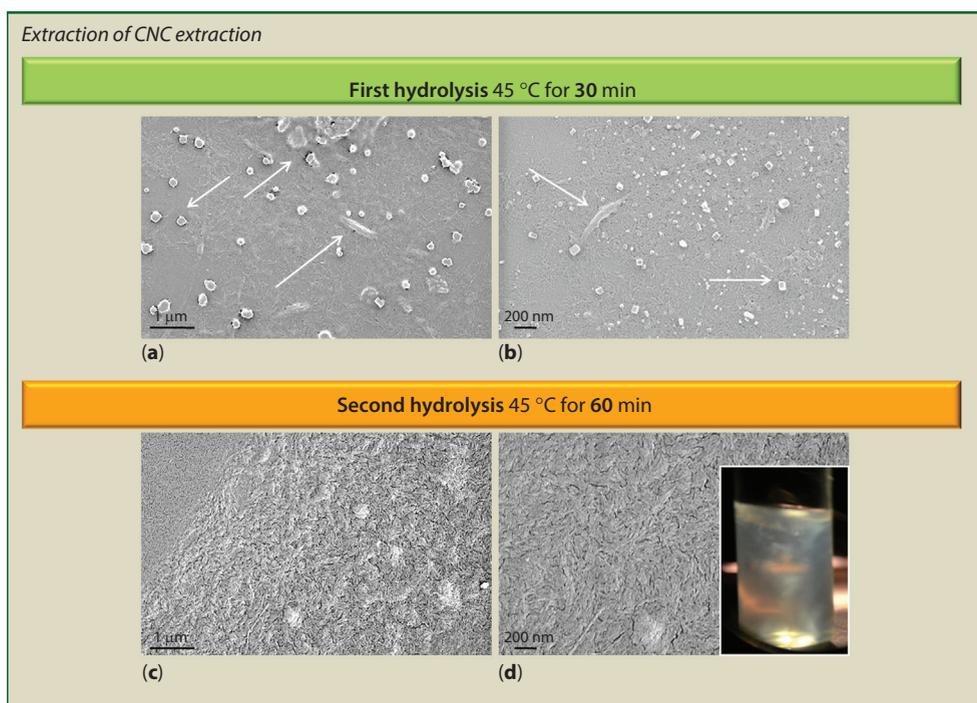


Figure 2 FESEM investigation of CNCs after acid hydrolysis at 45 °C for 30 min and at 45 °C for 60 min at two different magnifications. The inset in (d) shows the shear-induced polychromatic birefringence of cellulose nanocrystals in aqueous solution.

with the isotropic phase [35]. Birefringence is an important optical property of a material having a refractive index and gives an indication of well-dispersed nanocrystals obtained by sulphuric acid hydrolysis.

On the basis of the results obtained by the two different extraction procedures, the second hydrolysis was selected as the best process to be applied, and the CNCs derived from 60 min of treatment were chemically and thermally characterized.

3.3 Thermal and Spectroscopic Analysis of *Posidonia Oceanica* Fibers and Cellulose Nanocrystals

Thermal properties of unbleached and bleached leaves of *Posidonia oceanica* and hydrolyzed cellulose nanocrystals were investigated by thermogravimetric analysis, while the chemical properties were assessed by infrared spectroscopy in ATR mode.

Figure 3 shows the weight loss (TG, a) and their derivative (DTG, b) curves of unbleached leaves, bleached leaves and hydrolyzed cellulose nanocrystals obtained from *Posidonia oceanica* leaves. As already observed in the literature, the natural fibers show a multistep thermal degradation. The thermal degradation of the unbleached and bleached materials shows several stages, indicating the presence of different components that decompose at different temperatures. The first step of the thermal degradation of the unbleached, bleached and hydrolyzed materials is characterized by moisture and volatile removal at a temperature below 100 °C [2]. The second peak of degradation at around 255 °C was attributed to hemicellulose degradation, which disappears completely in the case of CNC (Figure 3b). The hemicellulose decomposes before

lignin and cellulose and the lower thermal stability of hemicellulose is due to the presence of acetyl groups [36]. The main third peak of degradation reaches its peak at 333 °C for the unbleached fibers, at 320 °C for the bleached cellulose and at 344 °C for the hydrolyzed specimen of nanocellulose. The higher thermal stability of CNC with respect to bleached cellulose materials was previously observed [37]. The last peak centered at 450 °C for the unbleached materials is due to the degradation of lignin component [36, 38], while a small broadening or shoulder at the same temperature is also visible in bleached fibers and CNC signal that could be due to a residual content of hemicellulose or a broad distribution of molecular mass from cellulose, respectively [37].

