CHEMICAL ENGINEERING TRANSACTIONS
VOL. 75, 2019

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Sauro Pierucci, Laura Piazza

ISBN 978-88-95608-72-3; **ISSN** 2283-9216
DOI: 10.3303/CET1975096

Morphological Analysis by Scanning Electron Microscopy of *Dictyota menstrualis* in Natura and Following an Extraction Process

Ingrid E. S. Lima[a], Sabrina T. Martinez[b], Valéria L. Teixeira[c,d*], Wilma A. Gonzalez[a*]

[a]Postgraduate Program in Chemistry, Military Engineering Institute, Rio de Janeiro, RJ, Brazil
[b] INCT de Energia e Ambiente, UFBA, 40170-290 Salvador, BA, Brazil
[c] Postgraduate Program in Neotropical Biodiversity, Institute of Biosciences , Federal University of the State of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[d] Postgraduate Program in Science and Biotechnology, Fluminense Federal University, Niteroi, RJ, Brazil
valerialaneuville@gmail.com, d5wilma@gmail.com

The seaweeds are important sources for generating new products with biotechnological potential. The secondary metabolites found in macroalgae are obtained by extraction processes using organic solvents. However, there is scant data in the literature about the morphology of the macroalgae before and after the extraction process. The aim of this work was to verify the morphology and chemical composition of macroalgae – *Dictyota menstrualis* (brown algae), before and after the extraction process with organic solvents using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). Based on micrographic analysis, it was possible to verify the morphological differences of the biomass samples. Before the extraction process, *Dictyota menstrualis* showed dense morphology, and after the extraction process, it presented lamellar morphology, whose chemical composition consisted of the elements carbon, oxygen, sodium, magnesium, aluminum, silicon, sulfur, chlorine, calcium, potassium, and iron.

1. Introduction

The biomasses of seaweed are composed of substances with nutritional value and biological applicability. These primary and secondary metabolites are proteins, lipids, vitamins, sterols, polysaccharides (alginates, fucoidans, and sulfated galactans) and inorganic elements. It is important to emphasize that variations of these natural products exist among the classes of algae (brown, red, and green), in different species and even in macroalgae of the same species due to the influence of external factors (Wells et al., 2016).
These phytochemical constituents include, for example, glycolipids, sulfated polysaccharides, terpenes, phenolic compounds, and acetogenins (Nogueira and Teixeira, 2016). These substances are extracted by organic solvents with different polarities.
The cell walls of brown marine macroalgae are composed mostly of cellulose (Siddhanta et al., 2009), hemicellulose, and an amorphous matrix. This amorphous part consists of alginates and sulphated polysaccharides (Davis et al., 2003).
Marine algal metabolites account for 13% of marine natural products, with terpenoids prevailing at a percentage over 50% (Maschek & Baker, 2008, Young et al., 2015).The genus Dictyota includes species morphologically similar of the marine brown algae producers of biologically active substances, in particular, diterpenes whose skeletons are almost exclusively found in marine organisms (Chen et al., 2018). In Brazil, studies on diterpenes of *Dictyota menstrualis* demonstrated antiviral activity against the human herpesvirus type 1, HIV-1 (Pereira et al., 2004; Cavalcanti et al., 2011) and against the acquired immunodeficiency virus type 1, HSV-1 (Abrantes et al., 2010), and were promising to anticoagulant action front of snake venoms (Moura et al., 2014).

Paper Received: 22 March 2018; Revised: 6 August 2018; Accepted: 20 January 2019

Please cite this article as: Lima I., Martinez S., Teixeira V., Gonzalez W., 2019, Morphological Analysis by Scanning Electron Microscopy of Dictyota Menstrualis in Natura and Following an Extraction Process, Chemical Engineering Transactions, 75, 571-576
DOI:10.3303/CET1975096

However, several articles have reported on the morphology of macroalgae biomasses *in natura* compared to macroalgae submitted to different treatments. For example, the micrographs and EDX spectra helped character the *Sargassum filipendula* after double alginate extraction (Costa et al., 2016).
The scanning electron microscopy confirms for characterization of biomass both *in natura,* and biomass that underwent extraction process. The micrographs generated in this step allow the fresh seaweed morphological verification and your waste, so the possible changes in the ultrastructure of seaweed can be observed. The technique of EDX detects the chemical elements that make up the samples in different conditions of analysis.
The objective of this work was to verify the *in natura* morphology and after the dichloromethane extractive process from the brown macroalgae *Dictyota menstrualis* (Hoyt) Schnetter, Hörning, & Weber-Peukert using scanning electron microscopy and technique EDX.Through the obtained results of morphological and chemical composition these residues will be used as source of raw material of generation of new renewable products due to the advantage of absent lignin from wall cell.

