The stranded macroalga Ulva lactuca as a new alternative source of cellulose: Extraction, physicochemical and rheological characterization

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Mohamed Amine Jmel [a], Nico Anders [b], Ghazi Ben Messaoud [a], Med Nejib Marzouki [a], Antje Spiess [b,c], Issam Smaali [a,*]

[a] Laboratoire D'ingénierie des Protéines et des Molécules Bioactives, Université de Carthage, INSAT, BP 676, Centre Urbain Nord, 1080, Cedex, Tunisia

[b] Next Generation Processes and Products, NGP^2, RWTH Aachen University, Forckenbeckstr 51, 52074, Aachen, Germany

[c] Braunschweig Integrated Centre for Systems Biology, BRICS, Braunschweig, Rebenring 56, 38106, Braunschweig, Germany

* Corresponding author: Issam.smaali@insat.rnu.tn

Abstract

Cellulose was successfully extracted from *Ulva lactuca*, an abundant and unexploited green macroalga that causes several environmental disorders by its excessive eutrophication. The extracted cellulose purity was checked by acid hydrolysis and high performance anion exchange chromatography coupled to pulsed amperometric detection, showing mainly glucose, and by Fourier transform infrared spectroscopy analysis, revealing the same functional groups as the commercial cellulose AVICEL and the absence of uronic esters from hemicellulosic contaminants. The crystallinity index was 59% compared to 82% for the AVICEL cellulose, showing the semi crystalline character of the extracted cellulose. The thermal behavior of both celluloses was the same as they showed a degradation peak at 360 °C as demonstrated by the thermogravimetric analysis. Beside the physicochemical properties, the alga cellulose presented several interesting rheological properties such as a high specific surface area (5.74 m^2/g of dry matter) and consequently a higher water retention (43.07 g/100g of dry matter). These results show that alga can be an alternative source of cellulose to the conventional ones such as wood.

1. Introduction

The growing concern about providing raw materials for polymer production has caused researchers to focus their attention on polymers extracted from renewable sources. Biopolymers extracted from abundant bio-resources can solve several problems caused by the steady decrease in petrochemical and fossil resources (Alemdar and Sain, 2008).

Several resource-based alternative polymers have been recently used to substitute chemically-synthetized fibers, producing green and environment-friendly materials. These biopolymers present numerous advantages for composites industry mainly because they present interesting rheological and mechanical properties. In addition, their abundance and availability makes them an ideal candidate for the future of bio-composites industry (Trache et al., 2016).

Cellulose represents the most abundant biopolymer on earth, synthetized by plants and by microorganisms (Klemm et al., 2005). Renewable, biodegradable and environment-friendly, cellulose is a glucose ($C_6H_{10}O_5$) homopolymer composed of amorphous and crystalline domains. The glucose monomers, also called anhydroglucose unit, contain three hydroxyl (OH) functions. Because of their ability to make stable hydrogen bonds, these hydroxyl groups give

the cellulose different properties, such as its crystalline organization (Amorphous and crystalline regions) its cohesive nature (Chandra et al., 2016). The three hydroxyl groups of each glucose unit possess a different reactivity representing then a wide range of derivative compounds that can be produced from cellulose by replacing these free OH functions by chemical functions, such as methyl, carboxymethyl, or acetyl functions (Haafiz et al., 2014).

The main conventional sources of cellulose are cotton and wood. They have been used for years for the production of several cellulose based products and in different industries especially textile and paper (Klemm et al., 2005).

The overuse of wood and cotton by competing industries, such as energy, construction, and textile industries, makes it impossible to provide the necessary amounts of wood and cotton for all the industries with reasonable prices, taking into account that many regions do not produce them. Therefore, there has been an increasing interest in other sources, such as aquatic and herbaceous plants and agricultural crops (Yousefi Shivyari et al., 2016).

In recent years, researchers have focused on non-woody plants for cellulose extraction since these sources contain less lignin (Tyagi and Suresh, 2016), which makes cellulose extraction and purification easier. Moreover, the extraction process from these sources is not harmful for cellulose as it does not need a delignification step and consumes less energy (Arvidsson et al., 2015). In this context, algal biomass represents an interesting alternative as it grows sustainably. It can be collected or cultivated in several aquatic media without interfering with arable lands and without affecting any food source (Jmel et al., 2016).

Previous works dealing with *Ulva lactuca* focused mainly on its pretreatment and energetic conversion (Jmel et al., 2018) or its application as a food supplement (Suryaningrum et al., 2017). No previous studies dealt with *Ulva lactuca* cellulose extraction and characterization despite the abundance of this biomass and its content in extractable polysaccharides. Bhutiya et al. (2018) have only described cellulose extraction and characterization from the green alga *Ulva fasciata* aimed at synthesis of zinc nanoparticules of cellulose with antibacterial effect.

