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# Characterization and Application of *Agave salmiana* Cuticle as Bio-membrane in Low-temperature Electrolyzer and Fuel Cells

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**Featured Application:** The present work it can be applied to obtain electric energy in fuel cells and the obtaining of H<sub>2</sub> in electrolyzer cells.

**Abstract:** This work describes the application of the *Agave salmiana* cuticle as a new protonic exchange biological membrane (0.080 ± 0.001 mm thickness). Different chemical, electrochemical and mechanical treatments were evaluated to stimulate the ionic exchange properties of the cuticle. Thermal treatment was adequate for its application in a two-chamber electrolyzer. Under optimal conditions an ionic conductivity value of 10 ± 3 mS cm<sup>-1</sup> was obtained; this value is similar to the value achieved using a Nafion membrane. The thermally-activated bio-membrane was also evaluated in a fuel cell, where the highest potential was obtained using methanol and hydrogen (0.46 ± 0.01 V). This result makes the *Agave salmiana* cuticle a competitive choice to replace the commercial membrane. Its surface morphology and their functional groups were evaluated through scanning electron microscopy (SEM), infrared spectroscopy and impedance spectroscopy. This thermally-treated *Agave salmiana* cuticle is an ecofriendly alternative to replace Nafion membranes in electrolyzer and fuel cells.

**Keywords:** Bio-membrane; *Agave salmiana*; cuticle; electrolyzer cell; fuel cell; conductivity

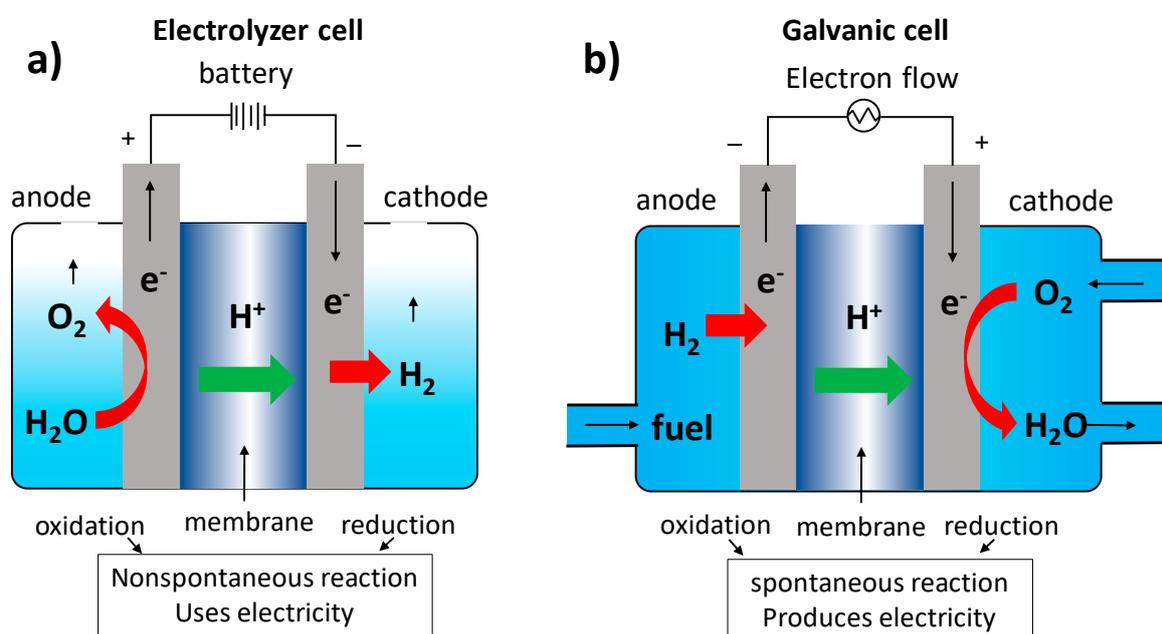
## 1. Introduction

Nowadays alternative energy source technologies have attracted great attention, due the depletion of fossil fuels and the consequences that combustion gases have upon global warming. The excessive use of fossil fuels has a large impact in the environment and poses a great risk for human health. Over the last decades, several research groups have focused their efforts on developing new alternative energy sources, such as solar cells [1], wind power [2], biogas [3], marine energy [4] and electrochemical cells [5]. These latter, particularly the electrolyzer and hydrogen fuel cells, are considered to be the main alternatives to fossil fuels used in automobiles [6–8].