2. Materials and methods

2.1 Algal material

Specimens of *Dictyota menstrualis* specimens were collected in November of 2016 at Enseada do Forno (Lat. 22° 45' 31.77" S and Longitude: 41° 56' 35.57" W), Armação dos Búzios, in the northern area of the state of Rio de Janeiro State of Rio de Janeiro, Brazil, in shallow water at a depth of 2.0 to 5.0 m by snorkeling. The algae were washed with local seawater and separated from sediments, epiphytes, and other associated organisms. Voucher specimens (HUNI 5019) were deposited in the Herbarium of the Universidade Federal do Estado do Rio de Janeiro (UNIRIO)-(Herbarium Professor Jorge Pedro Pereira Carauta). The algae cleaned were dried in air at room temperature.

2.2 Extraction process

The previously dried algae were exhaustively extracted with dichloromethane (CH_2Cl_2) for a week. The algae were kept in the shade, under room temperature. After this period, the biomass obtained was dried at room temperature.

2.3 Biomass Characterization utilizing the Scanning Electron Microscopy with Energy Dispersive Spectroscopy

Initially these samples of dried algae (both *in natura* and after the extraction process) were previously macerated and were sputter coated with platinum (LEICA ACE-600) and analyzed with the equipment FEI Field Emission Scanning Electron Microscope and energy-dispersive X-ray spectroscopy (EDX), Bruker. (Electron Microscopy Laboratory at the Military Engineering Institute).

3. Results and discussion

Modifications in the morphology of these seaweeds were viewed with scanning electron microscopy (SEM). After the extraction process, the biomasses presented significant changes and this suggested that dichloromethane had removed natural products generating a structural change in the biomass.
The *Dictyota menstrualis* is a brown marine macroalga found on the coast of the state of Rio de Janeiro. The micrographs of Figure 1(a-b) correspond to the sample of the marine macroalga *D. menstrualis in natura*. The analysis these images showed that this biomass had dense morphology with probable absence of porosity.

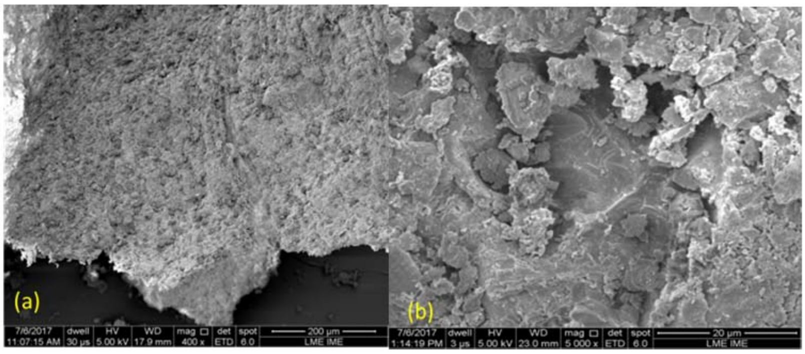

Figure1: Micrographs of the in natura and after extracted process biomass from the Dictyota menstrualis. (a-b) In natura macroalgae (c) extracted macroalgae (d) the material with oval form found

