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Preparation and characterization of new cellulose nanocrystals from marine biomass *Posidonia oceanica*

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ABSTRACT

In the recent decades, the rational management of agricultural residues and industrial byproducts presented an interesting approach. In this context, the valorization of *Posidonia oceanica* leaves and balls, a widely available marine residue in Tunisia, was carried out. This biomass was found to be an interesting source of cellulose. Thus, the cleaned and ground raw material was delignified and bleached. The extraction with alkali solution (yield 60% of cellulose) followed by bleaching gave 45% of pure cellulose. The resulting extracted cellulose was characterized by various techniques. Then, different nanocrystal suspensions were prepared from the *P. oceanica* cellulose and holocellulose and characterized by different methods such as TEM and nano-sizer, which gave their morphological parameters (length and size). The obtained nanocrystals were compared with those usually prepared from wood and annual plants, and it was shown that promising nanomaterials can be prepared.

1. Introduction

Cellulose, the most abundant polymer on earth, has received increasing interest because of the emerging potential associated with nanomaterials and nanotechnology and the legal and society incitation on using sustainable raw materials (Belgacem and Gandini, 2008; Dufresne, 2012). Cellulose is a natural high-molecular-weight linear homopolymer constituted of repeating β-D-glucopyranosyl units joined by 1–4 glycosidic linkages in a variety of arrangements. In the cell wall of lignocellulosic materials, cellulose exists as aggregates of nano-size fibrils. Different terms are used to describe these nano-fibrils depending on their width, namely, elementary fibrils (~3–5 nm width), microfibrils (~10–30 nm), and microfibrillar bands (~100 nm). The length of these nano-fibrils is of the order of several hundreds of nanometers. These nano-fibrils are embedded in a matrix of non-cellulosic materials (Belgacem and Gandini, 2008; Dufresne, 2012; Siqueira, 2010). Isolation of cellulose from different lignocellulosic materials such as agricultural wastes and marine plants is of both industrial and scientific importance due to the large variety of lignocellulosic raw materials all around the world.

During the past twenty years, several reports on the preparation of cellulose nanocrystals (CNCs) from various sources have been extensively developed such as softwood kraft pulp (Araki et al., 1998, 1999; Pu et al., 2007; Revol et al., 1994), softwood sulfite pulp (Beck-Candanedo et al., 2005), hardwood ECF (elemental chlorine free) pulp (Beck-Candanedo et al., 2005), recycled pulp (Filson et al., 2009), cotton fiber (Araki et al., 2000; Cao et al., 2009; Dong et al., 1996, 1998; Hasani et al., 2008; Pei et al., 2010; Revol et al., 1994; Tang and Weder, 2010; Wang et al., 2010), sisal fiber (De Rodriguez et al., 2006), flax fiber (Cao et al., 2007), ramie fiber (Habibi et al., 2007; Habibi and Dufresne, 2008; Zoppe et al., 2009), wheat straw (Helbert et al., 1996), bamboo residue (Liu et al., 2010a,b), bacterial cellulose (Grunert and Winter, 2002), grass fiber (Pandey et al., 2009), tunicate cellulose (Angles and Dufresne, 2000; Favier et al., 1995; Samir et al., 2004; Ljungberg et al., 2006; Habibi et al., 2007; Sturcova et al., 2005; Tang and Weder, 2010),

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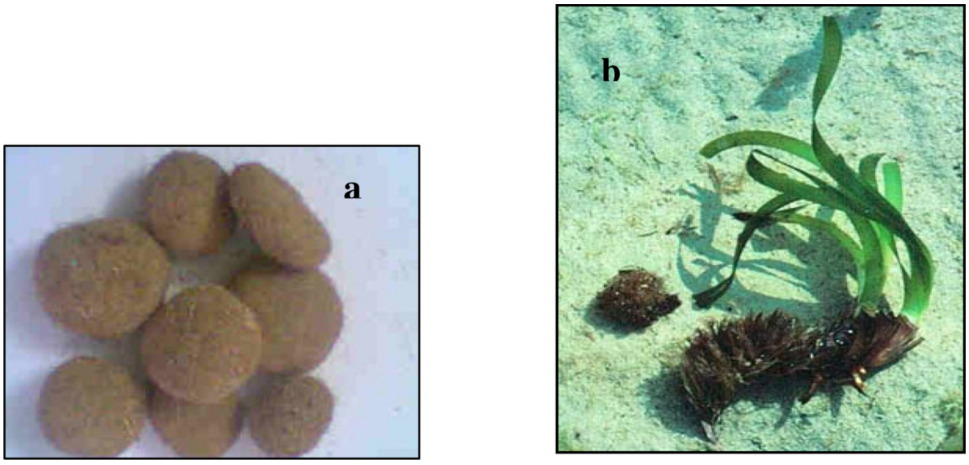

Fig. 1. *Posidonia oceanica* balls (a) and leaves (b).

and microcrystalline cellulose (MCC) (Auad et al., 2010; Bai et al., 2009; Bondeson et al., 2006; Bondeson and Oksman, 2007; Oksman et al., 2006; Liu et al., 2010a,b). These studies showed the important potential associated with the preparation of these nanoelements and explored their use in several promising applications. In these studies, the most common preparation method used is acid hydrolysis, mostly with sulfuric and hydrochloric acids. It is worth noting that other methods, such as enzymatic hydrolysis or TEMPO oxidation pretreatments followed by mechanical disintegration have also been used, but they produce bigger nanoparticles.