The residual mass, calculated at 550 °C, of unbleached and bleached leaves of *Posidonia oceanica* and hydrolyzed cellulose nanocrystals, was also considered and reported in Figure 3. The higher residual mass observed for CNC (43% measured at 550 °C) (Figure 3a) is attributed to the lower degradation ability of cellulosic materials at higher temperature. These results could be ascribed to the increased amount of sulfated groups, which acted as flame retardants [36, 39].

Figure 4 shows the FT-IR spectra of unbleached, bleached leaves and CNCs extracted from *Posidonia oceanica*. All the spectra of unbleached, bleached leaves and CNCs show the signal at 3364 cm^{-1} and 2926 cm^{-1} due to the stretching vibrations of OH and CH and by signals in the C-O stretching region between 1200–950 cm^{-1} [40]. After chemical treatment, the band at 1511 cm^{-1} assigned to the aromatic C-O stretching mode for the guayacyl ring of lignin was absent [41, 42], confirming the removal of the lignin fraction.

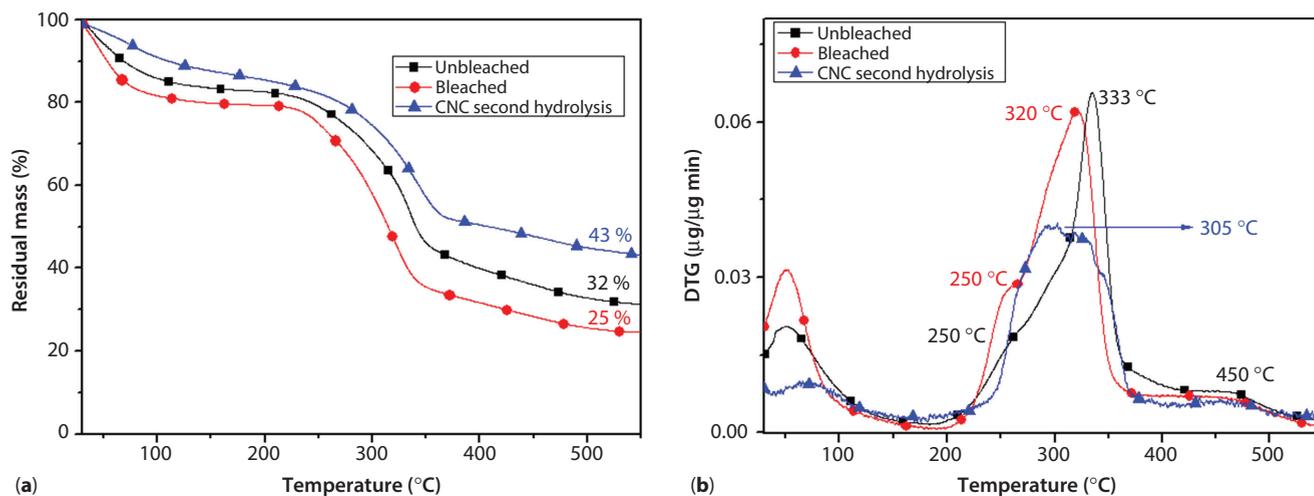


Figure 3 Residual mass (TG) (a) and differential residual mass (DTG) (b) curves of unbleached, bleached and hydrolyzed (CNCs) *Posidonia oceanica* leaves. The residual mass of different materials (a) was calculated at 550 °C.

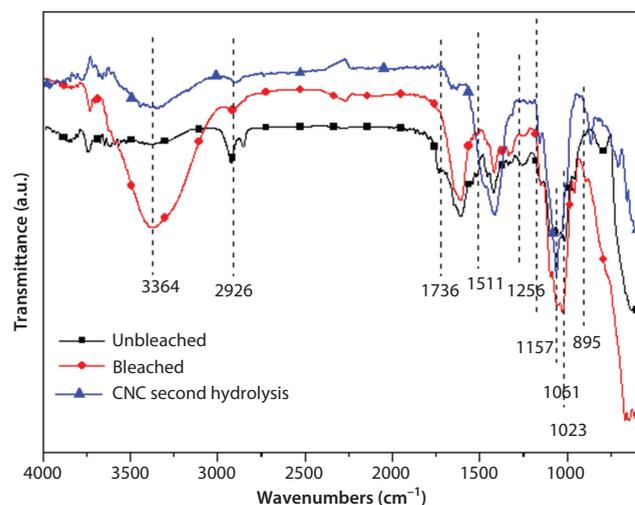


Figure 4 FT-IR spectra of unbleached, bleached and hydrolyzed (CNCs) *Posidonia oceanica* leaves in the range of 4000–600 cm^{-1} .