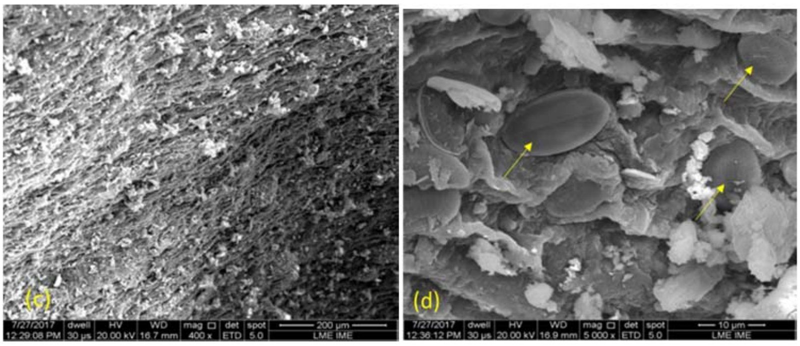

Figure1: Micrographs of the in natura and after extracted process biomass from the Dictyota menstrualis. (a-b) In natura macroalgae (c) extracted macroalgae (d) the material with oval form found

These micrographs of the biomass after the extraction process with an organic solvent showed significant morphological change, presented lamellar morphology with oval-shaped material aggregated in the algae biomass as shown in Figure 1(c-d).

The material found in the surface of the biomass was analyzed by SEM and EDX. Silicon was detected by EDX and together SEM images characterized as diatoms. The diatoms are organisms formed by resistant silicon shell (Souza et al., 2007, Moreno-Ruiz and Carreño, 1994). In addition to the chemical element Si, others including C, O, Na, Mg, Al, S, Cl, K, Ca, and Fe were detected, as shown in Figure 2(The unmarked peaks are relative platinum). In a study Costa et al. (2016) show through SEM-EDX results from *Sargassum filipendula* seaweed presence of diatoms shells composed by Si, Na, Mg, Al, S, K, Ca and Fe (Costa et al., 2016).

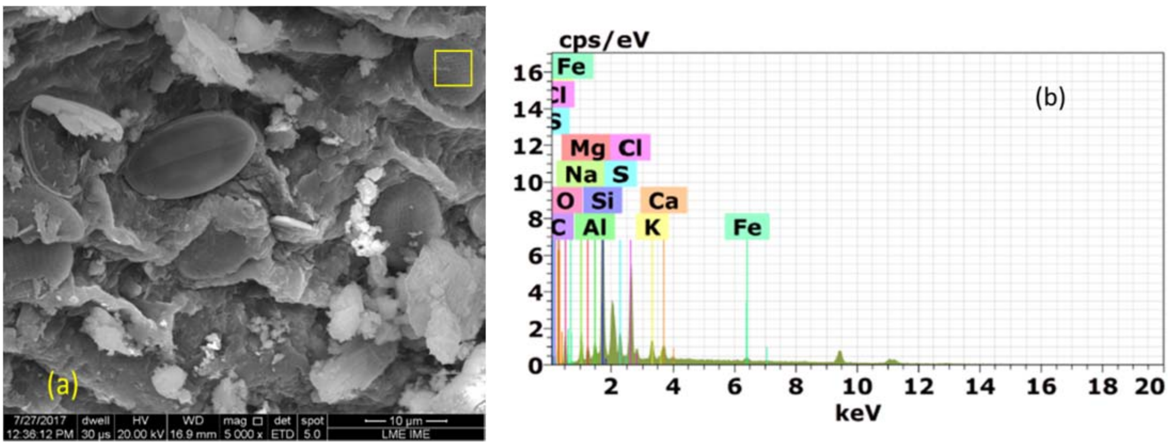

Figure 2: SEM of the diatom and EDX spectra. (a) diatom present in the brown macroalgae with rectangle marking the analyzed area by EDX. (b) EDX spectrum and the chemical elements detected.

Comparation of the morphology and chemical composition in natura and residue biomass is displayed exhibited in the Figure 3.

The EDX analysis was able to qualitatively identify chemical elements present on the surface of the algae biomass. This analysis was conducted at several regions on the macroalgae biomass. This analysis on different parts of the biomass allowed the verification of compositional heterogeneity. The regions presented different concentrations of chemical elements. On the *in natura* sample of *D. menstrualis*, the following elements were observed: carbon, oxygen, sodium, magnesium, aluminum, silicon, sulfur, chlorine, calcium, and potassium (Figure 3b). The extracted biomass algae presented the same chemical elements as in the *in natura* sample but had more of the element iron.

