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Preparation of Chitosan Membrane derived from Crustaceans residues Forproton Exchange Membrane Application

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Abstract

Proton exchange membrane (PEM) is used in fuel cells, as an alternative to synthetic fuel cell membranes, currently widely used, a natural biopolymer chitosan is one of the promising membrane materials, it is the N-deacetylated derivative of chitin, and chitin has been found in a wide range of natural sources. Shrimp and crab crustacean exoskeletons are regarded as organic solid wastes by fishing industry and food processing in Ecuador. Thus, the raw material is cheap, biodegradable, and renewable source of chitin and chitosan. The general objective of this study was to obtain a cost effective and eco-friendly chitosan membrane derived from shrimp and crab residues and to estimate various, physicochemical parameters. The membrane chitosan preparation from shrimp and crab consisted of cleaned, grained to a finely powder. Chitin was extracted from the powder sample by deproteinization and demineralization process. Chitosan membrane was obtained from chitin by thermo alkaline deacetylation process and cross-linked in sulfuric acid. Ash content values of shrimp chitosan are lower than crab chitosan suggesting that crab chitosan contain large amounts of mineral material, such as calcium carbonate. The shrimp chitosan has a higher degree of acetylation and lower values of viscosities than crab chitosan. TGA and DTG measurements exhibit shrimp chitosan weight loss in three stages, whereas crab chitosan shows six stages of weight loss. This is associated to the evaporation of water present in the sample and degradation of compounds within crab chitosan that occurs over a large temperature interval with the final stage beginning at about 400°C. FT-IR spectroscopy results showed the collapse of shrimp and crab chitin to chitosan, strong decrease in intensity decarbonization and were compared to data from thermo gravimetric analysis conducted in oxygen atmosphere. XRD pattern of shrimp and crab chitosans exhibited two crystalline peaks at 2θ = 9.25° and 19.19°. In conclusions, chitin and chitosan were prepared from shrimp and crab residues, which are thrown as waste by the seafood industry, causing environmental menace in Ecuador. The results demonstrate the potential to produce shrimp and crab biopolymer as proton exchange membrane for fuel cells.

Keywords: Preparation, Chitosan, Membrane, Crustaceans, Forproton, Exchange, Membrane, Application.

Introduction

Proton exchange membrane (PEM) is used in fuel cells. The membrane serves to separate hydrogen gas or methanol, provide the electrolyte in the presence of water, which is readily absorbed by the membrane, the negative ions are rigidly held within the structure, and the positive ions (protons) contained within the membrane are mobile and are free to transport from the anode to the cathode (Figure-1), which is essential to fuel cell operation[1,2].

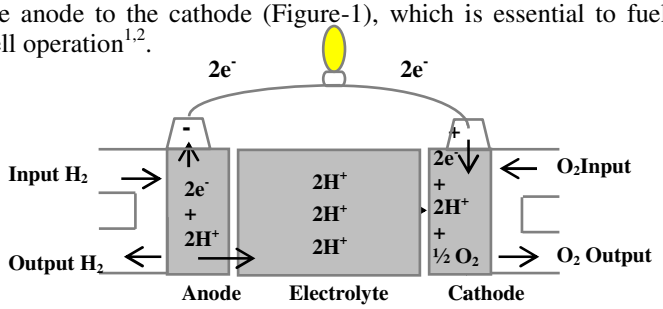

Figure-1
Proton exchange membrane fuel cell (PEMFC)

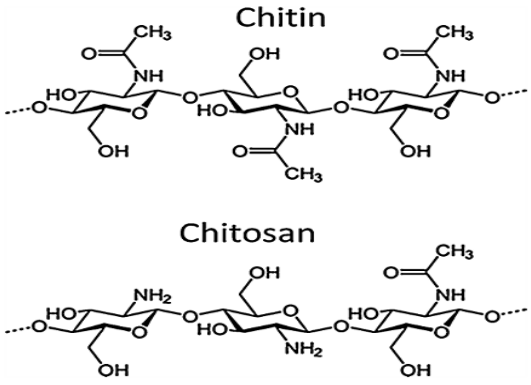

Figure-2
Structure of chitin and chitosan

(DDA) of chitosan influences the properties of chitosan and controls the degree of cross-linking in the presence of any suitable cross linker. For application as proton exchange membrane in fuel cells, the chitosan membranes should be

cross-linked in order to ensure physical and chemical stability in the presence of water. The chemical structure of chitosan membrane cross-linked is illustrated in Figure-3[2]. Currently, Nafion® manufactured by DuPont (Figure-4) is widely used as fuel cell membrane, which is consisted of a perfluorinated backbone with side chains that ended by strongly acid groups.[5] As an alternative to Nafion, a natural biopolymer chitosan is one of the promising membrane materials, it is the *N*-deacetylated derivative of chitin, and chitin has been found in a wide range of natural sources (crustaceans, fungi, insects, molluscs, etc.).