To propose a new sustainable source of cellulose feedstock, and to suggest a solution for the eutrophication caused by green macroalgae proliferation, this study aimed at the extraction and characterization of cellulose from the green macroalga *Ulva lactuca.* The different physicochemical and rheological properties of the extracted cellulose such as the thermal stability, the behavior towards water, the crystallinity and the specific surface area were

investigated and compared to the commercial AVICEL cellulose ones in order to turn a waste biomass into a source of valuable products, especially cellulose.

2. Materials and methods

2.1. Biological material and sample handling

The green macroalga *Ulva lactuca* was collected from the lagoon of Tunis (GPS: 36.813095.10.192673, salinity: 33.8 psμ). Samples were transported to the laboratory and were immediately washed with distilled water, air dried, hand milled and stored in plastic bags until use.

2.2. Cellulose extraction

The extraction of the green macro alga cellulose was realized as described in our previous work (Jmel et al., 2016) with few modification. Extractives and lipids were first of all eliminated by Soxhlet extraction with ethanol as solvent. A solution of ammonium oxalate (0.05% v/v) allowed the elimination of ulvan after a 1 h incubation at 100 °C. The following step consisted in bleaching the alga at 60 °C in a solution of acetic acid (5% v/v) and $NaClO_2$ (2% v/v) for one batch until the residual biomass turned white. Finally, the obtained powder already washed to neutrality, was subjected to a NaOH (0.05 M) bath overnight at 60 °C, washed to neutrality, heated to boiling in a hydrochloric solution (5%) and kept overnight at 30 °C. The extracted cellulose was washed to neutrality and oven dried at 105 °C.

2.3. X-ray diffraction (XRD)

Cellulose crystallinity was measured using an Empyrean X-ray Diffractometer (PANalytical) with CuK_α Radiation at 40 kV and 40 mA. The recorded range was from 1° to 50° with a step size of 0.013.

The crystallinity index (CrI) was then calculated using the formula proposed by (Segal et al., 1951)

$$CrI = \frac{I_{0.02} - I_{am}}{I_{0.02}} \times 100$$

with: $I_{0.02}$: The intensity of the diffraction plane 0.02 at $2\theta = 22.5°$, I_{am}: The intensity at about $2\theta = 18°$.

2.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra were recorded using a FT-IR spectrometer model: Nexus 470 from Thermo Nicolet. Samples were measured using the ATR-method with no further sample preparation (400 scans). The ATR-unit implemented in the spectrometer was “Smart SplitPea” (Thermo Nicolet) fitted with a silicon crystal. Spectral resolution was 4 cm^{-1}.

2.5. Scanning electron microscopy (SEM)

The surface morphology characteristics of the native and pretreated biomass were observed using a scanning electron microscope model S-3000 from Hitachi. Before the observation, samples were spread on a conductive adhesive and then coated with gold. The same procedure was applied to the commercial AVICEL cellulose in order to assess its surface morphology and compare it to the extracted cellulose.

2.6. Thermogravimetric analysis

TGA studies were carried out using a TG 209 C thermogravimetric analyzer instrument. About 10 mg of the sample were placed in a crucible and the TGA spectra were recorded in an ambient nitrogen atmosphere from 25 to 900 °C at a heating rate of 10 °C/min.

2.7. Rheological characterization

The water vapor adsorption kinetics from cellulose samples were determined by a high precision gravimetric technique (Dynamic Vapor Sorption DVS). Measurements were started at 0% humidity and then samples were introduced (2–10 mg). Specific surface area was determined using the Brunauer–Emmett–Teller (BET) method using a BELSORP-max specific surface area measurement instrument.

3. Results and discussion

3.1. Extraction, purity analysis and morphological characterization

A sequential process was conducted in order to successfully extract cellulose from the green macroalga *Ulva lactuca* as, mentioned previously in our work (Jmel et al., 2016). The process shown in Fig. 1 consisted first of eliminating lipids and extractives. This step was followed by the extraction of ulvan and pigments, aiming for further valorization, and finally the alkali and acid baths were extracted.

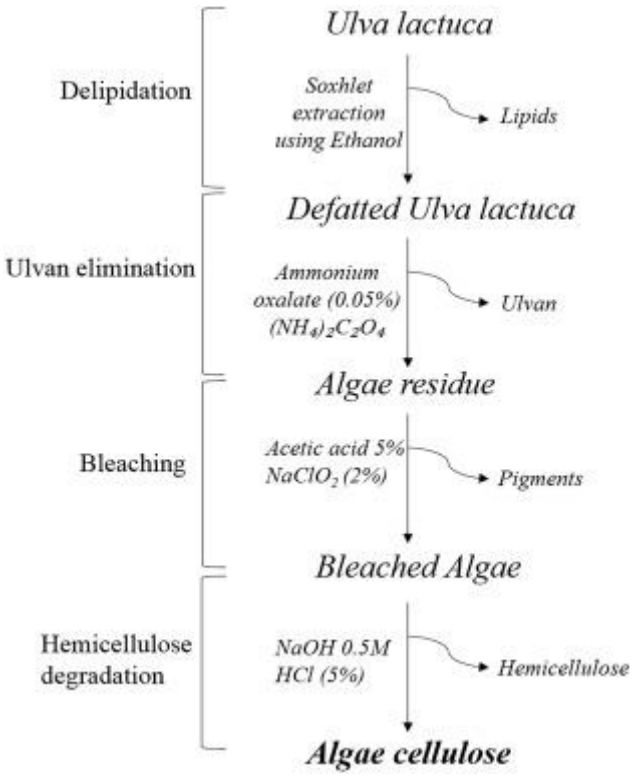

Fig. 1. Cellulose extraction process from *Ulva lactuca*.