Depending on the membrane type, electrochemical cells can be classified as: Proton exchange membrane fuel cells (PEMFC), alkaline fuel cells (AFC), phosphoric acid fuel cells (PAFC), molten carbonate fuel cells (MCFC) and solid oxide fuel cells (SOFC) [9].

PEMFC are considered an important prospect, since the temperature required for energy production is within 25–100 °C [10]. The amount of reagents, products and residues generated

from electrolyzer and hydrogen fuel cells is much less compared to that produced by other energy sources. Furthermore, components used in these cells are simple and robust, as shown in Figure 1.



**Figure 1.** Components of electrochemical cells. (a) Electrolyzer cell (electrolytic) and (b) hydrogen cell (galvanic cell).

During energy production the membrane is deemed to play a very important role in the efficiency of the electrochemical cell, as it is responsible for separating the electrodes and allowing the free flow of  $H^+$  ions between the anode and the cathode [11]. The membrane consists of a film that contains adequate functional groups that allow ion exchange, for example ( $-COOH$ ,  $-SO_3H$ ) [12].

Perfluorosulfonic acid-type membranes (Nafion) are commonly used in PEMFC applications. Nafion membranes have been demonstrated to have high proton conductivity, flexibility and excellent chemical and mechanical stability in their soaked state [13]. Over the past few years, Nafion has been used in direct ethanol and methanol fuel cells, hydrogen cells and electrolyzer cells [14]. The main drawbacks of Nafion membranes are their methanol permeability, high cost and loss of proton conductivity at approximately  $100\text{ }^\circ\text{C}$  [15]. In this respect, some modifications with graphene oxide have been made, but it was discovered that these hybrid membranes could not replace Nafion membranes completely [16]. Other studies have been proposed, with inorganic membranes and bio-membranes obtaining similar performances to the Nafion membrane [17,18].

In recent years different authors have characterized and assessed the efficiency of membranes through different techniques such as impedance spectroscopy (Nyquist diagram). The resistance of membrane pores is associated with their ionic conductivity, so at a lower resistance the membrane will offer better efficiency [19,20]. Different comparisons of new membranes to existing ones have also been made, and this has simultaneously led to the development of innovative fuel and electrolytic cells [21,22].

*Agave salmiana* is an arid and semi-arid plant commonly found in Mexico; it can survive extreme conditions, thanks to its cuticle. The cuticle epidermis is mainly responsible for gas exchanging through stomata, which allows the cuticle to act as an interface between the cell and the external environment [23]. Some researches have used the agave plant with different purposes, for instance bioethanol production [24], as an additive in cement mortars [25], bio-polymer composite formation [26], etc. The chemical characterization of the *Agave salmiana* cuticle has demonstrated the presence of  $-OH$  and  $-COOH$  functional groups [27–29].

For bio-membranes, ion mobility activation processes are required because this step negatively affects the efficiency of the electrochemical cell. There are different treatments types that allow ion movement; the most common ones are chemical, electrical, mechanical and thermal [30]. The present article describes the use of this *Agave salmiana* cuticle as a proton-exchange bio-membrane integrated into electrolyzer and fuel cells, and this as an alternative to the Nafion membrane