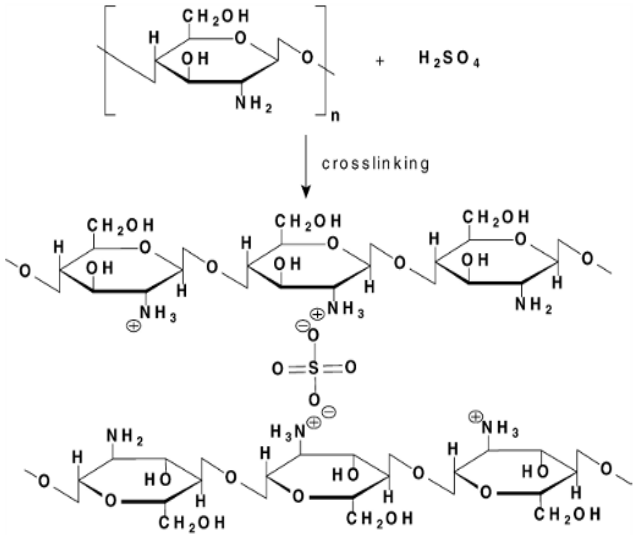

Figure-3
Chemical structure of ionically cross-linked chitosan. The amino groups in chitosan and sulfate ions have a coulombic interaction which makes possible the ionic crossing of the main chains

Shrimp and crab crustacean exoskeletons are regarded as organic solid wastes by fishing industry and food processing in Ecuador. Thus the raw material is cheap, biodegradable, and renewable source of chitin and chitosan[5]. The purpose of this research is to: obtain a cost effective and eco-friendly chitosan membrane derived from shrimp and crab residues; to develop the membrane preparation method and; to estimate various, physicochemical parameters.

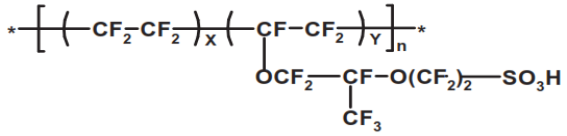

Figure-4
Chemical structure of Nafion®

Materials and Methods

Preparation of shrimp and crab chitosans: Figure-5 describes the process of preparation for shrimp chitosan. Shells residues were collected from the food market, samples were washing with water, dried until constant weight at 60°C and milled using a Wiley knife mill, and sieved to (-250µm, +180µm) and store for further experiments. Shrimp chitin was obtained by deproteinization, 2±0.0005 g of shrimp sample was soaked in 25 mL 6M sodium hydroxide at 65°C, mixed thoroughly for 3 h, and then filtered through Whatman #1, and rinsed to neutrality in a funnel with distilled water. The process was followed by demineralization, the alkali treated shrimp sample was soaked 25 mL 1 M hydrochloric acid at 65°C, and mixed thoroughly for 3 h, and then filtered through Whatman #1, and rinsed to neutrality in a funnel with distilled water. Chitin, thus obtained was converted into chitosan by the process of deacetylation as described in Ramirez Perez[6]. Chitin was deacetylated in 70% NaOH by heating and mixed thoroughly for 5 h. The obtained precipitate was washed to neutrality with distilled water. To purify, chitosan samples were dissolved in 25 mL 1.5% acetic acid solution, at 60°C, mixed thoroughly for 4 h, and then the pH was adjusted to 10 with 40% NaOH solution, and placed in a flat glass container, dried at 40°C, 24 h to obtain a chitosan membrane. The dried chitosan membrane was later cross-linked and protonated by submersion in 4% H_2SO_4 for 24 h, to promote interaction among the amino groups in chitosan and sulfate ions and a three dimensional structure of polymeric cross-linked chains, which increase chitosan membrane resistance. The membrane was then washed and stored in de-ionized water at room temperature to obtain a proton exchange membrane. Whereas the shrimp chitosan membrane was colorless and transparent, the crab chitosan membrane exhibited a pale white color. However, both chitosan membranes were flexible and showed mechanical resistant. Due to manual casting of the chitosan membranes their size and thickness were variable. The membrane chitosan preparation from crab followed the same procedure as shown in Figure-6.