This extraction procedure presented several advantages in comparison with the previously mentioned extraction from *Enteromorpha* sp. The main advantage of using *Ulva lactuca* is that the bleaching step permitted the elimination of pigments after only one cycle. In our previous works, the bleaching step was repeated up to three times for a complete elimination of pigments. This is mainly due to the specific composition of *Ulva lactuca* and allows an important economy of time and chemicals. In general, a such extraction procedure could be scaled up to an industrial level since there is no supplementary costs and environmental impacts compared to woody celluloses like AVICEL. In the other hand, it opens new perspectives for utilization that requires a cellulose with similar properties found for the *U. lactuca* one. More depth and data for the industrial application of this cellulose could be justified with a Life cycle assessment evaluation. A similar proof was demonstrated for the rice straw cellulose extraction (Boonterm et al., 2016).

In order to check its purity, the extracted cellulose was subjected to acid hydrolysis, followed by HPAEC-PAD analysis, with glucose as the main component. (Data not shown). The gravimetric measurement showed an extraction yield of 12.4% (w/w).

The cellulose structural analysis is an important step not only for the cellulose manufacturing but also for exploring and developing functional applications (Henrique et al., 2013). The

cellulose surface was analyzed using scanning electron microscopy in order to assess the surface properties of the extracted alga cellulose in comparison with the AVICEL commercial one (Supplementary materials).

The SEM observations shows a flat morphology for the cellulose fibers. The AVICEL cellulose (Fig. 2) revealed a smooth surface and a uniformity in fibers' length. On the other hand, the extracted cellulose from *Ulva lactuca* exhibited a relatively rough surface and no uniformity in fibers' size. In addition, the fibers were swollen because of the alkali treatment during the extraction (Wang et al., 2017). Moreover, alga cellulose fibers were disrupted, and the sides were cracked. In fact, during the extraction process, the bleaching and the alkali treatment caused the removal of ulvan, hemicellulose and other components that provide a stability and a rigidity to the algal biomass structure. Their elimination caused the cracked and disrupted effect of the extracted cellulose. Moreover, the treatment with an acid solution caused the generation of different sizes of cellulose fibers (Henrique et al., 2013).

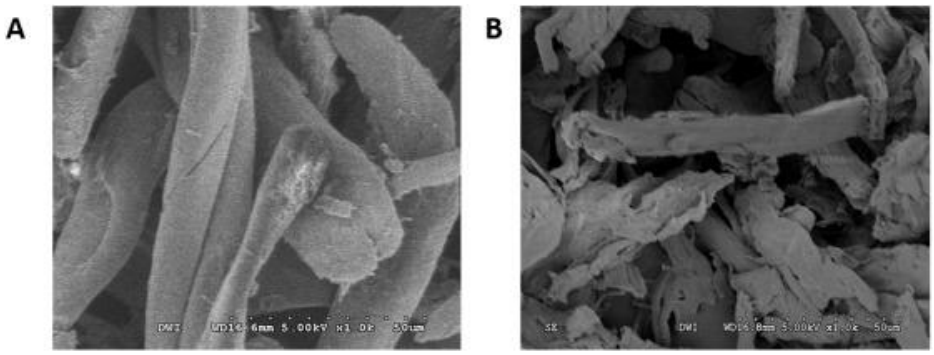

Fig. 2. SEM micrographs of (A) AVICEL cellulose (×1000 magnification) and (B) *Ulva lactuca* cellulose (×1000 magnification).

3.2. FTIR spectroscopy analysis

Typical cellulose spectra was obtained for both celluloses using FTIR. The comparison of the two celluloses using FTIR spectra showed a similar appearance for both cellulose spectra patterns, indicating the presence of the same functional groups and confirming the purity of the extracted cellulose. However, the intensity of the peaks was higher for the AVICEL cellulose (Fig. 3), indicating a higher crystallinity (Kalita et al., 2013).