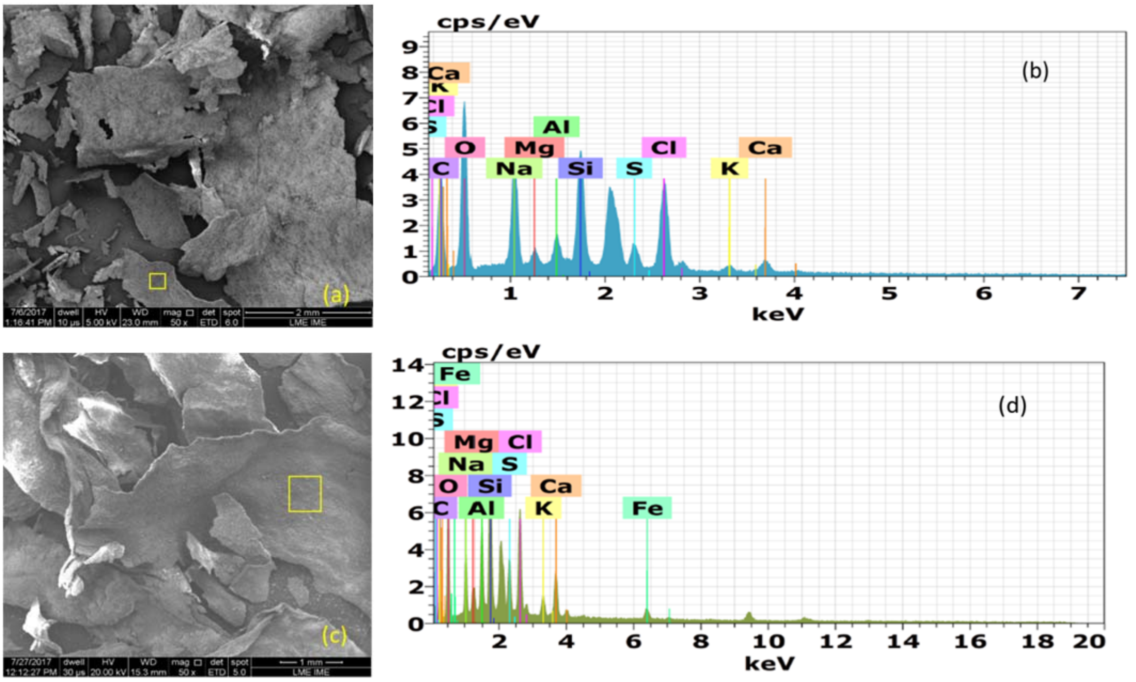

Figure 3: Micrographs and EDX of the in natura and after extracted process macroalga (a) in natura biomass (b) EDX spectrum from in natura biomass. (c) extracted biomass (d) EDX spectrum of the extracted biomass

Sulfur presence has been still observed, probably due to the existence of sulfated polysaccharides. Fucoidan is the polysaccharide found in the extracellular medium of brown algae (Davis et al., 2003). These substances have sulfur in your structure which would explain the presence of sulfur in the two samples of the *Dictyota menstrualis.*

Carbon and oxygen can be found in different substances in the seaweed, for example, in the polysaccharides stored in the algae biomass as mannitol and laminarin (Davis et al., 2003). The acidic alginic or alginate is found in the amorphous matrix or as extracellular material of the biomass from the brown algae.

Presence of metals can be explained by the ability of polysaccharides to “sequester” these elements. Andrade and other researchers (2010) concluded in their study that *P. gymnospora* seaweed synthesizes more polysaccharides in environments contaminated by heavy metal as a defense mechanism (Andrade et al., 2010).

Some light metal ions are acquired by ambient marine and usually are bound to the sites present in acid functional groups of the polysaccharides. In the *in natura* biomass of the brown algae it is possible to find K^+, Na^+, Ca^+ and Mg^+ ions found abundantly in the marine environment (Schiewer and Volesky ,1995). Several factors can influence this process, such as the type of biomass from the algae, pH, concentration of heavy metals, presence of competitive ions, temperature (Zeraatkar et al., 2016) and amount of alga biomass (Lau et al., 2003)

Marine macroalgae can be rich in Ca, Mg, Na, K, Fe, Mn, Zn, Cu, Ni, Co, Cr, Cd, and carbohydrates, where these elements vary among different species, and many of these elements have important nutritional value (El-Said and El-Sikaily, 2013). . After the extraction process of the biomass from *D. menstrualis,* the presence of iron was found in its composition.

