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Physicochemical analysis of cellulose from microalgae *Nannochloropsis gaditana*

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Nannochloropsis gaditana **is a microalgae belonging to the class of Eustigmatophyceae. This particular microalga is the most studied species. For its richness in lipids, it is used for the biodiesel production. The aim of this work is to advance another important metabolite which is cellulose. This represents 25% of the dry weight of the *N. gaditana.* A low concentration of NaOH (2%) and sodium hypochlorite (6%) allowed the obtainment of relatively pure cellulose analyzed by Fourier transform infrared (FT-IR) and X-Ray Diffraction (XRD). The progress of the microalgae mass production could help in the substitution of the cellulose of microalgae for the vegetal cellulose, as seen in the simple technical extraction, the yield and the procurement of uncontaminated molecule with lignin. This substitution will contribute in protecting the environment.**

Keywords: Cellulose, *Nannochloropsis gaditana*, procedure extraction, structural characterization.

INTRODUCTION

Nannochloropsis gaditana is a microalgae that belongs to the class of Eustigmatophyceae (Andersen, 1998). It has five species and they are: *N. gaditana, N. salina, N. oculata, N. oceanica and N. limnetica* (Hibberd, 1981; Lubian,1982; Karlson et al.,1996; Krienitz et al.,2000 Suda et al.,2002) . The *gaditana* and *salina* species are recently reported as two strains belonging to the same species (Shawn et al., 2014). *N. gaditana* is rich in lipids and produces mass biodiesel (AttilioConverti et al., 2009; Rodolfi et al., 2009; Pal et al., 2011; Bondioli et al., 2012). The cell wall of *N. gaditana* is comprised a bilayer structure consisting of a cellulosic inner wall (~75% of the mass balance), protected by an outer hydrophobic algaenan layer (Scholz et al., 2014). To meet with the global needs of cellulose (the textile, paper, plastic, paint, chemistry, pharmaceutical and cosmetic field) and vegetable, a wide deforestation and environmental damage is involved. The substitution of the parietal compounds of the plant source by those microalgae is possible and can present several advantages. Reasons being that: (i) the growth of microalgae is quick and harvesting is done continually throughout the year (Gouveia, 2011); (ii) they can grow in saline aqueous areas, waste water and brackish water (Gouveia, 2011)

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or even in wasteland (Demirbas, 2011); (iii) their production contributes to a better CO_2 sequestration than plants (Gouveia, 2011).

The mechanical properties of the cellulose determine its use. These properties depend on the spatial structure obtained after extraction by chemical treatment (Morán et al., 2008). The aim of this study is to extract the main parietal compound of *N. gaditana* (cellulose) at different concentrations of extraction reagents. The quality of cellulose is analyzed by Fourier transform infrared (FT-IR) and by X-Ray Diffraction (XRD). Cellulose is a linear homopolymer consisting of anhydroglucopyranose units (AGU), connected by glycosidic linkages in β - (1-4). The chains are linked by hydrogen bonds fashioned by covalent bonds formed between C1 of one monomer in one strand and a C4 in an adjacent strand.

MATERIALS AND METHODS

Biological material

N. gaditana is mass produced by the company: PARTISANO BIOTECH Algeria in Oran and the samples are provided in dried powder form.

Cellulose extraction

The extraction method of algae cellulose was a modification of Jayme-Wise method (Leavitt and Danzer, 1993; Gaudinski et al., 2005; Rosli et al., 2013), initiated with the de-waxing of algae by treating micralgae with toluene: ethanol. Five gram (5 g) of micro algae dry powder were treated with a mixture of toluene/ ethanol (68:32) (v/v) for 24 h using magnetic stirring. After filtration, the parietal residue was separately treated with 2 or 4% NaOH for 2 h at 80°C, to eliminate Hemicelluloses. Bleaching is performed with sodium hypochlorite (6 or 10%) at pH 4.8, stirred for 2 h at 70°C. The cellulose pellet recovered by centrifugation was dried using lyophilization and weighed. The extraction was carried out in triplicate.