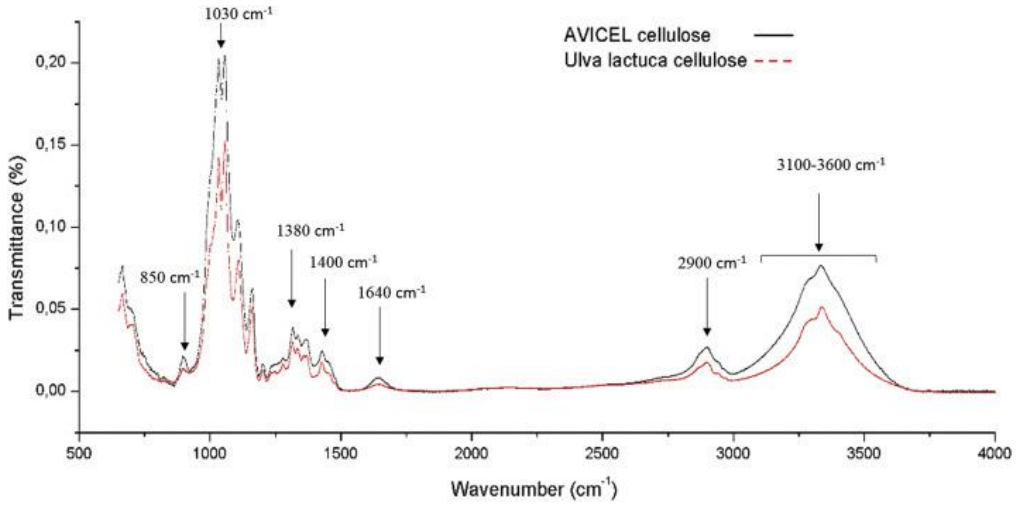

Fig. 3. FTIR spectra of the AVICEL and *Ulva lactuca* celluloses.

The wide band in the 3100-3600 cm^{-1} region is due to the OH stretching vibration. The peaks observed at 2900 cm^{-1} are due to the C–H stretching vibration in both celluloses (Khawas and Deka, 2016). Another indicator of the extracted cellulose purity is the absence of peaks in the 1730 cm^{-1} wavelength that corresponds to the vibrations of acetyl groups and uronic ester groups existing in hemicellulose (Leite et al., 2017). These carbonyl-stretching vibrations are generally absent in products from algal biomass because of the absence of lignin (Boonterm et al., 2016)

The small peak at 1640 cm^{-1} was attributed to the adsorbed water in both celluloses and was higher for the AVICEL one since the extracted alga cellulose was dried before all the characterization tests.

In fact, the water retention of the studied molecule have important consequences on the clearness of the FTIR spectra. Water generates FTIR peaks at around 1640 cm^{-1} and in the region between 3100 and 3600 cm^{-1} (Abidi et al., 2014). The peak at 1640 cm^{-1} is assigned to the O–H bending of the adsorbed water to the studied molecule. The second broad peak between 3100 and 3600 cm^{-1} is caused by the water as part of the crystal structure of molecule. In other terms, the OH groups responsible of hydrogen bonding in the cellulose molecule. The smoothness of the broad peak is generally affected by several factors and the main important one is the strength or the weakness of water bonding to the OH groups of the cellulose (Olsson and Salmén, 2004). The strongly bonded water to the cellulose exhibits a peak at around 3250 cm^{-1}, In the other hand, the weakly bound water is responsible for the sharp shape of the peak observed between 3100 and 3600 cm^{-1}. This water is generally bound to the cellulose OH

groups by another intermediate water molecule and causes noises in the FTIR spectra (Olsson and Salmén, 2004)

The band at 850 cm^{-1} was explained by the glycosidic C–H deformation and the O–H bending ring vibration. It highlighted the glycosidic linkages of the glucose units of both celluloses (Alemdar and Sain, 2008). The peaks at 1430, 1380 and 950-1030 cm^{-1} are characteristic peaks of cellulose I (Tang et al., 2013).

The FTIR analysis showed that the extracted cellulose (Fig. 3) contained the main characteristic bands, and the extraction process had no effect on its structure. However, the intensity of the peaks was different, and the surface morphology of the extracted cellulose compared to the AVICEL one was also different, indicating a possible difference in their crystallinity (Haafiz et al., 2014).

3.3. X-ray diffraction

Cellulose crystallinity remains a key factor for its application and for determining of its main properties (Khawas and Deka, 2016). As well as the AVICEL one, the cellulose extracted from *Ulva lactuca* displayed four cellulose characteristic peaks at 14.6°, 16.5°, 22.9° and 34.7° respectively corresponding to the crystallographic planes (1 1^{-} 0), (1 1 0), (2 0 0) and (0 0 4) (Lu et al., 2005; Voronova et al., 2015).

The peaks displayed by the cellulose extracted from *Ulva lactuca* revealed that the cellulose ultrastructure remained unchanged. and there was not a transformation into cellulose II (Chandra et al., 2016). The crystallinity indexes (CrI) for both celluloses were 82 and 59% for AVICEL and *Ulva lactuca* cellulose respectively. Compared to the AVICEL one (Fig. 4), the *Ulva lactuca* cellulose was more amorphous and showed a crystallinity index characteristic of a semi-cristalline cellulose, opening a wide range of applications of the alga cellulose such as bio composite synthesis. A decline in the crystallinity of the cellulose decreases the toughness of the extracted alga cellulose, producing a more flexible fiber with a higher abundance of amorphous regions and consequently an easily manipulated cellulose for applications such as encapsulation or the biocomposites production (Jmel et al., 2016).