The same strategy could be applied to a marine waste *Posidonia oceanica* (*P. oceanica*) balls and leaves (Fig. 1), which is the dominant Mediterranean Sea grass. Important quantities of *P. oceanica* fragments accumulate on Tunisian coasts, which impose the cleaning of the beaches every summer. The valorization of this available and renewable lignocellulosic biomass can be considered as a suitable solution for this problem. Nowadays, *P. oceanica* is studied as a low cost and renewable adsorbent for removing dyes or phenol (Ncibi et al., 2006, 2008; Gezguez et al., 2009) or as a source of cellulose (Aguir and Mhenni, 2006; Khiari et al., 2010, 2011, 2012). To the best of our knowledge, no report on the preparation of CNCs from *P. oceanica* is available in the literature. This work is focused on the use of *P. oceanica* as a source of two cellulose qualities and the extraction of CNC from them. The prepared nanoelements were carefully characterized.

2. Materials and methods

2.1. Raw material: Posidonia oceanica

P. oceanica balls and leaves used in this study were collected in Monastir, Tunisia, in September 2012. These wastes were washed in order to eliminate sand and contaminants, and then dried under natural conditions during October 2012 (average relative humidity = 65%; average temperature = 25 °C).

2.2. Preparation of holocellulose, cellulose fibres and CNC from Posidonia oceanica

2.2.1. Preparation of holocellulose

The holocellulose from *P. oceanica* balls and leaves was prepared according to Wise et al. (1946) method. Concretely, previously extracted *P. oceanica* raw material was dispersed into 160 mL of water in which 1.5 g of sodium chlorite and 0.5 mL of acetic acid was added. The mixture was refluxed under stirring during 1 h. The same protocol was repeated (addition of the same quantities of sodium chlorite and acetic acid after each 1 h and under reflux) several times until the residue of *P. oceanica* observed was perfectly white. The yield of holocellulose corresponds to the mass of the obtained dry residue divided by the initial mass of material. Several preparations were also carried out at least in triplicate. Holocellulose obtained from the balls and leaves of *P. oceanica* was coded as HPB and HPL, respectively.

2.2.2. Preparation of cellulose from Posidonia oceanica

Cellulose was obtained following a two-step process, namely: (i) delignification–bleaching of the fibrous suspension, and (ii) elimination of the hemicellulose from the fibrous suspension. In our case, the operation of delignification consisted in extracting and isolating the cellulosic fibers by adopting a chemical soda-anthraquinone process. This process is usually recommended for annual plants (Alcaide et al., 1990; Fiserova et al., 2006; Jiménez and Lopez, 1990; Jiménez et al., 1993, 2008; Schott, 2000). The delignification of *P. oceanica* balls and leaves was carried out according to the procedure described by Khristova et al. (2005) and Khiari et al. (2010) with a total alkali charge of 20% expressed in NaOH (based on w/w o.d. *P. oceanica* balls), an anthraquinone concentration of 0.1% (w/w with respect to o.d. material) and a cooking time at constant temperature of 120 min. The liquor to solid ratio was changed and fixed at 10. Different cooking temperatures were tested ranging from 150 to 170 °C. All the experiments were conducted in a 1 L reactor, in which the heating time to reach the constant temperature was 1 h. The ensuing fibers were then extensively washed with water, before being bleached using 100 mL of sodium hypochlorite solution (12% of active chlorine) under a basic pH (ca. 12), for 15 min. Finally, the bleached fibers were extensively washed with water until neutrality and air dried before further use. Each cooking condition was carried out, at least in duplicate, and the difference between the various values obtained was within an experimental error of 5%. Finally, a last stage which consisted in eliminating hemicellulose from the isolated fibers was realized. This step aimed at obtaining fibers with high purity (Dufresne, 2012; Khiari et al., 2011). The bleached fibers were mixed in 350 mL of a sodium hydroxide solution (4%). The reaction was performed in water at 80 °C, and the suspension was stirred mechanically. After 2 h, the suspension was filtered and washed with distilled water, until reaching a neutral pH or equal to that of distilled water. This treatment was repeated twice. Several experimental tests were realized for each tested raw material. Cellulose obtained from the balls and leaves of *P. oceanica* was coded as CPB and CPL, respectively.