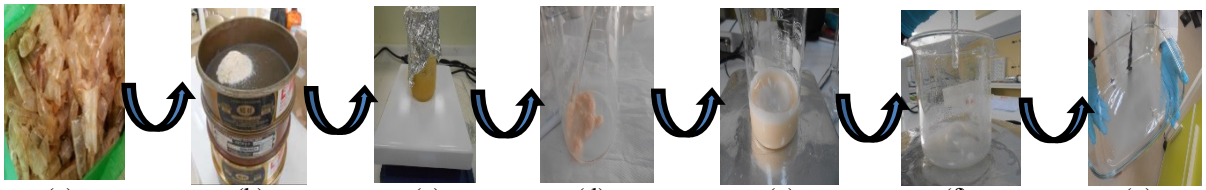

Figure-5
Preparation of shrimp chitosan membrane:
(a) Shrimp waste residues, (b) Milled and sieved (c) Deproteinization and demineralization, (d) Shrimp chitin, (e) Deacetylization, (f) Chitosan shrimp biopolymer and cross linked, (g) Chitosan shrimp membrane

Viscometry measurements: The viscosity of shrimp and crab chitosan samples were measured in the pseudo plastic fluid state using a Sine-wave Vibro Viscometer (SV-100). The viscosity was measured immediately after chitosan and crab samples underwent the purification process. Thus, the sample was hot to start the measurement and it was recorded each 5 sec until the sample cooled down to room temperature.

Thermogravimetry.

Thermogravimetric measurements were performed on a TA instrument SDT Q600. Small chitin (chitosan) sample powder was crimped in a standard container and heated gradually from room temperature to 900^{o}C at heating constant rate of 20^{o}C/min, under oxygen dynamic flow rate of 100 mL/min. The temperature of the decomposition onset, temperature at maximum process rate, and percentage weight loss were evaluated from the thermogravimetric (TG) and derivative thermogravimetric (DGT) curves.

FT-IR spectroscopy: The Fourier Transform Infrared (FT-IR) spectra of shrimp chitosan and crab chitosan samples were recorded on a Perkin Elmer GX2000, samples were between 95% and 5% transmittance, from 370 to 10,000 cm^{-1} with a resolution of 8 cm^{-1} from 9 scans at room temperature (RT). Each sample was vacuum-dried at 80^{o}C for 6 h and then cooled in a desiccator with color silicone. A pellet was formed from a sample and KBr at pressure of 8 Ton/cm^{2}. Data analysis was carried out using Perking Elmer Spectrum.

X-ray diffraction (XRD): The structural characteristics of chitosan (chitin) was determined by using X-ray diffract meter (XRD) Bruker-D8- Advance. The intensity of the diffracted X-rays is measured as a function of the diffraction angle 2θ and the specimen orientation. The wavelength used was 1.5418 A^{o} corresponding to Cu, at 0.01oA at 40kV and 40 mA.

An experimental factorial design was applied in order to design the experimental assays to enhance the DDA in chitosan membrane, the selected factors were, temperature, % acetic acid, stirring and washing time each one at five levels, as a response independent tests of, qualitative mechanical tests and viscosity.

Results and Discussion

Shrimp chitosan samples were visibly cleaner than samples of crab chitosan. The crab chitin samples dissolved small fragments of red-pink exoskeletons that may contain carotenoids, whereas solutions of shrimp chitin were colourless. Table-1 shows same physical and chemical parameters of shrimp and crab chitosans. Ash content values of shrimp chitosan were lower than crab chitosan suggesting that crab chitosan contain large amounts of mineral material, such as calcium carbonate. The shrimp chitosan has a higher degree of acetylation and lower values of viscosities than crab chitosan. It was reported that the viscosities and the non-Newtonian flow properties of chitosan solutions increase with the degree of deacetylation of chitosan and additional salt decreases the viscosities and the non-Newtonian flow properties of the solutions of chitosan[7]. This suggests that shrimp chitosans have lower degree of acetylation than crab chitosan. Moreover, the mineral contents in crab chitosan decrease the values of viscosities.