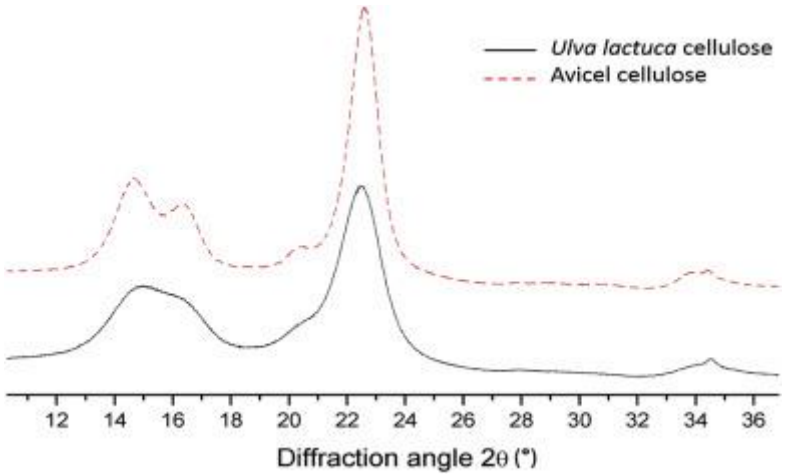

Fig. 4. X-Ray diffractograms of AVICEL and *Ulva lactuca* celluloses.

3.4. Rheological characterization

Determining the cellulose behavior towards water becomes necessary in order to exploit it correctly and avoid any undesirable effects during its transformation (Sinija and Mishra, 2008). Determining the cellulose sorption isotherms gives a lot of information about its accessibility since we can predict this biopolymer stability by determining the availability of water molecules through the variation of different factors such as temperature or humidity (Jamali et al., 2006).

In this context, sorption isotherms, the mononuclear layer of hydration and the specific surface area of the AVICEL and *Ulva lactuca* celluloses were determined. The sorption isotherms shown in Fig. 5 revealed a similar appearance for a humidity of 90%. However, the cellulose extracted from *Ulva lactuca* presented a higher water retention of 43.07 g/100g DM compared to 27.01 g/100g DM for the AVICEL cellulose. The water retention of *Ulva lactuca* cellulose is close to the water retention of the cellulose extracted from another green macroalga namely *Enteromorpha* sp. (42.87 g/100g DM) that we reported in our previous work (Jmel et al., 2016).

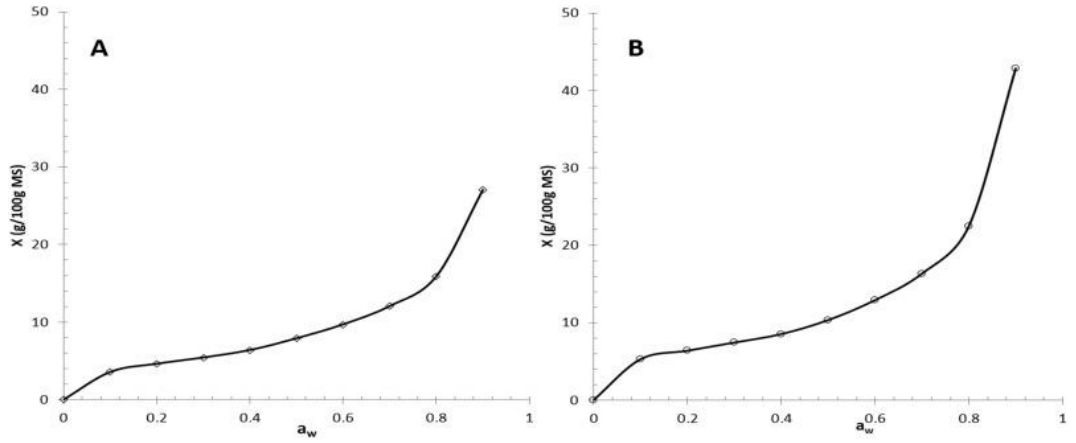

Fig. 5. Sorption isotherms of the AVICEL (A) and *Ulva lactuca* (B) celluloses.

The sorption isotherms can be divided into three different regions. The first region corresponds to the monolayer moisture, which is bound to the cellulose. The second region, generally close to linearity, corresponds to the multilayered water. Finally, the third region indicates the free moisture generally available for cellulose chemical reactions (Rhim and Lee, 2009). To consolidate the information provided by the sorption isotherms, the monolayer moisture and the specific surface area of both celluloses were determined and summarized in Table 1:

Table 1. Monolayer moisture and specific surface area of the AVICEL and *Ulva lactuca* cellulose.