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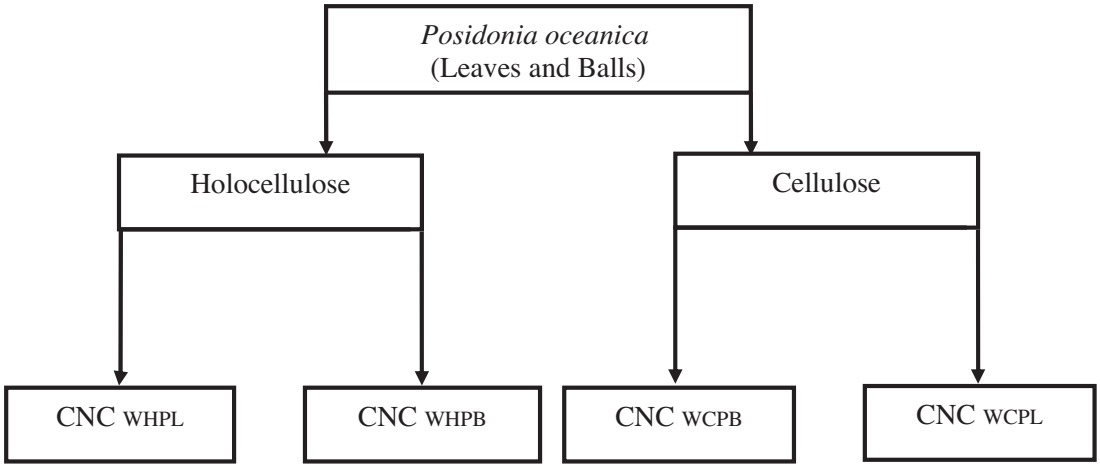

Fig. 2. Schematic representation of the different CNC prepared from holocellulose and cellulose fibers obtained from *Posidonia oceanica* leaves and balls.

2.2.3. Preparation of CNC from Posidonia oceanica

Fig. 2 describes the different types of CNC prepared from cellulose and/or holocellulose, which were extracted from *P. oceanica*. As reported in several publications (Alloin et al., 2011; Anglès and Dufresne, 2000; Araki et al., 1999; Beck-Candanedo et al., 2005; Ben Mabrouk et al., 2009; Bendahou et al., 2009; Bras et al., 2011; Cao et al., 2007; Chen et al., 2009; Garcia et al., 2006; Helbert et al., 1996; Johar et al., 2012; Kargarzadeh et al., 2012; Martins et al., 2011; Orts et al., 1998; Roohani et al., 2008; Siqueria et al., 2010; Teixeira et al., 2012), the preparation of CNC was carried out as follow: about 10 g of prepared cellulosic material (holocellulose or cellulose fibers) was dispersed in 200 mL of 6.5 mol L^{-1} sulfuric acid in a flask containing a mechanical stirrer and a thermometer. Hydrolysis was performed at 55 °C under vigorous stirring for 20 and 40 min. The excess of sulfuric acid was removed from the ensuing suspension by centrifugation at 8000 rpm for 15 min. The suspension was then submitted to dialysis against distilled water for 72 h using a cellulose acetate membrane (Sigma–Aldrich) until neutral pH, i.e., 6–7. The suspension was then submitted to an ultrasonic treatment for 5 min to reduce the aggregate size and stored in a refrigerator. The hydrolysis yield was calculated as the ratio between the weight of the freeze-dried hydrolyzed residue and the initial weight of starting material. The different CNC preparations were carried out at least in duplicate. CNC obtained from cellulose extracted from the balls and leaves of *P. oceanica* was coded as WCPB and WCPL, respectively. CNC obtained from holocellulose extracted from the balls and leaves of *P. oceanica* was coded as WHPB and WHPL, respectively.

2.3. Characterization of cellulosic materials from Posidonia oceanica

Several methods were used to characterize the raw materials or the prepared cellulosic materials from *P. oceanica* balls and leaves.

2.3.1. Chemical analysis

The chemical composition of the prepared materials was established. Thus, the extraction yield (holocellulose or cellulose fiber), Klason lignin, Kappa number as well as ash amount were assessed by using different standards or methods. As there are many standards used to determine the chemical composition of the studied raw materials, we decided to describe them very briefly:

- Ash yield (T211 om-07): The standard method TAPPI T211 om-07 was used in order to determinate the ash content. It was calculated as the solid residue remaining after combustion of the tested material at 525 ± 25 °C for at least 4 h. The characterization of this residue was also carried out by micro-elementary analysis using a detector coupled to scanning electron microscope device.
- Klason lignin: The Klason lignin content was established according to the standard Tappi method (T222 om-06). The acid-insoluble lignin was measured by subjecting the holocellulose and/or cellulose fibers to acid hydrolysis and filtering the obtained insoluble lignin residue, which was then dried and weighed.
- Kappa number (T 236 om-06): The Kappa number was determined as the amount (in milliliters) of 0.1 mol L^{-1} potassium permanganate solution consumed by 1 g of moisture-free fiber at 25 °C. This value provides an estimation of the residual lignin in pulp, i.e., the degree of delignification. This measurement is applicable to all types of chemical and semi-chemical pulps and gives Kappa numbers ranging from 1 to 100.

