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Cellulose fibers extraction from *Ulva lactuca* from the Black Sea

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Abstract. Cellulose fibres are known for their good mechanical properties, therefore they are used as fillers in structural composite materials, including as nanofibrils in nanomaterials. Also, they are biocompatible, non-toxic and biodegradable, reason for their use in the food industry as packaging materials or in obtaining medical materials. One source of cheap, easy- to- extract cellulose is the algal mass of *Ulva lactuca*, one of the most frequent species found in the Black Sea. In this study, cellulose extraction from *Ulva lactuca* was achieved by a simple low cost physical-chemical treatment. Freshly harvested seaweed was dried at 45 °C for 48 hours, transformed into a fine powder in order to increase the contact surface between the solvents and the alga. Extraction of lipids and chlorophyll took place in Soxhlet apparatus with ethanol. Successive steps of chemical treatment, having in view removal of hydrosoluble ulvans, pigments and hemicellulose lead to a yield of 15.36% in dry matter (DM) of cellulose-rich insoluble fraction proving that *Ulva Lactuca* species is a viable alternative resource in cellulose production.

Keywords: cellulose extraction, *Ulva lactuca*, ulvan.

1. Introduction

In modern society, synthetic polymers are used in abundance in various fields due to their good mechanical properties and low price. However, the non-degradable behavior of polymers obtained from fossil fuels leads to environmental damage. Therefore, many scientists focused on natural biopolymers due to their ecological and economic role [1, 2]. Biopolymers are produced by organisms (plants, animals, bacteria, fungi) through biosynthesis and prone to separation by enzymatic or chemical treatment [3]. In the last decades, many biopolymers have replaced chemically synthesized fibers, leading to the development of the bio-composite materials industry [4-8].

Algae are a sustainable source for the global demand of biopolymers without affecting food supply. Biopolymers obtained from algae seem to have great perspectives due to their high photosynthetic efficiency. Also, algae have a high growing rate and can be grown almost anywhere, even in wastewaters or salt waters.

Ulva lactuca (Phylum *Chlorophyta*, Class *Ulvophyaceae*, Order *Ulvales*, Family *Ulvaceae*), commonly known as sea lettuce, is a green alga arguably rich in cellulose like fibers, which grows abundantly along the sea coasts [9, 10]. According to the study carried out by the Grigore Antipa Institute [11], for Romanian Black Sea *Ulva*, the biochemical composition of the algal powder of the is the following: carbohydrates (54.95% ± 1.43%), mineral substances (24 ± 8.25%), proteins (14.58% ± 1.30%), lipids (0.69% ± 0.06%). Comparatively, following other experimental studies, polysaccharides in Ulva are reported up to 45% [12], but it depends on the climatic region and the season of harvesting. Polysaccharides are found mainly in the cell wall with a role in structural reinforcement of the algae. They are also present in the intercellular space, in small proportion. In *Ulva*, there are two major polysaccharides: ulvans (made-up mainly of rhamnose, glucuronic acid, xylose, glucose and sulphate [13]) and cellulose, and two minor: xyloglucan and glucuronan. Xyloglucan, glucuronan and part of ulvans are hydrosoluble, but cellulose is insoluble and can be separated from the other polysaccharides. Separation of cellulose from *Ulva* is easier to perform in the absence of lignin so there is no delignification in the separation scheme.

This biopolymer shows many advantages for the composite materials production because it can be used in the food industry as packaging materials, provided that the biopolymer is compatible with foodstuffs, but also in the areas of biomaterials or structural materials, depending on the properties of the material obtained [14]. In this study, cellulose extraction from *Ulva lactuca* species was performed in a chemical treatment, following a scheme in [4], modified.