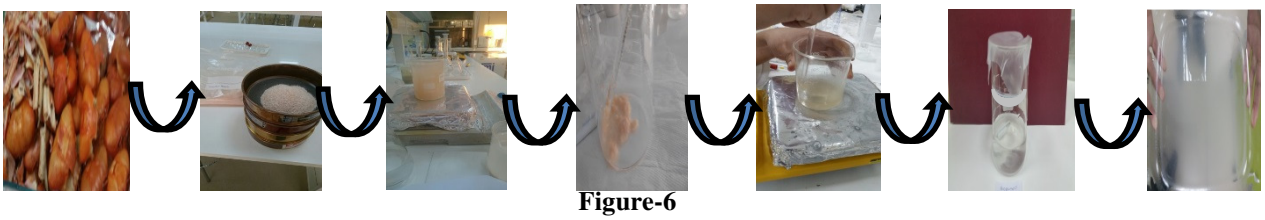

Figure-6

Preparation of crab chitosan membrane: (a) crab waste residues, (b) milled and sieved (c) deproteinization and demineralization, (d) crab chitin, (e) deacetylization, (f) chitosan crab biopolymer and cross linked, (g) chitosan crab membrane

Table-1

The Ash, moisture, nitrogen and acetyl content of shrimp and crab chitosans

%Moisture	% Ash	% Nitrogen	%DA*	Viscosity (Pa.s)
	Shrimp chitosan			
7.78+/-0.55	0.56+/-0.05	7.84+/-0.24	47.2	0.91 - 3.35
	Crab chitosan			
5.57+/-0.65	0.63+/-0.05	8.20+/-0.04	27.4	1.1 - 4.25

*Degree of acetylation (DA)=[(8.69-%nitrogen content)/(8.69-6.89)]

The purification process optimization of shrimp chitosan was achieved at the following operation conditions: 80°C, 1.5% acetic acid, 120 rpm of stirring, and 4 h under continuous washing.

Thermogravimetric analysis, weight loss (TGA) and derivative of weight loss (DTG)curves are overlaid so as to obtain a better understanding of thermal decomposition mechanism is given for shrimp chitosan (Figure-7) and crab chitosan (Figure-8) in the presence of oxygen (in air atmosphere). Shrimp chitosan exhibits three stages of weight loss. The first stage takes place between 5 and 50°C with almost 15% loss of the initial weight. This is probably due to the presence of water, which is evaporated at low temperature. This is followed by a further 59% weight loss around 200°C and ends around 300°C (stage 2). Perhaps, strongly hydrogen-bonded water is released.

The third stage of decomposition is observed at around 475°C, which indicates about 35% drop of shrimp chitosan. Some researchers have attributed this loss to depolymerization of chitosan chains, decomposition of pyranose rings through dehydration and deamination and finally ring-opening reaction[8,9]. In the final stage an additional loss of the sample decomposition is observed starting around 475°C and ending at about 850°C. TGA of crab chitosan shows six stages of weight loss (Figure-8). The first stage takes place between 5 and 50°C with almost 10% loss of the initial weight. This is followed by a further, 7% (180°C), 14% (240°C), then 11% (350°C), 30% (400°C), 7.5% (715°C) drop of crab chitosan, respectively. This sequential stages of crab chitosan weight loss is probably associated to the evaporation of water present in the sample and degradation of compounds within crab chitosan that occurs over a large temperature interval. Also, it could be to the same reasons attributed above to shrimp chitosan.

FT-IR spectroscopy was used to determine the structure of chitin and chitosan of shrimp and crab, respectively. The FT-IR spectra of chitin and chitosan were recorded within the wavelength region of 400-4000 cm^{-1}. Table-2 demonstrates the FT-IR peak assignments of shrimp and crab chitosan in oxygen (air atmosphere). Shrimp chitin and chitosan IR spectra are shown in Figure-9. The difference between shrimp chitosan IR spectra Figure-9 (B and C) was the purification method under 1 and 1.5% of acetic acid. In the spectra of shrimp chitin extracted from shrimp exhibited the band at 3446 cm^{-1} corresponds attributed to the H-bonded OH stretching, the band at 2900 cm^{-1}corresponds to aliphatic CH stretching.

The band at 1430 to 1380 attributed to NH bending, ant the band at 850 cm^{-1} is associated to ring skeletal vibration. The FTIR spectrum of shrimp chitosan exhibited bands around 3450 to 3420 cm^{-1}corresponds to H-bonded NH_2 and OH stretching. Further, the bands1400 and 1550 $cm^{-1}$are assigned to amide groups stretching of C=O and the well defined peak at 899 cm^{-1}corresponds to saccharide structure (Figure-9B and 9C). The results are consistent with FT-IR spectrum characteristics reported in the literature[10,11]. It also confirms our earlier observations of shrimp and crab chitosan, where the significant changes in loss of weight were monitored by TGA/DTG within the temperature range.