As recommended by the various standards used, all the experiments were duplicated and the differences between the two values were within an experimental error of 5%.

2.3.2. Morphological and spectroscopy analysis

2.3.2.1. Transmission electron microscopy (TEM). About 0.5 μL of each CNC suspension was loaded onto a 300-mesh carbon coated copper grids using a Labnet micropipette. Water from the suspension on the carbon coated grid was allowed to evaporate. Additional drop of each CNC suspension obtained from *P. oceanica* was added onto their respective grids to increase the amount of cellulose particles and the process was repeated. CNC-coated grids were examined using a JEOL 200CX transmission electron microscope at 80 kV. The dimensions of the imaged CNC were determined by transmission electron microscope software.

2.3.2.2. Atomic force microscopy (AFM). The AFM measurements were performed with a NanoScope IIIa, multimode SPM from Veeco. Calibration was performed by scanning a calibration grid with precisely known dimensions. All scans were performed in air with commercial Si Nanoprobes SPM tips. Height and phase images were obtained simultaneously in tapping mode at the fundamental resonance frequency of the cantilever with a scan rate of 0.5 line s^{-1}, using a *j*-type scanner. The free oscillating amplitude was 3.0 V, while the set point amplitude was chosen individually for each sample. For AFM analysis of CNC, a droplet of the aqueous CNC suspension was allowed to dry on a freshly cleaved mica surface.

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2.3.2.3. Particle size measurements. Particle size measurements of prepared CNCs were performed at 25 °C with a commercial nano-Zetasizer (Zetasizer NanoZS, Malvern, France). The particle radius was controlled by light scattering. For each test, the suspension was diluted to a concentration of 0.01 wt%. Then, a given volume of the diluted solution was injected in the Zetasizer cell after 30 s homogenization with ultrasonic bath. The size was measured after reaching stable values.

2.3.2.4. X-ray diffraction (XRD). X-ray diffraction experiments were conducted to determine the crystallinity of the cellulosic materials (holocellulose, cellulose fiber and CNC) obtained from *P. oceanica* after different treatments. Each material in the form of milled powder was placed on the sample holder and leveled to obtain total and uniform X-ray exposure. The prepared samples were examined using an X-ray diffractometer (D8-Advance Bruker AXS GmbH) at room temperature with a monochromatic CuKα radiation source ($\lambda = 0.154$ nm) in the step-scan mode with a 2θ angle ranging from 5° to 60° with a step of 0.04 and scanning time of 5.0 min. In order to evaluate the crystallinity of the different samples, the crystallinity index C_I, was determined based on the reflected intensity data following the method of Segal et al. (1959):

$$C_I(\%) = 100 \times (I_{002} - I_{am})/I_{002} \quad (1)$$

where I_{002} is the maximum intensity of the (0 0 2) lattice diffraction peak and I_{am} is the intensity scattered by the amorphous part of the sample. The diffraction peak for plane (0 0 2) is located at a diffraction angle around $2\theta = 22°$ and the intensity scattered by the amorphous part is measured as the lowest intensity at a diffraction angle around $2\theta = 18°$.

Table 1
Chemical composition of *Posidonia oceanica*.

	Posidonia balls	*Posidonia* leaves	Standards methods
Extractives ethanol/toluene	10.7	19.2	T224 om-07
Lignin (Klason lignin)	29.8	29.3	T222 om-06
Hollocellulose	61.8	57.1	Wise et al., 1946
α-Cellulose	40.0	31.4	T203 cm-99
Ash	12.0	10.5	T221 om-07

3. Results and discussion

3.1. Collection of the raw materials and their chemical analysis

P. oceanica (balls and leaves) was collected and extracted using toluene/ethanol mixture. Then, the chemical analysis was carried out according to the standard Tappi methods and the results are listed in Table 1.

The amount of cellulosic material in *P. oceanica* balls and leaves is quite high, which is in agreement with the data reported by Khiari et al. (2010). Lignin content is also somewhat high compared to other agricultural residues such as bagasse, rice straw, palm rachis, and wheat straw (Alcaide et al., 1990; Khiari et al., 2010; Schott, 2000). The ash amount is also present in high quantity and it is close to that reported for bagasse and rice straw (Alcaide et al., 1990; Fiserova et al., 2006; Jiménez and Lopez, 1990; Jiménez et al., 1993, 2008; Schott, 2000). As expected, the ash content is higher than that found commonly in wood.