Cellulose	**Monolayer moisture (g H_2O/g DM)**	**Specific surface area (m^2/g DM)**
AVICEL	3.99	0.79
Ulva lactuca	5.22	5.74

The cellulose extracted from *Ulva lactuca* revealed a higher monolayer moisture than the AVICEL cellulose. In addition, the green alga cellulose revealed a high specific surface area (5.74 m^2/g DM) compared to the AVICEL cellulose. The high specific surface area is an important parameter for the cellulose bonding. The recent trend in research about bio composites goes towards replacing the conventionally used polymers by natural fibers, exhibiting high specific surface area exactly like the extracted alga cellulose. The use of *Ulva*

lactuca cellulose for bio composites production allows the creation of low cost, low weight and extremely high- performing bio composites (Cao et al., 2016).

3.5. Thermal stability

The thermogravimetric curves allow the study of the thermal degradation of biopolymers and materials. Therefore, the thermal behavior of *Ulva lactuca* cellulose (Fig. 6) was investigated and compared to the AVICEL one (Fig. S4).

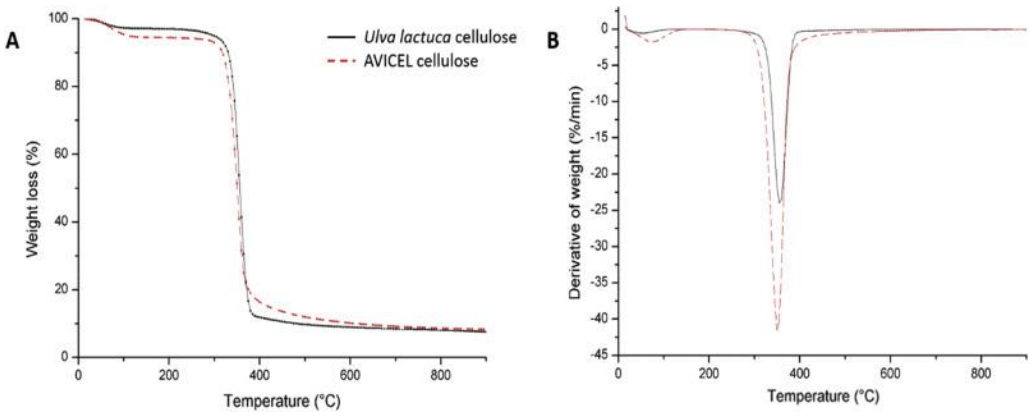

Fig. 6. TGA (A) and DTG (B) curves of AVICEL and *Ulva lactuca* celluloses.

For the two types of cellulose, a degradation pattern of two steps was observed. The first degradation step was the minor one and occured between 30 and 110 °C. This initial weight loss was due to the evaporation of moisture bound to the cellulose. The moisture evaporation was more important in the case of the AVICEL cellulose, confirming the FTIR result which indicated a more important peak at 1640 cm^{-1}, showing a more important water retention (Tang et al., 2013). The second major weight loss occurred at 360 °C for the AVICEL cellulose and at 365 °C for the *Ulva lactuca* one. For the temperatures above 400 °C, no remarkable weight loss was observed and the thermal decomposition patterns were stable because of the cleavage of the C–H and C–C bonds and the carbonization of the polysaccharidic chains (Wang et al., 2017). The thermal properties of the cellulose extracted from *Ulva lactuca* were similar to those of the AVICEL cellulose. Both celluloses were stable at high temperature. A possible application of these cellulose fibers is its use as a reinforcing and a processing agent of bio composites. These applications require high processing temperatures, going above 200 and 250 °C (Roman and Winter 2004). Therefore, the cellulose extracted from *Ulva lactuca* can be used in this context as a reinforcing agent for packaging industries or in the biocomposite synthesis (Khawas and Deka, 2016).

3.6. A proposition for cellulose extraction process

The cellulose extracted from *Ulva lactuca* presented several interesting properties, showing the necessity of scaling the extraction process to an industrial scale. The extraction process (Fig. S1) consists mainly of five steps namely, alga delipidation, ulvan elimination, bleaching, acid hydrolysis and finally alkaline treatment. The detailed process is illustrated in Fig. S1, including intermediate units of filtration and washing.

The first step consists of crushing the green macroalgal biomass with ethanol in order to extract lipids and extractives. The mix is then filtered, and the defatted alga is collected and transferred to the Ulvan elimination reactor. The collected ethanol can be recycled and reused for the first step of the process. The second step is the removal and the recuperation of ulvan from the defatted alga, using an ammonium oxalate (0.05%) solution. The algal residue is then transferred to the bleaching reactor where the pigments are removed, using a mixture of Acetic acid (5%) and $NaClO_2$. The bleached alga is then transferred to the mild acid hydrolysis tank followed by the alkaline treatment tank. The extracted cellulose is then washed, and the alkaline washing solution can be recycled again in the alkaline treatment tank. The different waste produced during this process can be recycled and valorized in order to get a circular processing unit.