2. Experimental

2.1. Materials

Fresh *Ulva lactuca* species was collected from the north of the Romanian Black Sea coast (Latitude: 44°13'04.8"N; Longitude: 28°38'24.0"E) in August 2019 (Figure 1.a. and 1.b). The algae were dried at 45 ^{o}C in a dehydrator for fresh vegetable, then preserved in a dry place until processing. Ethanol with 96% purity was purchased from "S.C. Chimreactiv S.R.L.", Romania. Ammonium oxalate and glacial acetic acid

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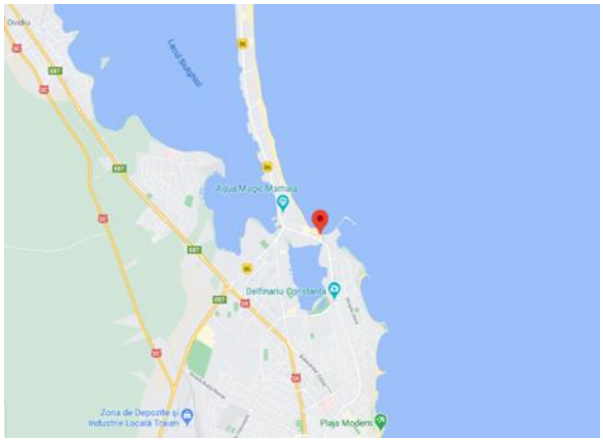
were purchased from "S.C. Chemical Company S.A.", Romania. Also, for this experiment sodium hypochlorite provided by "S.C. Plantaria S.R.L." and sodium hydroxide provided by S.C. Merck Romania S.R.L. were used.

a.



b.

Figure 1. a) Sea lettuce (*Ulva lactuca* species); b) Satellite view of harvest area.

2.2. Equipment and methods

Determination of the moisture content in fresh samples of *Ulva lactuca* species was made with the accurate device named OHAUS thermobalance, model MB45. Humidity and dry mass were measured by drying the samples following the drying programme for fresh vegetables: 7 minutes at 200 ^{o}C, 1 minute at 150 ^{o}C and 12 minutes at 105 ^{o}C.

Determination of dry matter after each step of separation scheme was performed by measuring the constant weight at 105 ^{o}C, using a laboratory oven.

Dried algae powder was observed by optical microscope with transmission IOR ML-4M (IOR, Romania), at 60x magnification.

Identification of functional groups in algae was performed with Nicolet 6700 FT-IR Spectrometer from Thermo Scientific. The applied method is based on the technique of attenuated total reflection (ATR) in tablet samples. The extraction with solvent (ethanol) from vegetable material was made in an experimental Soxhlet extraction apparatus. This method is based on the large difference between the boiling points of solvent and those of the extracted analytes. Therefore, the extract is brought to the boiling temperature of the solvent, which will condense in a refrigerant and return to the cartridge. The process was repeated by performing several extraction cycles until complete extraction takes place. The process yield can be controlled so the extraction yield is maximum.

Other treatments, such as ulvan removal, bleaching and hemicellulose removal were performed in a round bottom flask with ascendant condenser, kept in a water bath, at desired temperature, or boiling the sample in solution, for a certain time, using the same equipment.

After each step of the separation scheme, the insoluble material was brought at constant weight in the oven at 105 ^{o}C, with special care for the material impregnated in ethanol which was previously dried in the draught cupboard, for 24 h.

3. Results and discussion

3.1. Pretreatment process

Freshly harvested seaweed was washed with seawater then distilled water in order to remove the sand and impurities. The moisture in fresh alga measured with the thermobalance was 84,96%±0.3%. Subsequently, the fresh alga was dried in a dehydrator at 45 °C for 48 h. The pretreatment process had in view the proper drying of the seaweed while avoiding to lose bioactive analytes needed in other experiments. The dry matter content in algae was 13.8%±0.2% measured at constant weight at 105 ^{o}C, comparing with alga already dried at 45 ^{o}C, so getting to work 15.1 g of alga dried at 45 ^{o}C, one can count on 13.02 g of dry matter. Next, the algae were grinded in a coffee miller, to obtain a fine powder, thus ensuring a high contact surface between the sample matrix and the solvent.

3.2. Optical microscopy test

Optical microscopy image of *Ulva lactuca* powder is shown in Figure 2 where one can observe filaments of different sizes but also some little round agglomerates, most likely lipids droplets up to 500 microns.