Characterization of cellulose

Analysis of cellulose by FTIR spectroscopy

FTIR spectra of the cellulosic samples were measured with FTIR alpha Brucker .The resolution of 26 scan were taken with the frequency range of 4000 to 400 cm^{-1} in the transmission mode.

Analysis of cellulose by DRX, (DRX D8 Advance Brucker)

The cellulose samples were analyzed from 4 to 70° (2θ) with a pitch of 0.02° and measuring 1s of acquisition time by an R-X diffractometer.

RESULTS AND DISCUSSION

Quantification of cellulose

Cellulose obtained after extraction and dried is 25% of the dry weight of the microalgae, *N. gaditana.*

Characterization of cellulose

Analysis of the cellulose by FT-IR

The infrared spectra of cellulose were studied in the literature (Nelson and O'Connor, 1964; Oh et al., 2005; Alvarez and Vazquez, 2006; Yang et al., 2007). The analysis by FTIR of the extracted cellulose from 2% NaOH and 6% $NaClO_2$ (Figure 1) is carried out to identify polysaccharide and to determine its contaminants such as: hemicelluloses, lipids and proteins.The spectrum obtained (Figure 1) revealed a broad band in the 3600 to 3100 cm^{-1}which correspond to the (- OH). The peak occurred at 3283 and 3272 cm^{-1}, which indicated the presence of hydroxyl groups in algae cellulose and carboxymethyl cellulose respectively. The peaks 2929/2857 and 2880 cm^{-1} corresponded to the (-CH) groups stretching vibration in cellulose algae and caboxymethyl cellulose (Oh et al., 2005; Nelson and O'Connor, 1964).

The peak present at 1069 cm^{-1} corresponding to 950 to 1200 cm^{-1} range is attributed to C-O vibration of carbon C_2, and also the 1632 cm^{-1} band corresponded to the carbonyl groups. In addition, the FTIR absorption bands at 1458/1378 and 1412/1320 cm^{-1} was assigned to O-H bending vibration.The occurrence of the peak at 1156 cm^{-1}, located in 1170 to 1082 cm^{-1} range corresponds to asymmetric C-O-C stretching vibration. The appearance of the 1524 cm^{-1} peak at interval (1600 to 1500cm^{-1}) corresponds to the aromatic rings. This group is a contaminant due to the existence of peptides or proteins. Proteins represent about 1% of the parietal content of *N. gaditana* (Scholz et al., 2014).

The peak presented at 1735 cm^{-1} in the spectrum corresponding to the cellulose obtained from 2% NaOH and 6% $NaClO_2$ could be, due to the presence of small amounts of hemicelluloses, which contain higher C=Olinkage at 1765 to 1715 cm^{-1}. Another possibility is that carboxyl or aldehyde absorption (1736cm^{-1}) could be arising from the opened terminal glycopyranose rings (Morán et al., 2008). The carboxyl may be due to the presence of lipids. The Cellulosic wall *N. gaditana* is protected by algaenan which is a lipid structure (Scholz et al., 2014). The current analysis of the cellulose extracted in our study by NMR reveals the presence of lipids (Stuart Jones, personal comm.).However, the extraction of cellulose in higher concentrations NaOH2 to 4% to get a better solubilization of hemicelluloses followed by bleaching with hypochlorite6% (Figure 2) does not reduce the peak intensity1735cm^{-1}.The hemicelluloses are principally extracted with 2% NaOH. This peak also corresponds with the functional groups aldehyde and carboxyl (Morán et al., 2008).

The aldehydes and carboxyl groups are more accessible at 4% NaOH. There is an increase in the peak intensity 1736cm^{-1}. The increase in the concentration of NaOH 4% did not only affect the peak 1736 cm^{-1}, but also the other peaks representing other functional groups. The