Scanning electron microscopy (SEM) observation was carried out on *P. oceanica* after its extraction, as shown in Fig. 3. The cellulose fibers obtained from the leaves present strong length

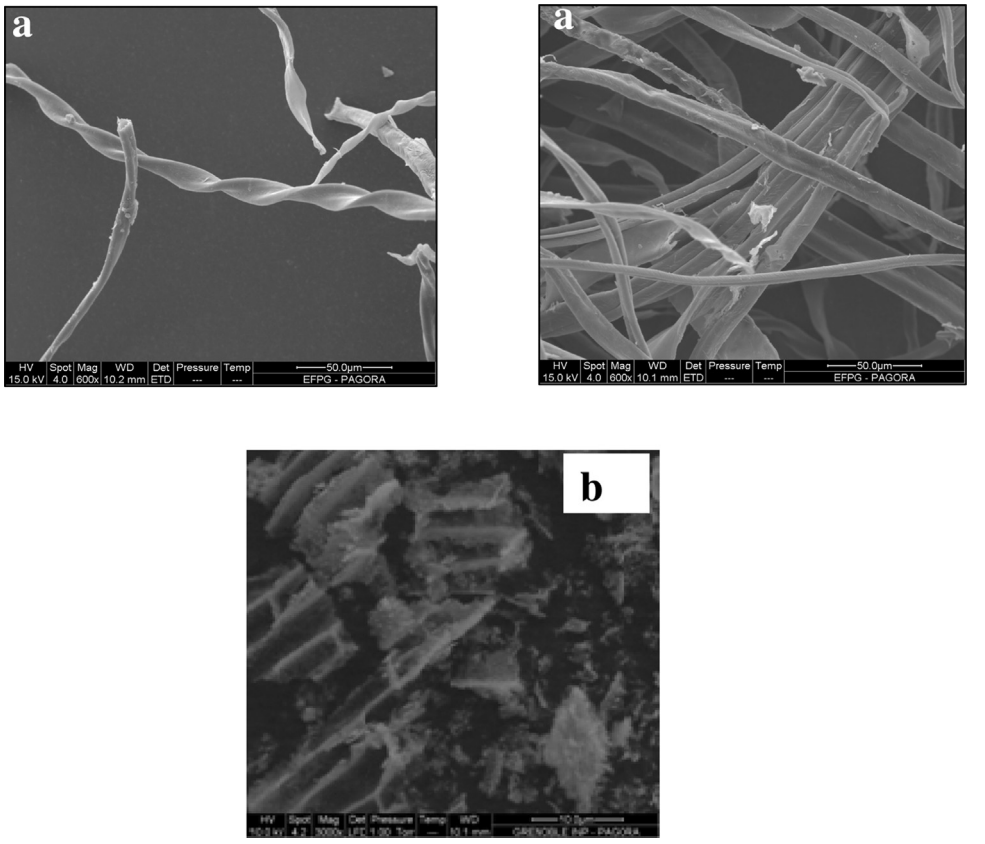

Fig. 3. SEM images of cellulose fibers prepared from *Posidonia oceanica* balls (a) and leaves (b).

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Table 2
Yield, Klason lignin, Kappa number and ash content of holocellulose and cellulose fibers from *Posidonia oceanica* leaves and balls.

Raw material	Designation	Yield (%)	Klason lignin	Kappa number	Ash %
Posidonia oceanica leaves	HPL	52.7	1.44	9.8	7.50
	CPL	28.7	0.31	2.1	5.20
Posidonia oceanica balls	HPB	59.0	1.14	7.7	7.40
	CPB	33.9	0.29	2.0	2.15