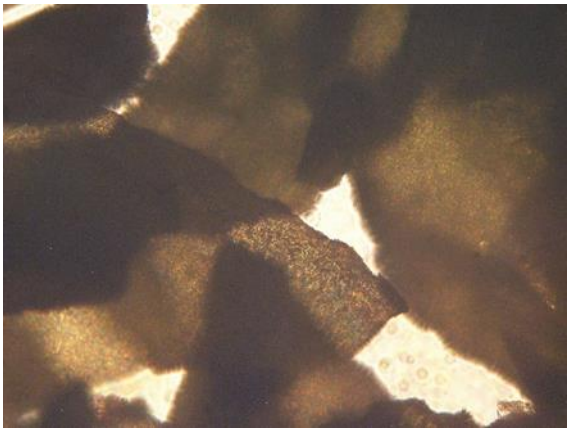


Figure 2. Microscopic image (60x magnification) of dry *Ulva lactuca* species

3.3. FT-IR spectrum of Ulva lactuca species

The FT-IR spectrometer analyzed the algal powder of *Ulva lactuca* species. The sample was introduced into the apparatus on germanium selenide crystal (GeSe), to achieve the spectrum on the wavelength range from 4000 to 600 cm^{-1}. The final spectrum was the average of

135 scans at a resolution of 4 cm^{-1}. The identification of the functional groups was performed by comparing the bands with those from the literature, characteristic of the main organic substances [15]. The FT-IR spectrum is plotted as intensity or transmittance (T) vs. wavenumber, cm^{-1}. The spectral analysis of *Ulva lactuca* powder can be seen in Figure 3, where a high abondance of absorption bands can be distinguished. A broad band in 3700-3200 cm^{-1} range with a wavelength of 3363 cm^{-1} can include the functional group -OH (υ_{OH} = 3200-3600 cm^{-1}) or amine (υ_{NH} = 3300-3500 cm^{-1}), also bending -NH being identified in the medium intensity band at 1610 cm^{-1}. The big width of the band at 3363.43 cm^{-1} indicates the association of molecules by hydrogen bonds. The confirmation of -OH structure proceeds from the high intensity of the band due to the valence vibration of the C-O bond. A medium intensity band in range of 3200-2700 cm^{-1}, at 2926.53 cm^{-1}, indicates a valence vibration of C-H bonds in alkane (υ_{CH} = 2850-2960 cm^{-1}) and its broadness may indicate the presence of a carboxylic acid whose molecules are strongly associated by hydrogen bonds (υ_{OH} = 2500-3200 cm^{-1}). Also, the appearance of intense bands in range of 1900-1450 cm^{-1} is due to the heterogeneous double bond C=O, a component part of several aldehydic functions ($\upsilon_{C=O}$ = 1680-1740 cm^{-1}) or carboxylic acids ($\upsilon_{C=O}$ = 1680-1725 cm^{-1}). The presence of -OH and carboxy groups is an indication of polysaccharides presence, containing glucose, mannuronic and guluronic acid as units in alginates and also of cellulose. The presence of groups -NH_2 and -COOH indicates the presence of aminoacids, constituents of proteins and long chains of alkanes associated with carboxy groups indicates the presence of lipids.

The study of seaweed composition is fundamental for the development of biopolymers obtaining technologies.

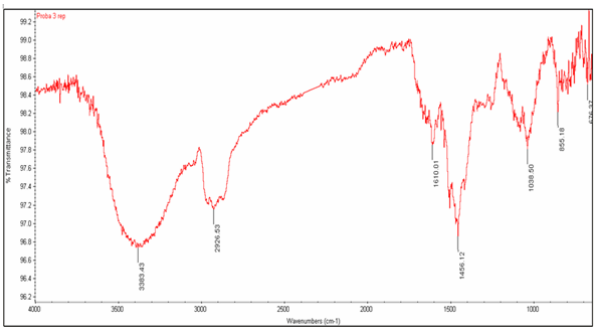


Figure 3. The FT-IR spectrum of dry *Ulva lactuca* algae

3.4. Procedure for extracting cellulose from algae

The scheme for extracting cellulose from algae is presented in Figure 4.