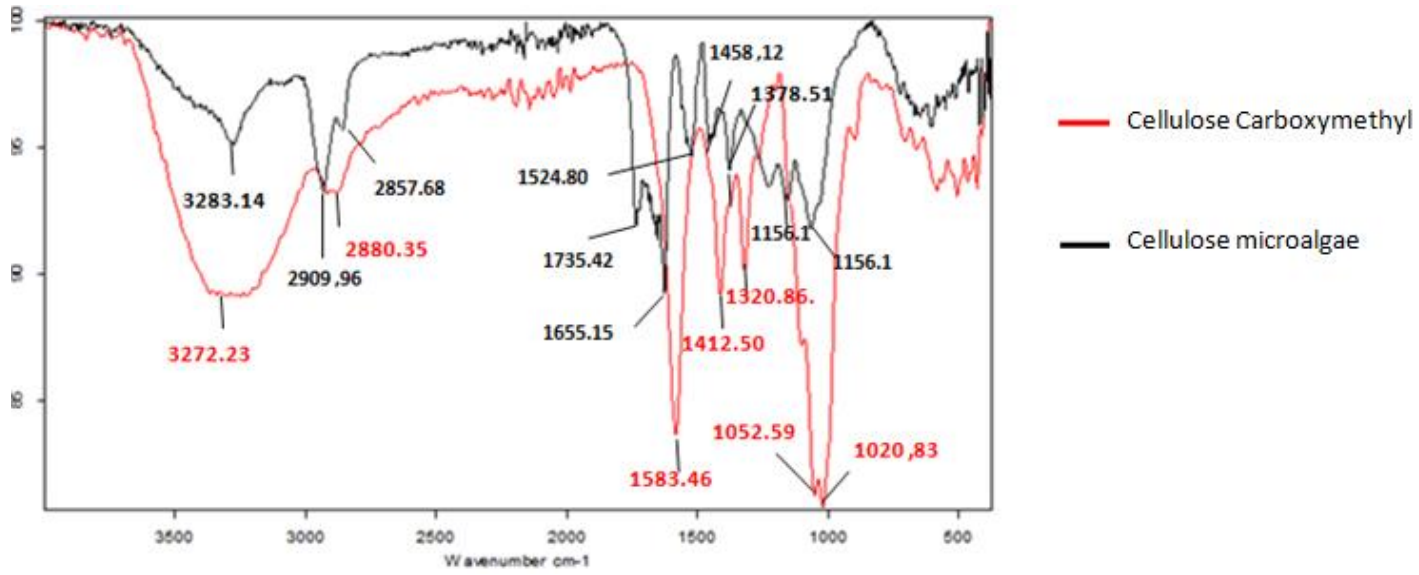

Figure 1. FT-IR spectra of carboxymethyl cellulose and cellulose extracted from *N. gaditama* by 2% NaOH and 6% $NaClO_2$.