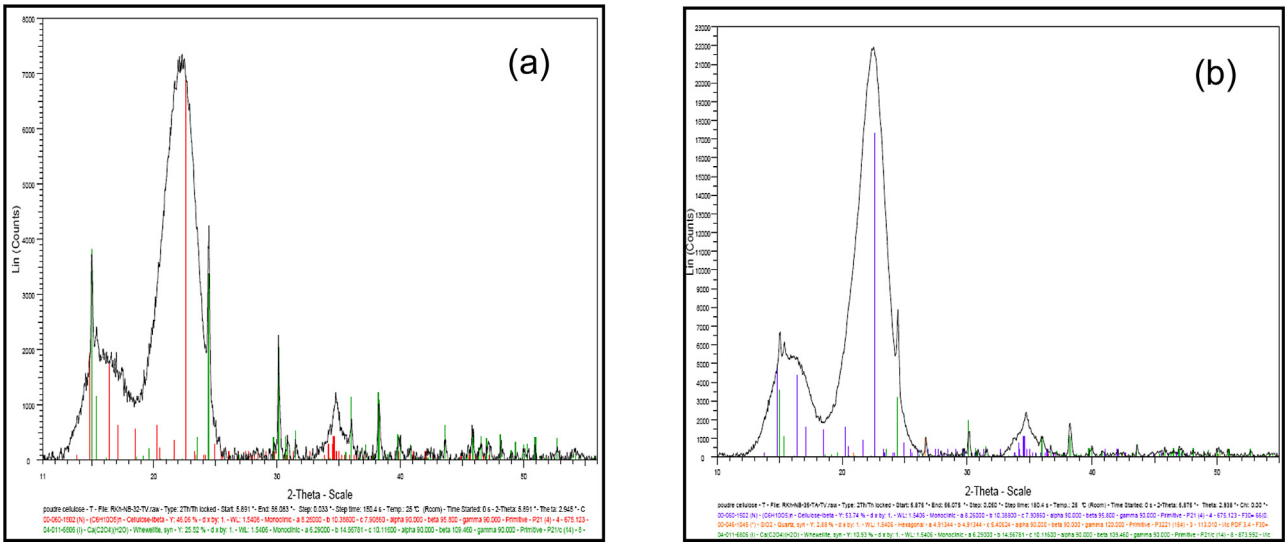

Fig. 4. X-ray diffractograms for *Posidonia oceanica* balls (a) and leaves (b).

heterogeneity, while the fibers from the balls have a spiral and cylindrical morphological structure.

3.2. Preparation and characterization of holocellulose and cellulose from Posidonia oceanica

The chemical composition of the four cellulosic materials qualities, in term of pulping yield, Klason lignin, Kappa number and ash content are summarized in Table 2.

From this table, one can draw the following concluding remarks:

- The amount of Klason lignin for holocellulose prepared from *P. oceanica* leaves and/or balls is five times higher compared with that found in cellulose fibers. On the other hand, these values are relatively weak and lower than those known for wood and non-wood plants.
- As expected, the Kappa number of the prepared cellulosic materials is proportional to the value of Klason lignin and varied from 1.7 to 9.8, which is similar to those known for wood and non-woody material.
- Finally, the ash content of *P. oceanica* is very high and comparable to certain annual plants such as rice, amaranth and the "pseudo-stem" of banana tree (Cordeiro et al., 2004). This relatively high ash content can result from a possible pollution by sand.

X-ray diffraction measurements were performed on both holocellulose and cellulose, as shown in Fig. 4, which shows the XRD patterns for the different *P. oceanica* derivative qualities. From this figure, it can be observed that the X-ray diffractograms show major intensity peak located at a 2θ value of around 22.7°, which is related to the crystalline structure of cellulose I for all samples, whilst the amorphous background is characterized by the low diffracted intensity at a 2θ value of around 18° (Segal et al., 1959). However, other peaks can be also observed. They correspond to silica and Weddellite ($CaC_2O_4{\cdot}2H_2O$), a mineral form of calcium oxalate. These impurities are the most commonly found in sea biomass.

Table 3
Crystallinity index of holocellulose and cellulose fibers from *Posidonia oceanica*.

Raw materials	Qualities	Crystallinity index, %
Posidonia oceanica leaves	HPL	41
	CPL	46
Posidonia oceanica balls	HPB	54
	CPB	60

The corresponding crystallinity index values for all prepared materials are listed in Table 3. The estimated crystallinity index for HPL, HPB, CPL and CPB was found to be 41, 54, 46 and 60%, respectively. These results clearly demonstrate that the crystallinity of the material progressively increases during the chemical extraction. This was ascribed to the progressive removal of amorphous hemicelluloses and lignin. It can also be noticed that the intensity of the presence of weddelite was also reduced. Moreover, the crystallinity index of the materials obtained from *P. oceanica* balls was higher than for materials prepared from leaves. All the deduced crystallinity indices were in agreement with those reported for annual plants, wood and non-woody material (Cordeio et al., 2004; Mansouri et al., 2012; Khiari et al., 2010, 2011).

3.3. Characterization of CNC from Posidonia oceanica leaves and balls

3.3.1. Effect of the vegetal specimen and the initial cellulosic substrate

Four qualities of CNC (WHPB, WCPB, WHPL and WCPL) were prepared, as described in Fig. 2. Table 4 presents the reaction yield and crystallinity index values associated with the prepared CNCs and shows that the reaction yield varied from 24.6 to 30.1% for *P. oceanica* leaves and between 20.1 and 27.9% for *P. oceanica* balls,

Table 4
Yield and crystallinity index of CNC prepared from *Posidonia oceanica* leaves and balls.