The infrared spectrum of crab chitin and chitosan in oxygen (air atmosphere) is depicted in Figure-10 (A and B). The FT-IR spectra of crab chitin is shown in Figure-10A, the band at 3375 cm^{-1}corresponds to H-bonded NH stretching, the band at 2950 cm^{-1} corresponds to CH stretching, the band at 2500 cm^{-1} is assigned to overlapping stretching vibration of CH and OH groups, the bands of 1475 and 1088 cm^{-1} indicating the NH_2 amide II and CO stretching, and the band at 899 cm^{-1} corresponds to the saccharide structure. The FT-IR of the crab chitosan showed bands of 3450 cm^{-1} attributed to H-bonded OH stretching.

The bands between 2950 and 2881 cm^{-1} corresponds to CH_2 and CH stretching vibration. The bands of 1700 and 1600 cm^{-1} correspond to CO of NH-CO stretching, and the bands of 1597, 1412, 1323, 1100 and 1088 cm^{-1} indicating the CO of COONa antisymmetric and symmetric stretching, CN stretching, and CO stretching, respectively. Therefore, the drop in intensity of shrimp chitosan and crab chitosan bands indicated above could be attributed to dehydration, deacetylation and depolymerization and thus degradation reactions.

Table-2
FT-IR analysis of shrimp and crab chitosans

Material	Wave number (cm^{-1})	Peak assignment
Shrimp chitosan	3450 to 3420	Overlapping Stretching of C-H and O-H groups
	1400 to 1550	Amide III band and Amide II band
	899	Saccharide structure
Crab chitosan	3450	-OH stretching and N-H stretching
	2950 to 2900	-CH_2 stretching vibration
	2881	C-H stretching
	1700 to 1600	C=O of NH-C=O stretching
	1597	C=O of –COO Naantisymetric stretching
	1412	C=O of –COO Nasymetric stretching
	1323	C-N stretching
	1100 to 1088	C-O stretching