In a study conducted by Deniaud-Bouët et al. (2014) the researchers suggested the existence of a cell wall model of brown algae of the order Fucales. In this model, the majority of the cell wall is composed of alginates and sulfated polysaccharides that are connected between cellulose microfibrils. A few cellulose microfibrils exist in the cell wall with tape form. The hemicelluloses are binding the cellulose with the sulfated polysaccharides that contain fucose. There are still phenols that are associated with alginates and proteins. Also has iodide, but its relationship with the other components of the cell wall has not been elucidated (Deniaud-Bouët et al., 2014). In this work it was not possible to observer microfibrills in the biomasses.

The natural products produced from the algae are hydrocarbons, polyphenols, carotenoids, sterols, and terpenoids (Teixeira, 2013). Andrade et al. (2004) conducted a study with the objective of evaluating the alginates and fucans sulfated present in the cell wall of the brown algae. Among the materials and methods used for this study were transmission electron microscopy (TEM) and EDXA technology. The alginates presented a sponge-like appearance and fucans-sulfated polygonal forms, and the EDXA spectrum found heavy metals present in these polysaccharides (Andrade et al., 2004).

4. Conclusions

The SEM images made possible the visualization of the morphology of the biomass from *Dictyota menstrualis* before and after extraction process. To complement this study, EDX analyses were used to verify the biomass chemical composition. The sample from *Dictyota menstrualis in natura* revealed dense morphology while the sample after extraction with dichloromethane presented lamellar morphology and the chemical elements detected were carbon, oxygen, sodium, magnesium, aluminum, silicon, sulfur, chlorine, calcium, potassium, and iron. In this study, diatoms were found that were characterized by SEM micrographs and the presence of the element Si in the EDX spectra.

Acknowledgments

The authors are grateful to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support and for Productivity Fellowships to VLT (301420/2010-6). VLT (E-26/103.176/2011) also thank the FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro) for the Cientista do Nosso Estado Fellowship. IESL thanks CAPES for the DSc fellowship.

Reference

Abrantes J.L., Barbosa J., Cavalcanti D.N., Pereira, R. C., Fontes C.L.F., Teixeira V.L., Souza T.L.M., Paixão I.C.N.P., 2010, The Effects of the Diterpenes Isolated from the Brazilian Brown Algae *Dictyota pfaffii* and *Dictyota menstrualis* against the Herpes Simplex Type-1 Replicative Cycle, Planta Medica, 76, 339-344.

Andrade L.R., Leal R.N., Noseda M., Duarte M.E.R., Pereira M.S., Mourão P.A.S., Farina M., Filho G.M.A., 2010. Brown algae overproduce cell wall polysaccharides as a protection mechanism against the heavy metal toxicity, Marine Pollution Bulletin, 60, 1482-1488.

Andrade L.R., Salgado, L.T., Farina M., Pereira M.S., Mourão P.A.S., Filho, G.M.A., 2004, Ultrastructure of acidic polysaccharides from the cell walls of brown algae, Journal of Structural Biology, 145, 216-225.

Cavalcanti, D.N., Oliveira M.R., De Paula J.C. Barbosa L.S., Fogel T. Pinto M.A., Paixão I.C.N.P., Teixeira V.L., 2011, Variability of diterpenes with potential anti- HIV activity isolated from the Brazilian brown alga *Dictyota menstrualis*, Journal of Applied Phycology, 23, 873-876.

Chen J., Li H., Zhao Z., Xia X., Li B., Zhang J., Yan X., 2018, Diterpenes from the Marine Algae of the Genus Dictyota, Marine Drugs, 16, 159- 184.