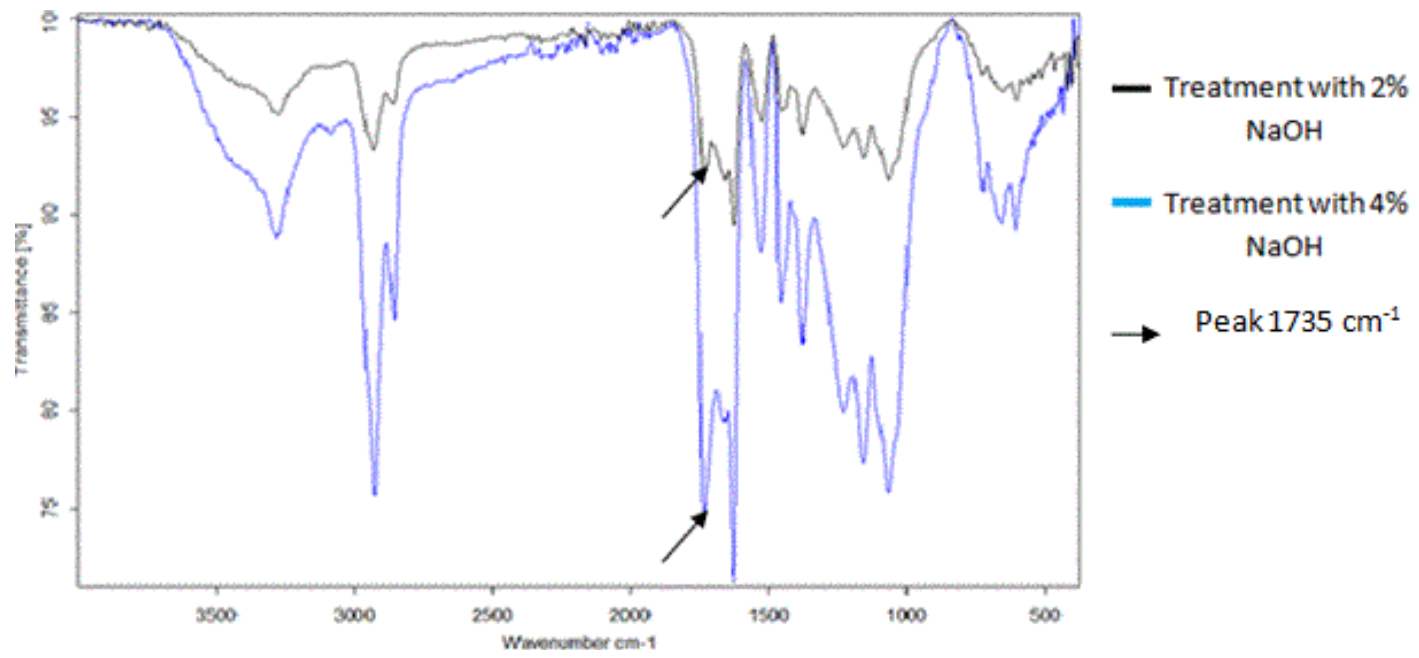

Figure 2. Cellulose FT-IR spectrum extracted from *N. gaditama* by 2 and 4% NaOH followed by 10% of $NaClO_2$.

concentration of 4% NaOH transforms the semi crystalline structure of cellulose to amorphous structure. The increase in the concentration of $NaClO_2$ from 6 to 10% (Figure 3) highlights the functional groups of the cellulose expressed by the increase of peaks intensity. The concentrations of 10% $NaClO_2$ would act on the cellulose structure while making its functional groups more accessible to the infrared rays. The semi crystalline structure of the cellulose became more amorphous.

Analysis of cellulose by XRD

In order to identify the crystalline and amorphous areas of celluloses obtained in this work, a diffractometry X-Ray was carried out. Cellulose samples obtained at different concentrations of NaOH (2 and 4%), followed by bleaching with 6% sodium hypochlorite, and another obtained after treatment with 2 and 4% NaOH, followed by 10% hypochlorite bleach were analyzed (Figure 4).