Starting materials	Qualities	Yield, %	Crystallinity index, %
Posidonia oceanica leaves	WHPL	24.6	62
	WCPL	30.1	62
Posidonia oceanica balls	WHPB	20.1	60
	WCPB	27.9	64

respectively. The crystallinity index followed also the same increasing trend, i.e., from 41 to 62% and from 54 and 64%, for *P. oceanica* leaves and *P. oceanica* balls, respectively. From this table, one can also observe that: (i) all the prepared CNC qualities from cellulose fiber materials present a higher yield and crystallinity index value that those prepared from holocellulose. This can be easily explained by the higher purity of the starting substrate used in the preparation of nanocellulose. It is worth to note that in all cases (*P. oceanica* leaves and/or balls), the obtained average yield presents roughly the same value, i.e., 30%, which is generally comparable to that obtained when using other annual plants (Alloin et al., 2011; Anglès and Dufresne, 2000; Araki et al., 1999; Beck-Candanedo et al., 2005; Ben Mabrouk et al., 2009; Bendahou et al., 2009; Bras et al., 2011; Cao et al., 2007; Chen et al., 2009; Dufresne, 2012; Garcia et al., 2006; Helbert et al., 1996; Johar et al., 2012).

Table 5
Geometrical characteristic of CNC prepared from *Posidonia oceanica*.

Starting materials		Width	Length	Length*	Aspect ratio
Posidonia oceanica leaves	WHPL	6.8	520	1018	76.5
	WCPL	7	338	977.5	48.3
Posidonia oceanica balls	WHPB	8.1	290	741.5	35.8
	WCPB	8	276	676.2	34.6

* Determined by nano-Zetasizer.

3.3.2. Characterization of the prepared CNCs from marine biomass

In this study, AFM and TEM were basically the two techniques used to investigate the morphology and size of the dispersed structures. The geometrical characteristics of the four CNC qualities ensuing from holocellulose and cellulose from *P. oceanica* have been investigated by TEM and AFM observation and using digital image analysis (Image J).

Fig. 5 shows the TEM micrograph of well-dispersed elongated rod-like *P. oceanica* nanoparticles and the results are summarized in Table 5. From this table one can also draw the following concluding remarks:

(i) The length determined by nano-Zetasizer (Length*) seems to be higher and double than the one obtained by TEM and AFM observation and using digital image analysis (Image J). This can be explained by the technique itself. In fact, the nano-Zetasizer method considers spherical particles which diameter

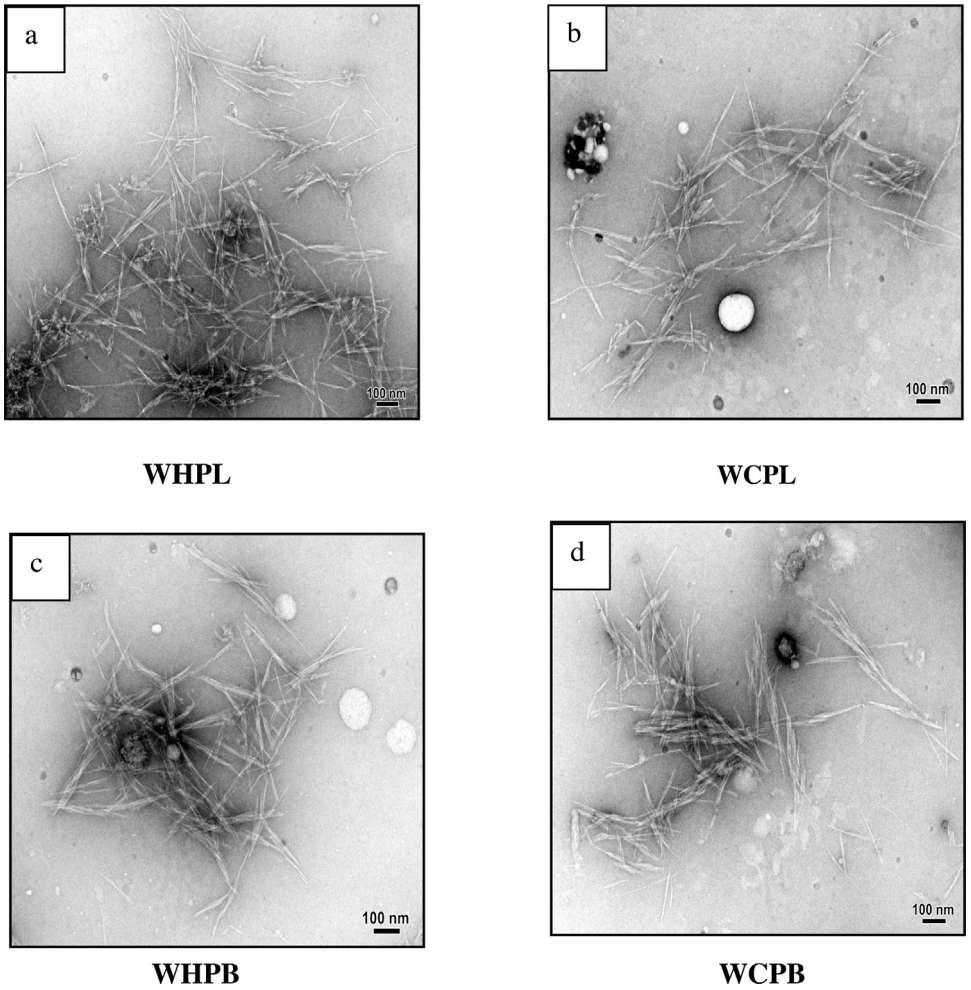

Fig. 5. Transmission electron micrographs from diluted CNC suspensions: (a) = WHPF; (b) = WCPF; (c) = WHPP and (d) = WCPP.