Costa S.D.C., Cardoso S.L., Nishikawa E., Viera M.G.A., 2016, Characterization of the Residue from Double Alginate Extraction from *Sargassum fiflipendula* Seaweed, Chemical engineering Transaction, 52, 133-138.

Davis T.A., Volesky B., Mucci, A., 2003, A review of the biochemistry of heavy metal biosorption by brown algae, Water Research, 37, 4311–4330.

Deniaud-bouët E., Kervarec N., Michel G., Tonon T., Kloareg, B., Hervé, C., 2014, Part of a special issue on plant cell walls. Chemical and enzymatic fractionation of cell walls from Fucales: insights into the structure of the extracellular matrix of brown algae, Annals of Botany, 114,1203-1216.

El-Said G.F., El-Sikaily, A., 2013, Chemical composition of some seaweed from Mediterranean Sea coast, Egypt, Environmental Monitoring Assessment, 185,6089-6099.

Lau T.C., Ang P.O., Wong P.K., 2003, Development of seaweed biomass as a biosorbent for metal ions, Water Science and Technology ,47, 49-54.

Maschek J.A., Baker B.J., 2008, The chemistry of algal secondary metabolism. In: Algal Chemical Ecology; Amsler, C.D., (Ed.), Springer: Berlin/Heidelberg, Germany, 1–24.

Moreno-Ruiz J.L.& Carreño A.L., 1994, Diatom biostratigraphy of Bahía Asunción, Baja California Sur, Mexico, Revista Mexicana Ciencias Geológicas, 11, 243-252.

Moura L.A., Almeida A.C.M., Domingos T.F.S., Ortiz-Ramirez F., Cavalcanti D.N., Texeira V.L., Fuly A.L., 2014, Antiplatelet and anticoagulant effects of diterpenes isolated from the marine alga *Dictyota menstrualis*, Marine Drugs, 12, 2471-2484.

Nogueira C.C.R. & Teixeira V.L., 2016. Seaweeds as source of new bioactive prototypes. In: Thajuddin, N, Dharumadurai, D (Ed) Algae - Organisms for Imminent Biotechnology, INTECH, Rijeka, pp.307-330.

Pereira H.S., Leão-Ferreira L.R., Moussatché N., Teixeira V. L., Cavalcanti D.N., Da Costa L.J., Diaz R., Frugulhetti I.C.P.P., 2004, Antiviral activity of diterpenes isolated from the Brazilian marine alga *Dictyota menstrualis* against human immunodeficiency virus type 1 (HIV-1), Antiviral Research, 64, 69-76.

Schiewer S., Volesky B., 1995, Modeling of the Proton-metal Ion Exchange in Biosoption, Environmental Science & Technology, 29, 3049-3058.

Siddhanta A.K., Prasad K., Meena R., Prasad G., Mehta G.K., Chhatbar M.U., Oza M.D., Kumar, S., Sanandiya, N.D., 2009, Profiling of cellulose content in Indian seaweed species, Bioresource Technology, 100, 6669- 6673.

Souza G.S., Koening M.L., Leça E.E., Coelho, M.P.C.A., 2007, Diatomáceas indicadoras de paleoambientes do Quartenário de Dois Irmãos, Recife, PE, Acta Botanica Brasilica, 21, 521-529.

Teixeira V.L., 2013, Produtos Naturais de Algas Marinhas Bentônicas, Revista Virtual de Quimica, 5, 343-362.

Wells M.L., Potin P., Craigie J.S., Raven J.A., Merchant S.S., Helliwell K.E., Smith A.G., Camire M.E., Brawley, S.H., 2016, Algae as nutritional and functional food sources: revising our understanding, Journal Applied of Phycology, 29, 949-982.

Young R.M., Schoenrock K.M., Von Salm J.L., Amsler C.D., Baker B.J., 2015. Structure and function of macroalgal natural products. In: Natural Products from Marine Algae: Methods and Protocols; Stengel, B.D., Connan, S., (Ed.), Springer: New York, NY, USA, 39–73.

Zeraatkar A.K., Ahmadzadeh H., Talebi A.F., Moheimani N.R., McHenry, M.P., 2016, Potential use of algae for heavy metal bioremediation, a critical review, Journal of Environmental Management, 30,1-15.