A basic economic approach is quite important for any proposed process. This approach is generally based on the main cost contributor. In our case, the main cost contributor is the algal biomass which is estimated by (Konda et al., 2015) to present 24% of the whole algal process cost (around $100/t). The algal biomass, which is considered as the main cost contributor, corresponds to an eutrophication product available in huge abondoned amounts on the Tunisian shores. Indeed, the algal biomass can help lower the process cost and avoid the high cost of the alga cultivation. It is important to note that the algal biomass availability is related to seasonal variability that may have an effect on cellulose content (Ben Yahmed et al., 2016).

The lack of economic data dealing with macroalga valorization to high-added value products represents a limiting factor for the complete economic study of the proposed process. However, an advanced economic study coupled with a simulation of the proposed process is an important perspective for determining the possible revenues.

4. Conclusion

In this study, we propose a valorization perspective of an abundant and unexploited biomass generally turning into waste. The results revealed that *Ulva lactuca* is an interesting alternative for cellulose production, especially for bio-composite industry. The cellulose was successfully isolated as demonstrated by acid hydrolysis followed by HPAEC-PAD. It exhibited the same cellulose characteristic bands as the AVICEL one according to FTIR spectra analysis. The crystallinity studies indicated a crystallinity index of 59% revealing a semi crystalline behavior of the green alga cellulose in comparison with the high crystalline AVICEL one (82%). Meanwhile, the two celluloses presented the same degradation temperature at about 360 °C. However, the alga cellulose showed an interesting behavior towards water. Its specific surface area was 5.74 m^2/g DM, and its water retention was 43.07 g/100g DM. *Ulva lactuca* cellulose, with its interesting properties, is a good candidate for an alternative source of cellulose production suitable for several applications especially bio-filters synthesis, reinforcement materials production or food packaging.

Acknowledgment

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References

Abidi, N., Cabrales, L., & Haigler, C. H. (2014). Changes in the cell wall and cellulose content of developing cotton fibers investigated by FTIR spectroscopy. *Carbohydrate Polymers*, *100*, 9-16.

Alemdar, A., & Sain, M. (2008). Isolation and characterization of nanofibers from agricultural residues–Wheat straw and soy hulls. *Bioresource technology*, *99*(6), 1664-1671. Arvidsson et al., 2015

Arvidsson, R., Nguyen, D., & Svanström, M. (2015). Life cycle assessment of cellulose nanofibrils production by mechanical treatment and two different pretreatment processes. *Environmental science & technology*, *49*(11), 6881-6890.

Yahmed, N. B., Jmel, M. A., Alaya, M. B., Bouallagui, H., Marzouki, M. N., & Smaali, I. (2016). A biorefinery concept using the green macroalgae Chaetomorpha linum for the coproduction of bioethanol and biogas. *Energy Conversion and Management*, *119*, 257-265.Bhutiya et al., 2018

Bhutiya, P. L., Mahajan, M. S., Rasheed, M. A., Pandey, M., Hasan, S. Z., & Misra, N. (2018). Zinc oxide nanorod clusters deposited seaweed cellulose sheet for antimicrobial activity. *International journal of biological macromolecules*, *112*, 1264-1271.

Boonterm, M., Sunyadeth, S., Dedpakdee, S., Athichalinthorn, P., Patcharaphun, S., Mungkung, R., & Techapiesancharoenkij, R. (2016). Characterization and comparison of cellulose fiber extraction from rice straw by chemical treatment and thermal steam explosion. *Journal of Cleaner Production*, *134*, 592-599.

Chandra, J., George, N., & Narayanankutty, S. K. (2016). Isolation and characterization of cellulose nanofibrils from arecanut husk fibre. *Carbohydrate polymers*, *142*, 158-166.

Cao, J., Zhang, X., Wu, X., Wang, S., & Lu, C. (2016). Cellulose nanocrystals mediated assembly of graphene in rubber composites for chemical sensing applications. *Carbohydrate polymers*, *140*, 88-95.

Haafiz, M. M., Hassan, A., Zakaria, Z., & Inuwa, I. M. (2014). Isolation and characterization of cellulose nanowhiskers from oil palm biomass microcrystalline cellulose. *Carbohydrate polymers*, *103*, 119-125.

Henrique, M. A., Silvério, H. A., Neto, W. P. F., & Pasquini, D. (2013). Valorization of an agro-industrial waste, mango seed, by the extraction and characterization of its cellulose nanocrystals. *Journal of environmental management*, *121*, 202-209.

Jamali, A., Kouhila, M., Mohamed, L. A., Jaouhari, J. T., Idlimam, A., & Abdenouri, N. (2006). Sorption isotherms of Chenopodium ambrosioides leaves at three temperatures. *Journal of Food Engineering*, *72*(1), 77-84.

Jmel, M. A., Anders, N., Yahmed, N. B., Schmitz, C., Marzouki, M. N., Spiess, A., & Smaali, I. (2018). Variations in physicochemical properties and bioconversion efficiency of Ulva lactuca polysaccharides after different biomass pretreatment techniques. *Applied biochemistry and biotechnology*, *184*(3), 777-793.