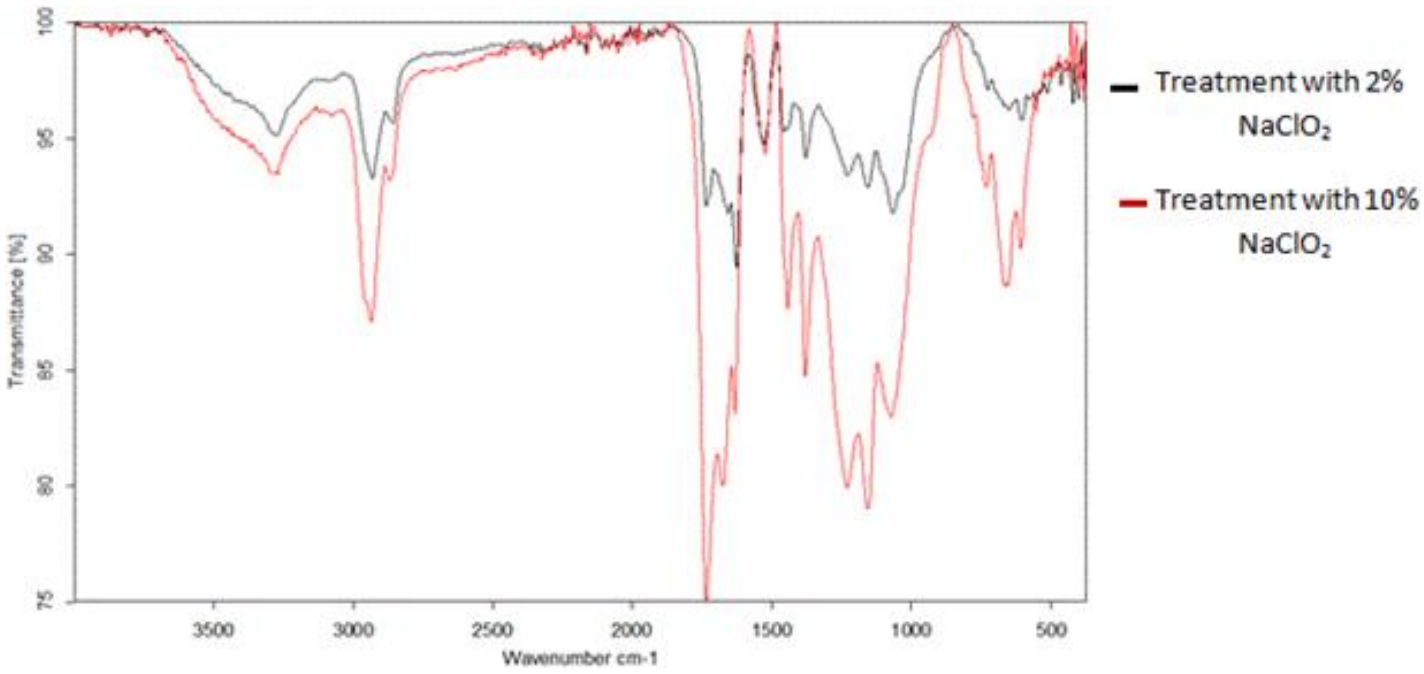

Figure 3. Cellulose FT-IR spectrum extracted from *N. gaditama* by 2% NaOH followed by 6 and 10% of $NaClO_2$.