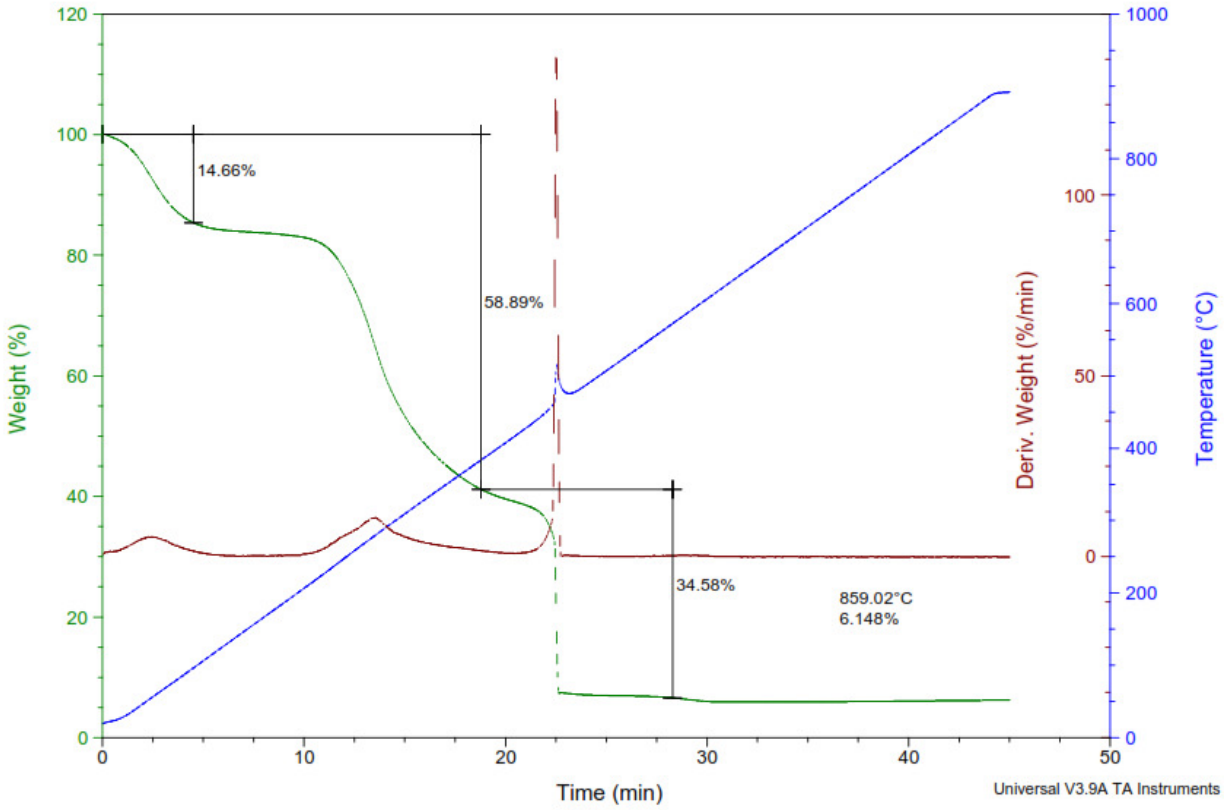

Figure-7
Thermal analysis curves TG/DTG of shrimp chitosan in the presence of oxygen

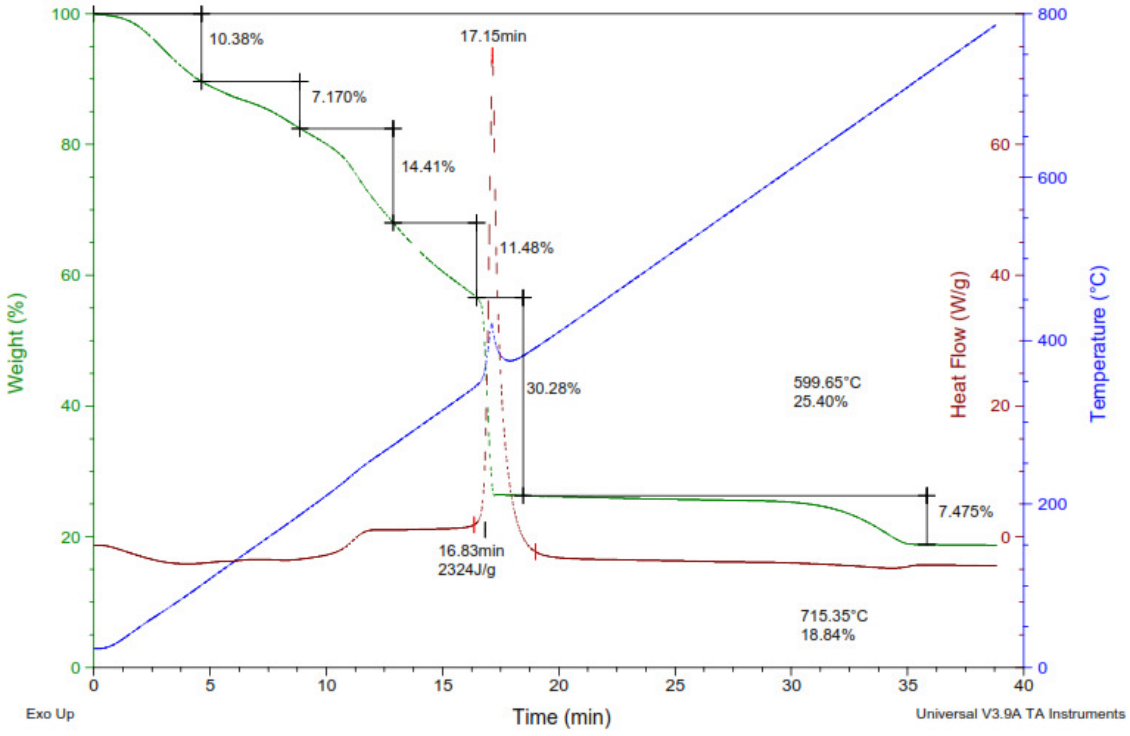

Figure-8
Thermal analysis curves TG/DTG of crab chitosan in the presence of oxygen