In general, Soxhlet extraction is an efficient method that applies to solid samples such as plants or parts of them. The extracted matter concentrates in the boiling flask where the extraction solvent is collected [16, 17]. For this experiment, 13.02 g DM of algae were introduced into the cartridge with 100 ml of ethanol as the extraction solvent in the flask. The extraction had a duration of 6 hours. After the first step, the dry matter decreased to 11.65 g, meaning that extractibles (mainly lipids and chlorophyll) represent 10.5% wt in DM. The next step was the removal of hydrosoluble ulvans. There are two types of ulvans, according to Ozcimen *et al*. [3]. The major part of them are water- soluble located in intercellular space and a cellulose- like insoluble type, present in cell walls. The hydrosoluble ulvans were removed with 100 mL of ammonium oxalate solution (0.05%) [4], after an hour of boiling the dried sample in the oxalate solution. The hydrosoluble ulvans represent 17.7% wt. (2.3 g) in DM. Afterwards, the algae were bleached in a solution consisting of 200 ml acetic acid (5% v/v) and 100 ml NaClO (2% v/v) heated to 60 °C for 12 hours, for removal of residual pigments. After the insoluble material was washed several times with distilled water and brought to pH = 7, then dried in the oven at 105 °C, it was kept into a NaOH solution (0.5 M) at 60 °C, for 12 hours. Finally, cellulose fibers were dried in the oven at 105 °C. Subsequently, it was washed to neutrality and heated to boiling point in a hydrochloric solution (5%), then cooled to 30 °C and maintained at this temperature for 12 h. This final step is intended to remove the *hemicellulose*, the random amorphous structure polysaccharides with shorter chain then cellulose. Thus, a yield of 15.36% wt (2.99 g) cellulose per DM was obtained proving that *Ulva lactuca* species is a viable alternative resource in cellulose production. This yield is comparable with data in the literature which stretches from 2% to 13.65% in Indian seaweeds species [18], 2.2% per DM in *Ulva* species along the Swedish coasts [19] or 12.4% in *Ulva* from Tunisian coast [20].

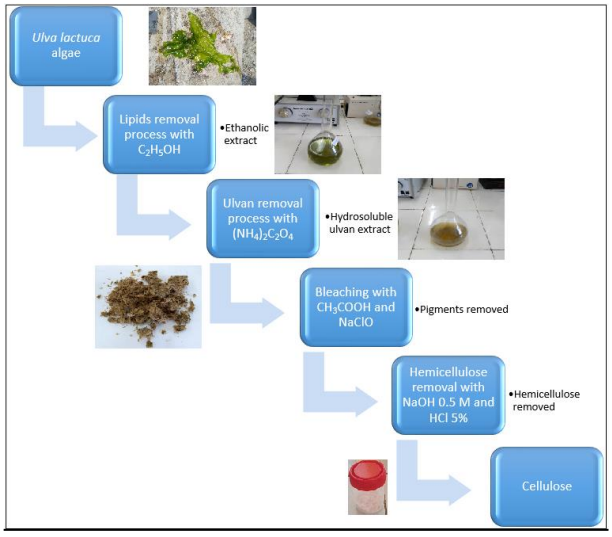


Figure 4. Cellulose extraction process from green macroalga *Ulva lactuca*

4. Conclusions

In this study, a brief characterization of *Ulva lactuca* species in Black Sea was performed: dry matter content, optical microscopy and chemical structure by functional group identification with a FT-IR spectrometer. Then, the extraction of cellulose was performed in a separation scheme including the removal of lipids, pigments, ulvans, and hemicellulose. The scheme includes operations for the removal of undesirable substances but

don't affect the cellulose which is obtained as a white bright powder. A yield of 15.36 % cellulose per dry matter was obtained proving that *Ulva lactuca* species is a viable alternative resource in cellulose production.

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Conflict of interest

Authors declare no conflict of interest.

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