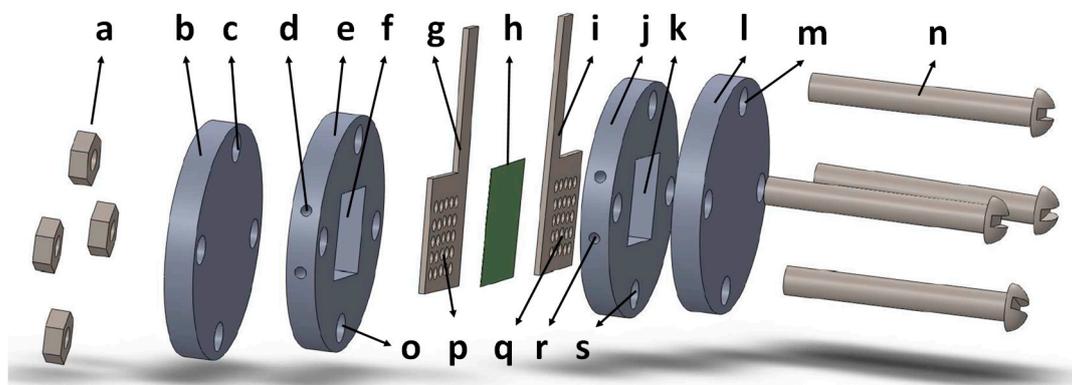
## 2. Materials and Methods

### 2.1. Reagents and Chemicals

All solutions were prepared by dissolving the respective analytical grade reagent in deionized water (resistivity not less than 18.0 M $\Omega$  cm) which was supplied from a Mili-Q system (Millipore, Bedford, MA, USA). The solutions employed for electrolyzer and fuel cell experiments were lye (NaOH) (0.1 M) and ethanol (0.5 M), respectively and they were prepared before each experiment. Sodium hydroxide, sulfuric acid, Nafion, chloroform, methanol, hexane, phenol, ethanol, diethyl ether and 1,2-ethylenediamine were obtained from Sigma (St. Louis, MO, USA).

### 2.2. Apparatus

All experiments involved the use of a conventional electrochemical cell of two chambers joined by a membrane, as shown in Figure 2. An adjustable AC-DC power supply (3B Scientific, model u33.35, Hamburg, Germany) was used in the electrolyzer analysis as a voltage source or current source. A galvanostat/potentiostat (Princeton Applied Research, Versa Stat 3, Ametek, Inc., Oak Ridge, TN, USA) was employed to measure the potential of the fuel cell. A pH/ion analyzer (Corning Science Products, model 450, NY, USA) was used to adjust the pH of the electrolyte solution to the desired value with intervals as short as 0.01 pH.



**Figure 2.** Conventional electrochemical cell of two chambers. a, nuts; b, l, Nylon covers; c, m, o, s, screw holes; d, r, oxidant and reducer agent input and products and excess reagent output, respectively; e, j, structures of the oxidation and reduction chambers; f, k, chamber cavities for oxidation and reduction; g, i, stainless steel electrodes of the electrolyzer cell, and which are both coated with Pt particles for the fuel cell; h, bio-membrane of the agave cuticle; n, screws; p, q, holes within both stainless steel electrodes (g and i) to achieve good contact among the reagent, electrode and membrane.

The bio-membrane (Figure 2h, 0.8 mm thick and 4.0 cm<sup>2</sup> large) was installed within the electrolyzer cell between the stainless steel electrodes (Figure 2, represented by g, i) and the chambers were filled with sodium hydroxide (NaOH) solution (Figure 2, denoted by letters f, k). The working temperature was 369 K at atmospheric pressure. On the other hand, in the fuel cell setup the bio-membrane (Figure 2, indicated as h, 0.8 mm thick and 4.0 cm<sup>2</sup> large) was installed between the stainless steel electrodes covered with Pt particles.

The anode chamber (Figure 2, as f) was filled with ethanol solution, whereas an air stream was pumped into the cathode chamber (as an oxygen source, Figure 2, given as k) was promoted by a peristaltic pump (ECOSHEL, model: RD100-01, Denver, CO, USA).

### 2.3. Bio-Membrane Treatment and Activation

In order to promote ion mobility, the bio-membrane must be previously activated. This step is critical, and the treatment employed depends upon the composition of the organic phase and the type of electrochemical cell used [31,32]. Therefore, this section describes the evaluation of several treatments for the proposed bio-membrane which were considered.