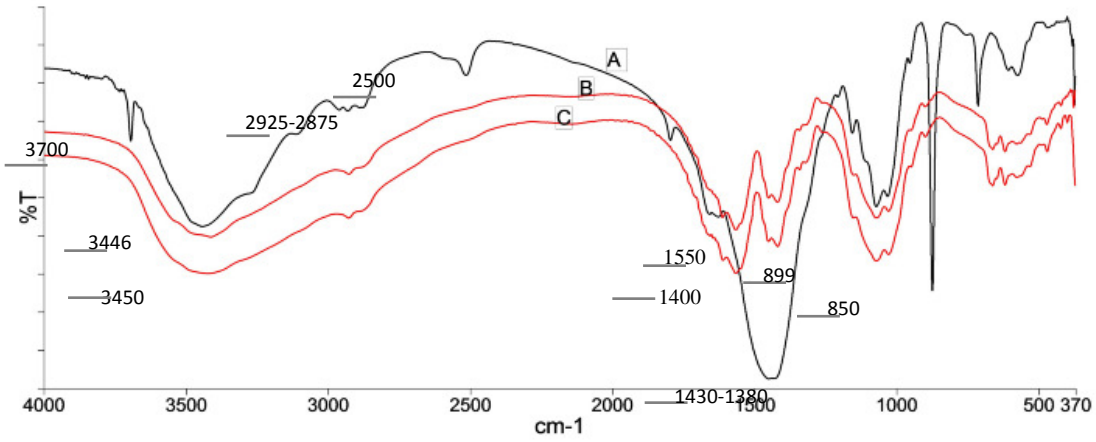

Figure-9
FTIR spectra of crab chitin (A) and shrimp chitosan (B) and (C)

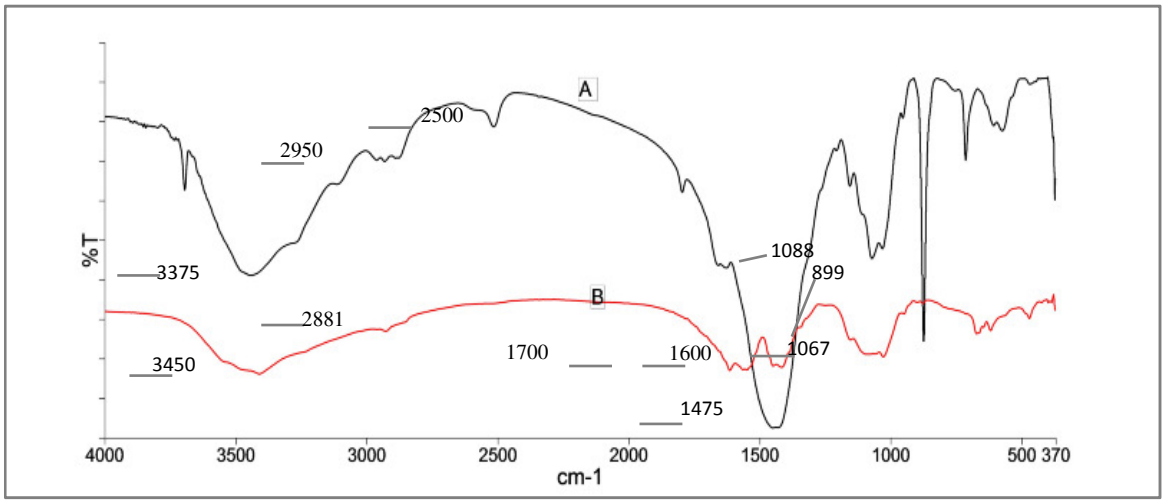

Figure-10
FTIR spectra of crab chitin (A) and crab chitosan (B)