Jmel, M. A., Ben Messaoud, G., Marzouki, M. N., Mathlouthi, M., & Smaali, I. (2016). Physico-chemical characterization and enzymatic functionalization of Enteromorpha sp. cellulose. *Carbohydrate Polymers*, *135*, 274-279.

Kalita, R. D., Nath, Y., Ochubiojo, M. E., & Buragohain, A. K. (2013). Extraction and characterization of microcrystalline cellulose from fodder grass; Setaria glauca (L) P. Beauv, and its potential as a drug delivery vehicle for isoniazid, a first line antituberculosis drug. *Colloids and Surfaces B: Biointerfaces*, *108*, 85-89.

Khawas, P., & Deka, S. C. (2016). Isolation and characterization of cellulose nanofibers from culinary banana peel using high-intensity ultrasonication combined with chemical treatment. *Carbohydrate polymers*, *137*, 608-616.

Klemm, D., Heublein, B., Fink, H. P., & Bohn, A. (2005). Cellulose: fascinating biopolymer and sustainable raw material. *Angewandte chemie international edition*, *44*(22), 3358-3393.

Konda, N. M., Singh, S., Simmons, B. A., & Klein-Marcuschamer, D. (2015). An investigation on the economic feasibility of macroalgae as a potential feedstock for biorefineries. *BioEnergy Research*, *8*, 1046-1056.

Leite, A. L. M. P., Zanon, C. D., & Menegalli, F. C. (2017). Isolation and characterization of cellulose nanofibers from cassava root bagasse and peelings. *Carbohydrate polymers*, *157*, 962-970.

Lu, W. J., Wang, H. T., Yang, S. J., Wang, Z. C., & Nie, Y. F. (2005). Isolation and characterization of mesophilic cellulose-degrading bacteria from flower stalks-vegetable waste co-composting system. *The Journal of general and applied microbiology*, *51*(6), 353-360.

Olsson, A. M., & Salmén, L. (2004). The association of water to cellulose and hemicellulose in paper examined by FTIR spectroscopy. *Carbohydrate research*, *339*(4), 813-818.

Rhim, J. W., & Lee, J. H. (2009). Thermodynamic analysis of water vapor sorption isotherms and mechanical properties of selected paper-based food packaging materials. *Journal of Food Science*, *74*(9), E502-E511.

Roman, M., & Winter, W. T. (2004). Effect of sulfate groups from sulfuric acid hydrolysis on the thermal degradation behavior of bacterial cellulose. *Biomacromolecules*, *5*(5), 1671-1677.

Segal, L., Nelson, M. L., & Conrad, C. M. (1951). Experiments on the Reduction of the Crystallinity of Cotton Cellulose. *The Journal of Physical Chemistry*, *55*(3), 325-336.

Sinija, V. R., & Mishra, H. N. (2008). Moisture sorption isotherms and heat of sorption of instant (soluble) green tea powder and green tea granules. *Journal of food engineering*, *86*(4), 494-500.

Suryaningrum, L. H., Dedi, J., Setiawati, M., & Sunarno, M. T. D. (2017). Nutrient composition and apparent digestibility coefficient of Ulva lactuca meal in the Nile tilapia (Oreochromis niloticus). *Aquaculture, Aquarium, Conservation & Legislation*, *10*(1), 77-86.

Tang, L., Huang, B., Lu, Q., Wang, S., Ou, W., Lin, W., & Chen, X. (2013). Ultrasonication-assisted manufacture of cellulose nanocrystals esterified with acetic acid. *Bioresource technology*, *127*, 100-105.

Trache, D., Hussin, M. H., Chuin, C. T. H., Sabar, S., Fazita, M. N., Taiwo, O. F., ... & Haafiz, M. M. (2016). Microcrystalline cellulose: Isolation, characterization and bio-composites application—A review. *International Journal of Biological Macromolecules*, *93*, 789-804.

Tyagi, N., & Suresh, S. (2016). Production of cellulose from sugarcane molasses using Gluconacetobacter intermedius SNT-1: optimization & characterization. *Journal of Cleaner Production*, *112*, 71-80.

Voronova, M. I., Surov, O. V., Guseinov, S. S., Barannikov, V. P., & Zakharov, A. G. (2015). Thermal stability of polyvinyl alcohol/nanocrystalline cellulose composites. *Carbohydrate polymers*, *130*, 440-447.

Wang, Z., Yao, Z., Zhou, J., & Zhang, Y. (2017). Reuse of waste cotton cloth for the extraction of cellulose nanocrystals. *Carbohydrate Polymers*, *157*, 945-952.

Yousefi Shivyari, N., Tajvidi, M., Bousfield, D. W., & Gardner, D. J. (2016). Production and characterization of laminates of paper and cellulose nanofibrils. *ACS applied materials & interfaces*, *8*(38), 25520-25528.