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Table 6
Geometrical characteristics (length, diameter, aspect ratio) of some cellulose CNCs.

Starting materials	References	Width (nm)	Length (nm)	Aspect ratio
Rice husk	Johar et al., 2012	15–20		10–15
Cotton	Martins et al., 2011	14.6	171.6	10.7
Cotton linter	Roohani et al., 2008	14.6 ± 3.9	171.6 ± 48.2	11–12
Bagasse	Bras et al., 2010	4–10	84–102	13
Kenaf bast	Kargarzadeh et al., 2012	12	158	13
Flax	Cao et al., 2007	10–30	100–500	15
Alfa	Ben Mabrouk et al., 2009	10	200	20
Eucalyptus wood pulp	Beck-Candanedo et al., 2005	6	145	24
Ramie	Alloin et al., 2011	7	200	28
Pea hull	Chen et al., 2009	7–12	240–400	34
Palm tree	Bendahou et al., 2009	6.1	260	43
Sisal	Siqueira, 2010	5	215	43
Wheat Straw	Helbert et al., 1996	5	225	45
Bleached softwood	Orts et al., 1998	5	180–280	33–47
Kraft pulp	Araki et al., 1999	3.5	180	50
Sisal	Garcia et al., 2006	4	250	60
Sugarcane bagasse	Teixeira et al., 2012	2–6	200–310	64
Capim dourado	Siqueria et al., 2010	4.5 ± 0.86	300 ± 93	67
Tunicin	Anglès and Dufresne, 2000	100–1000	10–20	50–200

corresponds to the length (Length*) of the nano-rods. However, the obtained values using this technique are wrong and then for the determination of the aspect ratio we considered just the length obtained by TEM and AFM analysis.

(ii) The CNC from *P. oceanica* leaves exhibits an average diameter of 6.8 nm and 7 nm and length of 520 nm and 338 nm determined from a minimum of 80 measurements, for WHPL and WCPL, respectively. Thus, it gives rise to an aspect ratio around 48.3 and 76.5, respectively. The last value is remarkable.

(iii) In the case of *P.oceanica* balls, CNC exhibits an average diameter of 8.1 nm and 8 nm and length of 290 nm and 276 nm for WHPB and WCPB, respectively. Corresponding aspect ratios are around 35.8 and 34.6.

Table 6 summarizes the values of geometrical characteristics for cellulosic nanomaterials derived from different species and collected from the literature. These values are typical for cellulose nanocrystals, regardless the cellulose source and the growth conditions (Dufresne et al., 1999; Dufresne, 2012). The aspect ratio observed for CNC from *P. oceanica* was found to be higher than the one of nanocrystals extracted from other annual plants such as ramie ($L/d = 28$ (Alloin et al., 2011)), bagasse pulp ($L/d = 13$ (Bras et al., 2011)), rice husk ($L/d = 10–15$, Johar et al., 2012), kenaf bast fibers ($L/d = 13$, Kargarzadeh et al., 2012), alfa ($L/d = 20$, Ben Mabrouk et al., 2009) and eucalyptus wood pulp ($L/d = 24$, Beck-Candanedo et al., 2005). For WCPL, WHPB and WCB, the aspect ratio is similar to values reported for pea hull ($L/d = 34$, Chen et al., 2009), palm tree ($L/d = 43$, Bendahou et al., 2009), sisal ($L/d = 43$, Siqueria et al., 2010), wheat Straw ($L/d = 45$, Helbert et al., 1996) and bleached softwood ($L/d = 33–47$, Orts et al., 1998). The aspect ratio reported in the literature for CNC varied from 10 to 70. The high aspect ratio of CNC obtained from *P. oceanica* indicates that these structures exhibit promising behavior as nanofillers for polymer matrices, providing valorization of this worldwide produced agricultural waste.

4. Conclusion

During this work the valorization of *Posidonia oceanica* leaves and balls, a widely available marine residue in Tunisia, was investigated. This biomass is an interesting source of cellulose. The valorization of such wastes can constitute interesting economic activities for the production of bio-products and biomaterials. Several qualities of cellulosic materials (holocellulose and extracted-bleached pulp) were prepared from *Posidonia oceanica*. The obtained materials were characterized by various techniques. The dimensions of extracted CNC were characterized and compared with those usually prepared from wood and annual plants and it was shown that promising CNC can be prepared from those materials. Moreover, in all cases high aspect ratio CNC from *Posidonia oceanica* were found. This indicates that these structures exhibit encouraging perspectives as nanofillers for polymer matrices.

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