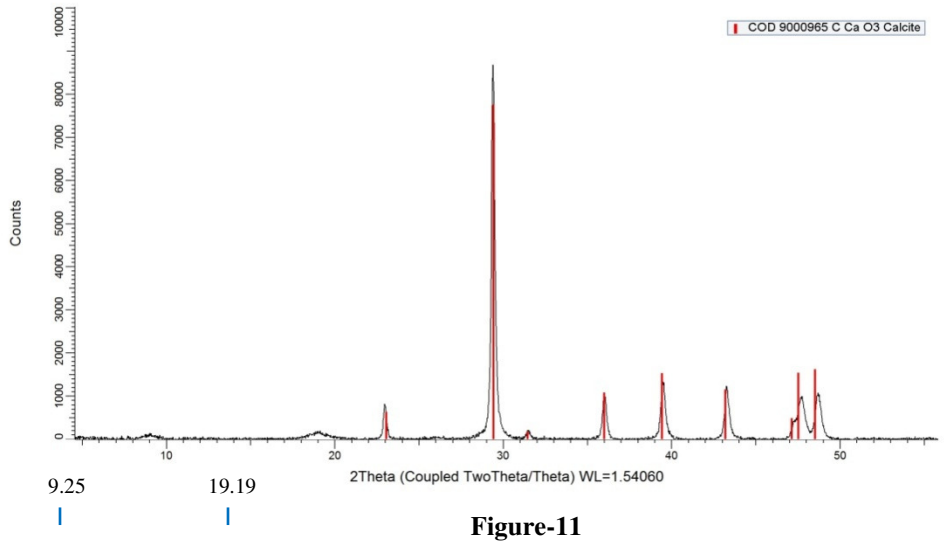

Figure-11
XRD pattern of crab chitosan

The XRD pattern of crab chitosan exhibits its characteristic crystalline peaks at $2\theta = 9.25^{o}$ and 19.19^{o} in Figure-8. In addition the wide angle X-ray diffraction pattern shows the presence of calcite, which is associated to crustacean species composition. Other researchers also reported that X-ray diffraction pattern of commercial crab chitosan as two crystalline peaks at $2\theta = 10.1^{o}$ and 19.8^{o}, whereas that of extracted crab chitosan showed only one crystalline peak at $2\theta = 19.4^{o}$[11]. When the same crab chitin was purified using different purification method found that the wide angle X-ray diffraction pattern of purified chitosan showed two peaks at $2\theta = 9.3^{o}$ and 19.1^{o}, which coincided with our findings.

This observation confirmed our earlier TGA observations of shrimp chitosan degradation in three stages and crab chitosan in six stages of loss of weight. In addition, the presence of calcite and magnesium, confirmed for both crustacean, the conversion from chitin to chitosan, respectively by a decarbonization.

Conclusion

In conclusions, chitin and chitosan were prepared from shrimp and crab residues, which are thrown as waste by the seafood industry and fisheries, causing environmental menace in Ecuador. The results of the present study demonstrate the potential to produce shrimp and crab biopolymer as proton exchange membrane for fuel cells, as well as for other applications. Shrimp chitosan showed a higher degree of acetylation and lower values of viscosities than crab chitosan. Crab chitosan exhibited more stages of weight loss than shrimp chitosan, which is associated to the evaporation of water that occurs over a large temperature interval monitored by TRGA/DTG. The drop in intensity of shrimp chitosan and crab chitosan bands monitored by FT-IR indicated dehydration, deacetylation and depolymerization and thus degradation reactions. The XRD pattern of crab chitosan exhibits its characteristic crystalline peaks at $2\theta = 9.25^{o}$ and 19.19^{o}. Work on proton conductivity measurements of shrimp and crab chitosan membranes and on the influence of acetylation on the properties of chitosan proton exchange membrane for fuel cells are in progress.

Acknowledgements

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References

1. Mekhilef S., Saidur R. and Safari A. (2012). Comparative study of different fuel cell technologies. *Renewable and Sustainable Energy Reviews*, 16, 981-989

2. P. Mukoma S., Jooste B.R. and Vosloo H.C.M. (2011). Synthesis and characterization of cross-linked chitosan membranes for application as alternative proton exchange membrane materials in fuel cells. *J. of Power Sources*, 136, 16-23.

3. Lambertus A.M. van den Broek et. al. (2015). Chitosan films and blends for packing material. Crabohydrate Polymers, 116, 237-242.

4. Park C.H., Lee C.H., Guiver M. and Lee Y.M. (2011). Sulfonated hydrocarbon membranes for medium-temperature and low-humidity proton exchange membrane fuel cells (PEMFCs). *Progress in Polymer Science*, 36, 1443-1498.

5. Ramirez-Perez Javier C. (2015). Development of novel hybrid materials from natural pigments and biopolymers for organic solar cells and fuel cells fabrication in Ecuador. Technical Report, SENESCYT, Quito-Ecuador.

6. Ramirez-Perez Javier C. (2013). Aerobic composting kinetics of biodegradation organic wastes, LAM. 1st ed., Saabrücken, Germany, 1-31, ISBN:13:978-3659461354.

7. Wang W. and Xu D. (1994). Viscocity and flow properties of concentrated solutions of chitosan with different degrees of deacetylation. *Int. J. Biol. Macromol.*, 16(3), 149-152.

8. Zawadzki J. and Kaczmarek H. (2009). Thermal treatment of chitosan in various conditions. *Carbohydrate Polymers*, 80, 395-401.

9. Wanjun T., Cunxin W. and Donghua C. (2005). Kinetic studies on the pyrolysis of chitin and chitosan. *Polymer Degradation and Stability*, 87, 389-394.

10. Kong Xiangping (2012). Simultaneous determination of degree of deacetylation, degree of substitution and distribution fraction of –COONa in carboxymethyl chitosan by potentiometric titration. Carbohydrate Polymers, 88, 336-341.

11. Subhapradha N., Ramasamy P., Shanmugam V., Madeswaran P., Srinivasan A. and Shanmugam A. (2013). Physicochemical characterization of β-chitosan from Sepioteuthislessonianagladius. *Food Chemistry*, 141, 907-913.