In the spectrum of unbleached leaves, the peak at 1736 cm^{-1} is assigned to the C=O stretching vibration of carbonyl and acetyl groups in the xylane component of hemicelluloses [43]. The absence of the 1256 cm^{-1} peak confirms the removal of the hemicellulose in the hydrolyzed materials. In the case of CNC, the signals at 1157 cm^{-1} (assigned to asymmetric C–O–C bridge stretching), 1061 cm^{-1} (anhydroglucose ring asymmetric stretching), 1023 cm^{-1} (C–O stretching), 895 cm^{-1} (associated with cellulosic β -glycosidic linkages in-plane C–H deformation and C–H deformation of cellulose), confirm the cellulosic I nature of the produced CNC [44].

4 CONCLUSIONS

In this article, cellulose nanocrystals were successfully extracted from *Posidonia oceanica* leaves by an optimized hydrolysis procedure. The CNCs were extracted after chemical pretreatment oriented to remove lignin and hemicellulose from leaves, followed by an acid hydrolysis treatment. The FESEM investigation of the first acid hydrolysis underlines the necessity to modify the hydrolysis parameters, since some unreacted leaf residues were detected. The CNCs obtained with the second hydrolysis (longer reaction time) were deeply characterized by TGA, FT-IR and FESEM; in addition, the birefringence of the CNC solution was tested and proven. The morphological investigation confirms that the parameters selected for the second hydrolysis permit an increase in the amount of reacted material, also reducing the dimension of CNCs. The CNCs obtained with the hydrolysis performed at $45 \text{ }^\circ\text{C}$

for 60 min show an acicular structure of 5–10 nm diameter and 200–450 nm length with a consequent aspect/ratio (length/diameter) of 40–45.

The properties of the cellulose nanocrystals extracted from *Posidonia oceanica* leaves could be of interest in a nanocomposite approach in which CNCs could be considered as reinforcement phase at the nanoscale level.

ACKNOWLEDGMENTS

The authors acknowledge the financial support of SEAMATTER: Revalorisation of coastal algae wastes in textile nonwoven industry with applications in building noise isolation, LIFE11 ENV/E/000600, Funding Program: LIFE+. Call 2011.

REFERENCES

1. A. Mihranyan, Cellulose from cladophorales green algae: From environmental problem to high-tech composite materials. *J. Appl. Polym. Sci.* **119**, 2449–2460 (2011).
2. D. Puglia, R. Petrucci, E. Fortunati, F. Luzi, J. M. Kenny, and L. Torre, Revalorisation of *Posidonia Oceanica* as Reinforcement in Polyethylene/Maleic Anhydride Grafted Polyethylene composites. *J. Renew. Mater.* **2**, 66–76 (2014).
3. B. Ferrero, T. Boronat, R. Moriana, O. Fenollar, and R. Balart, Green composites based on wheat gluten matrix and *Posidonia Oceanica* waste fibers as reinforcements. *Polym. Composite.* **34**, 1663–1669 (2013).
4. E. Fortunati, F. Luzi, D. Puglia, R. Petrucci, J. M. Kenny, and L. Torre, Processing of PLA nanocomposites with cellulose nanocrystals extracted from *Posidonia oceanica* waste: Innovative reuse of coastal plant. *Ind. Crop. Prod.* **67**, 439–447 (2015).
5. F. Bettaieb, O. Nechyporchuk, R. Khiari, M. F. Mhenni, A. Dufresne, and M. N. Belgacem, Effect of the oxidation treatment on the production of cellulose nanofiber suspensions from *Posidonia oceanica*: The rheological aspect. *Carbohydr. Polym.* 134664–134672 (2015).
6. F. Bettaieb, R. Khiari, M. L. Hassan, M. N. Belgacem, J. Bras, A. Dufresne, and M. F. Mhenni, Preparation and characterization of new cellulose nanocrystals from marine biomass *Posidonia oceanica*. *Ind. Crop. Prod.* **72**, 175–182 (2015).
7. R. Khiari, M. F. Mhenni, M. N. Belgacem, and E. Mauret, Chemical composition and pulping of date palm rachis and *Posidonia oceanica*—A comparison with other wood and non-wood fibre sources. *Bioresour. Technol.* **101**, 775–780 (2010).
8. Y. Liu, H. Wang, G. Yu, Q. Yu, B. Li, and X. Mu, A novel approach for the preparation of nanocrystalline cellulose by using phosphotungstic acid. *Carbohydr. Polym.* **110**, 415–422 (2014).
9. J. Kim, S. Yun, and Z. Ounaies, Discovery of cellulose as a smart material. *Macromolecules* **39**, 4202–4206 (2006).