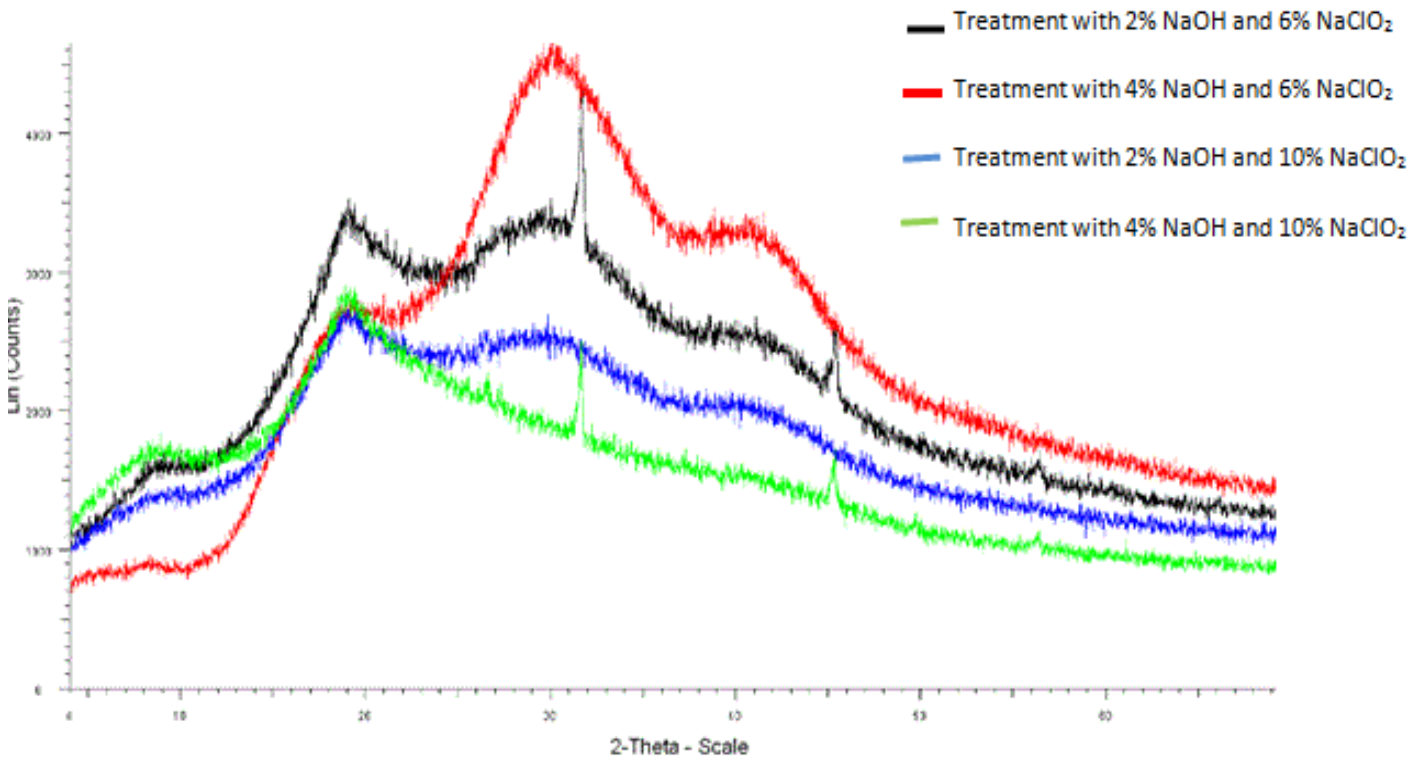

Figure 4. X-ray diffractogram of cellulose extracted from 2 and 4% of NaOH followed by bleaching 6 and 10% of $NaClO_2$

The XRD confirmed the hypothesis that the variable of NaOH concentration of 2 to 4% shows an amorphous structure, which becomes increasingly abundant to a maximum concentration of 10% sodium hypochlorite.

Conclusion

N. gaditana is rich in cellulose. This compound would represent 25% of the dry weight of the microalgae. The FTIR analysis of the cellulose extract identified the functional groups of the cellulose and its contaminants. The XRD analysis tracked the structural change (crystalline, amorphous) of the cellulose taken out based on the extraction mode. During extraction, the NaOH is used to remove hemicellulose and the $NaClO_2$ bleaching removes other contaminants. According to the results, 2% NaOH seemed sufficient to extract most of the hemicellulose. An increase to 4% NaOH did not diminish the intensity of the peak 1735 indicative of the hemicellulose carbonyl group. However, the intensity of the functional groups of the cellulose peaks is important. The concentration of 4% NaOH appears to have altered the semi-crystalline structure in amorphous structure making them a more accessible functional group. XRD analysis confirmed the hypothesis that Cellulosic extract obtained in 2% NaOH is semi-crystalline, and at 4% NaOH, the cellulosic extract is amorphous. Similarly, bleaching seemed to affect the cellulosic extract structure. Whereas, 2% NaOH followed by a bleach of 6% $NaClO_2$ cellulosic extract is semi-crystalline. An increase in the concentration to 10% $NaClO_2$ yielded the amorphous structure. These observations were concluded from the FTIR spectra and RXD diffraction patterns. This "amorphous" property has an industrial interest. The functional groups are more accessible and thus esterifiable. However, the cellulosic extract obtained remained contaminated with lipids and proteins. These two compounds are incorporated into the cell wall. Aggressive physicochemical treatment given to remove these contaminants, may damage the fragile spatial structure of the cellulose extracted. The extraction method must be gentle not to alter the structure of cellulose and should be strong enough to remove contaminants. *N. gaditana* is a unicellular organism. Its cell wall is less complex than that of higher plants known for their high lignin. Lignin is the major contaminant in the extraction of cellulose. Cellulose microalgae would be an alternative to replace the cellulose of higher plants. However, the choice of the extraction process is important. Improper process can damage the cellulose structure (Moon et al., 2011).

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Alvarez VA, Vazquez A (2006). Influence of fiber chemical modification procedure on the mechanical modification procedure on the mechanical properties and water absorption of MaterBi/sisal fiber composites. Compos. Part A Appl. Sci. Manuf. 37(10):1672-1680

Andersen RA, Brett RW, Potter D, Sexton JP (1998). Phylogeny of the Eustigmatophyceae based upon 18SrRNA gene, with emphasis on Nannochloropsis. Protist 149:61-74.

Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M (209). Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsisoculata and Chlorella vulgaris for biodiesel production. J. Chem. Eng. Process. 48:1146-1151

Bondioli P, Della Bella L, Rivolta G, ChiniZittelli G, Bassi N, Rodolfi L, Casini D, Prussi M, Chiaramonti D, Tredici MR (2012). Oil production by the marine microalgae Nannochloropsis sp. F&M-M24 and Tetraselmissuecica F&M-M33. J. Bioresour. Technol. 114:567-672.

Demirbas MF (2011). Biofuels from algae for sustainable development. J.Applied Energy, 88:3473-3480.

Gaudinski JB, Dawson TE, Quideau S, Schuur EA, Roden JS, Trumbore SE, Sandquist DR, Oh SW, Wasylishen RE (2005). Comparative analysis of cellulose preparation techniques for use with 13C, 14C, AND 18O isotopic measurements. J. Anal. Chem. 77:7212-7224.

Gouveia L (2011). Microalgae as a Feedstock for Biofuels. Springer, London. pp.1-69.

Hibberd DJ (1981). Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae (synonym Xanthophyceae). Bot. J. Linn. Soc. 82:93-119.

Morán JI, Alvarez VA, Cyras VP, Vázquez A (2008). Extraction of cellulose and preparation of nanocellulose from sisal fibers. Cellulose 15:149-159.

Karlson B, Potter D, Kuylenstierna M, Andersen RA (1996). Ultrastructure, pigment composition, and 18S rRNA gene sequence for *Nannochloropsisgranulata* sp. nov. (Monodopsideaceae, Eustigmatophyceae), a marine ultraplankter. Phycologia 35:253-260.

Krienitz L, Hepperle D, Stich HB, Weiler W (2000). *Nannochloropsislimnetica* (Eustigmatophyceae), a new species of picoplankton from freshwater. Phycologia 35:219-227.

Leavitt SW, Danzer SR (1993). Method of batch processing small wood samples to holocellulose of stable-carbon isotope analysis. Anal. Chem. 65:87-89.

Lubian LM (1982). Nannochloropsis gaditana spec. nov., una nueva Eustigmatophyceae marina. Lazaroa 4:287-293.

Moon RJ, Martini A, Nairn J, Simonsen J, Youngblood J (2011). Cellulose nanomaterials review: structure, properties and nanocomposites. Chem. Soc. Rev. 40(7):3941-3994.

Nelson ML, O'Connor RT (1964). Relation of certain infrared bands to cellulose crystallinity and cristal lattice type. Part II: a new infrared ratio for estimation of crystallinity in cellulose I and II. J. Appl. Polym. Sci. 8(3):1328-1341

Oh SY, Yoo DI, Shin Y, Seo G (2005). FTIR analysis of celluloses treated with sodium hydroxide and carbon dioxide. J. Carbohydr. Res. 340:417-428.

Pal D, Khozin-Goldberg I, Cohen Z, Boussiba S (2011). The effect of light, salinity, and nitrogen availability on lipid production by Nannochloropsis sp. Appl. Microbiol. Biotechnol. 90:1429-1441.

Rodolfi L, Chini-Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, Tredici M (2009). Microalgae for oil: strain selection,induction of lipid synthesis and outdoor mass cultivation in a low cost photobioreactor. J. Biotechnol. Bioeng 102:100-112.

Scholz MJ, Weiss TL, Jinkerson RE, Jing J, Roth R, Goodenough U, Posewitz MC, Gerken HG (2014). Ultrastructure and composition of the *Nannochloropsisgaditana* cell wall. Eukaryot. Cell 13(11):1450-

1464.
Starkenburg SR, Kwon KJ, Jha RK, McKay C, Jacobs M, Chertkov O, Twary S, Rocap G, Cattolico RA (2014). A pangenomic analysis of the *Nannochloropsis*organellar genomes reveals novel genetic variations in key metabolic genes. BMC Genomics 15:212.
Suda S, Atsumi M, Miyashita H (2002). Taxonomic characterization of a marine *Nannochloropsis*species, *N. oceanica*sp. nov. (Eustigmatophyceae). Phycologia 41:273-279.
Yang H, Yan R, Chen H, Dong HL, Zheng C (2007). Characteristics of hemicelluloses, cellulose and lignin pyrolysis. Fuel 86:1781-1788.