10. P. Lu and Y.-L. Hsieh, Preparation and properties of cellulose nanocrystals: Rods, spheres, and network. *Carbohydr. Polym.* **82**, 329–336 (2010).
11. K. Fleming, D. G. Gray, and S. Matthews, Cellulose crystallites. *Chem-Eur. J.* **7**, 1831–1836 (2001).
12. Y. Habibi, L. A. Lucia, and O. J. Rojas, Cellulose nanocrystals: Chemistry, self-assembly, and applications. *Chem. Rev.* **110**, 3479–3500 (2010).
13. M. Matos Ruiz, J. Y. Cavaillé, A. Dufresne, J. F. Gérard, and C. Graillat, Processing and characterization of new thermoset nanocomposites based on cellulose whiskers. *Compos. Interface.* **7**, 117–131 (2000).
14. L. Brinchi, F. Cotana, E. Fortunati, and J. M. Kenny, Production of nanocrystalline cellulose from lignocellulosic biomass: Technology and applications. *Carbohydr. Polym.* **94**, 154–169 (2013).
15. J.-s. Fan and Y.-h. Li, Maximizing the yield of nanocrystalline cellulose from cotton pulp fiber. *Carbohydr. Polym.* **88**, 1184–1188 (2012).
16. N. Lin and A. Dufresne, Nanocellulose in biomedicine: Current status and future prospect. *Eur. Polym. J.* **59**, 302–325 (2014).
17. F. Luzi, E. Fortunati, D. Puglia, M. Lavorgna, C. Santulli, J. M. Kenny, and L. Torre, Optimized extraction of cellulose nanocrystals from pristine and carded hemp fibres. *Ind. Crop. Prod.* **56**, 175–186 (2014).
18. E. D. Cranston and D. G. Gray, Morphological and optical characterization of polyelectrolyte multilayers incorporating nanocrystalline cellulose. *Biomacromolecules* **7**, 2522–2530 (2006).
19. H. A. Silvério, W. P. F. Neto, N. O. Dantas, and D. Pasquini, Extraction and characterization of cellulose nanocrystals from corncob for application as reinforcing agent in nanocomposites. *Ind. Crop. Prod.* **44**, 427–436 (2013).
20. M. A. Saïd Azizi Samir, F. Alloin, M. Paillet, and A. Dufresne, Tangling effect in fibrillated cellulose reinforced nanocomposites. *Macromolecules* **37**, 4313–4316 (2004).
21. X. Zhou, M. M. Sain, and K. Oksman, Semi-rigid biopolyurethane foams based on palm-oil polyol and reinforced with cellulose nanocrystals. *Compos. Part A: Appl. S.* (2015).
22. E. Fortunati, F. Luzi, D. Puglia, F. Dominici, C. Santulli, J. M. Kenny, and L. Torre, Investigation of thermo-mechanical, chemical and degradative properties of PLA-limonene films reinforced with cellulose nanocrystals extracted from *Phormium tenax* leaves. *Eur. Polym. J.* **56**, 77–91 (2014).
23. Y. Tang, S. Yang, N. Zhang, and J. Zhang, Preparation and characterization of nanocrystalline cellulose via low-intensity ultrasonic-assisted sulfuric acid hydrolysis. *Cellulose* **21**, 335–346 (2014).
24. K. B. R. Teodoro, E. d. M. Teixeira, A. C. Corrêa, A. de Campos, J. M. Marconcini, and L. H. C. Mattoso, Whiskers de fibra de sisal obtidos sob diferentes condições de hidrólise ácida: efeito do tempo e da temperatura de extração. *Polímeros* **21**, 280–285 (2011).
25. X. M. Dong, J. F. Revol, and D. G. Gray, Effect of microcrystallite preparation conditions on the formation of colloid crystals of cellulose. *Cellulose (Dordrecht, Neth.)* **5**, 19 (1998).
26. R. J. Moon, A. Martini, J. Nairn, J. Simonsen, and J. Youngblood, Cellulose nanomaterials review: structure, properties and nanocomposites. *Chem. Soc. Rev.* **40**, 3941–3994 (2011).
27. E. Fortunati, D. Puglia, F. Luzi, C. Santulli, J. M. Kenny, and L. Torre, Binary PVA bio-nanocomposites containing cellulose nanocrystals extracted from different natural sources: Part I. *Carbohydr. Polym.* **97**, 825–836 (2013).
28. C. Zhang, Y. Dan, J. Peng, L. S. Turg, R. Sabo, and C. Clemons, Thermal and mechanical properties of natural rubber composites reinforced with cellulose nanocrystals from southern pine. *Adv. Polym. Tech.* **33** (2014).
29. X. Cao, H. Dong, and C. M. Li, New nanocomposite materials reinforced with flax cellulose nanocrystals in waterborne polyurethane. *Biomacromolecules* **8**, 899–904 (2007).
30. T. Aires, N. Marbà, E. A. Serrao, C. M. Duarte, and S. Arnaud-Haond, Selective elimination of chloroplastidial DNA for metagenomics of bacteria associated to green algae. *J. Phycol.* **48**, 483–490 (2012).
31. E. Fortunati, D. Puglia, M. Monti, C. Santulli, M. Maniruzzaman, and J. M. Kenny, Cellulose nanocrystals extracted from okra fibers in PVA nanocomposites. *J. Appl. Polym. Sci.* **128**, 3220–3230 (2013).
32. F. Bettaieb, R. Khiari, A. Dufresne, M. F. Mhenni, J. L. Putaux, and S. Boufi, Nanofibrillar cellulose from *Posidonia oceanica*: Properties and morphological features. *Ind. Crop. Prod.* **72**, 97–106 (2015).
33. M. C. Ncibi, V. Jeanne-Rose, B. Mahjoub, C. Jean-Marius, J. Lambert, J. J. Ehrhardt, Y. Bercion, M. Seffen, and S. Gaspard, Preparation and characterisation of raw chars and physically activated carbons derived from marine *Posidonia oceanica* (L.) fibres. *J. Hazard. Mater.* **165**, 240–249 (2009).
34. C. Barrón, C. M. Duarte, M. Frankignoulle, and A. V. Borges, Organic Carbon metabolism and carbonate dynamics in a mediterranean seagrass (*Posidonia Oceanica*) meadow. *Estuaries and Coasts* **29**, 417–426 (2006).
35. D. Bondeson, A. Mathew, and K. Oksman, Optimization of the isolation of nanocrystals from microcrystalline cellulose by acid hydrolysis. *Cellulose* **13**, 171–180 (2006).
36. H. Kargarzadeh, I. Ahmad, I. Abdullah, A. Dufresne, S. Y. Zainudin, and R. M. Sheltami, Effects of hydrolysis conditions on the morphology, crystallinity, and thermal stability of cellulose nanocrystals extracted from kenaf bast fibers. *Cellulose* **19**, 855–866 (2012).
37. E. Fortunati, D. Puglia, M. Monti, L. Peponi, C. Santulli, J. M. Kenny, and L. Torre, Extraction of cellulose nanocrystals from phormium tenax fibres. *J. Polym. E.* **21**, 319–328 (2013).
38. F. M. Pelissari, P. J. do Amaral Sobral, and F. C. Menegalli, Isolation and characterization of cellulose nanofibers from banana peels. *Cellulose* **21**, 417–432 (2014).
39. M. Roman and W. T. Winter, Effect of sulfate groups from sulfuric acid hydrolysis on the thermal degradation behavior of bacterial cellulose. *Biomacromolecules* **5**, 1671–1677 (2004).

40. R. C. Sun and X. F. Sun, Fractional and structural characterization of hemicelluloses isolated by alkali and alkaline peroxide from barley straw. *Carbohydr. Polym.* **49**, 415–423 (2002).
41. C. Cocozza, A. Parente, C. Zaccone, C. Mininni, P. Santamaria, and T. Miano, Chemical, physical and spectroscopic characterization of *Posidonia oceanica* (L.) Del. residues and their possible recycle. *Biomass and Bioenergy* **35**, 799–807 (2011).
42. A. Coletti, A. Valerio, and E. Vismara, *Posidonia oceanica* as a Renewable Lignocellulosic Biomass for the Synthesis of Cellulose Acetate and Glycidyl Methacrylate Grafted Cellulose. *Materials* **6**, 2043–2058 (2013).
43. F. Monlau, A. Barakat, J. P. Steyer, and H. Carrere, Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks. *Bioresource Technol.* **120**, 241–247 (2012).
44. M. L. Nelson and R. T. O'Connor, Relation of certain infrared bands to cellulose crystallinity and crystal lattice type. Part II. A new infrared ratio for estimation of crystallinity in celluloses I and II. *J. Appl. Polym. Sci.* **8**, 1325–1341 (1964).