For this process, a 9 cm<sup>2</sup> (3 × 3 cm) *Agave salmiana* leaf was taken with the adaxial side facing down, and the membrane was carefully cut off with a scalpel. The external part was exfoliated with sandpaper (Grit designation of 500) until a 0.08 mm film was obtained [33].

Different treatments are proposed in this work, and an unmodified membrane serves as a control. The obtained bio-membrane was incorporated into an electrolyzer, while the bio-membrane with the best behavior was incorporated into a fuel cell. Five treatments were evaluated:

- i. Individual immersion (24 h) of the bio-membrane in 50 ml of: An acid solution (HCl 0.1 M), an alkaline solution (NaOH 0.1 M) and a neutral medium (deionized H<sub>2</sub>O).
- ii. Clean-up treatment of the abaxial face with a cotton ball soaked in organic solvents (chloroform, ethyl ether, hexane and methanol).
- iii. Polymeric coating of the bio-membrane by immersion in a Nafion (5 wt%) ethanol:water (1:2 v:v) solution during 60 minutes with agitation.
- iv. Scraping of the cuticle abaxial face to remove any wax from its surface.
- v. Thermal treatment by immersing the bio-membrane into boiling water for 4 h.

In all cases, the bio-membrane was washed twice with deionized water (20 mL) after its treatment. The bio-membrane was maintained at 4 °C in deionized water until its incorporation into the electrolyzer or fuel cell.

Before its use in different cells, the bio-membrane was placed between two stainless steel electrodes and immersed in NaOH 0.1 M solution. To stimulate ion exchange in the cuticle stomata, a potential of 15.0 V was applied for 3.0 min.

## 3. Results

### 3.1. Biomembrane Characterization in a Coupled Electrolyzer Cell

In order to evaluate the effect of each treatment, the bio-membrane was incorporated into the electrolyzer cell using NaOH (0.1 M) as the electrolyte solution in the two chambers. All experiments were performed at room temperature (298 K), an applied current of 4.4 mA was used while the potential (V) of the electrolyzer cell was being recorded. Electrical resistance ( $R$ ,  $\Omega$ ) was measured, and ion mobility ( $\sigma$ , mS cm<sup>-1</sup>) was then estimated according to Equation (1), where “ $l$ ” is the membrane thickness (cm), and “ $A$ ” is the cross-sectional area ( $A$ , 4.0 cm<sup>2</sup>) [34].

$$\sigma = \frac{l}{R * A} \quad (1)$$

A continuous evaluation of the bio-membrane was carried out under standard conditions in order to determine the membrane half-life without losing its efficiency in the electrochemical cell. These experiments were performed in triplicate in different solutions; results obtained in the electrolyzer cell are displayed in Table 1.

**Table 1.** Activation treatments of the bio-membrane.

Treatment		Potential (V)	$\sigma$ mS cm <sup>-2</sup>	Half-life (h)
<b>Without treatment</b>		9.3	1.39 ± 0.01	0.05
<b>Immersion</b>	H <sub>2</sub> O	3.9	0.03 ± 0.01	0.06
	NaOH 0.1 M	3.8	0.02 ± 0.03	0.08
	H <sub>2</sub> SO <sub>4</sub> 0.1 M	3.6	0.02 ± 0.06	0.08
	chloroform	4.4	0.11 ± 0.03	0.05
<b>Clean-up</b>	ethyl ether	3.5	4.26 ± 0.02	0.16
	hexane	3	6.25 ± 0.03	0.06
	methanol	3	0.07 ± 0.01	0.25
<b>Polymeric coating</b>	Nafion	2.7	0.02 ± 0.01	1.85
<b>Scraping</b>		3.4	5.13 ± 0.02	0.65
<b>Thermal</b>		2	10.00 ± 0.03	336

In all cases the potential obtained was lower to the one observed with the untreated membrane; this behavior is expected, and it helps minimize the energy used to promote the formation of H<sub>2</sub> and O<sub>2</sub>. Ion mobility was found to depend on the applied treatment (Table 1).

Immersion treatment is considered a gentle treatment for the *Agave salmiana* bio-membrane and for all studied media, and was considered an easy, economical treatment. The removal of water-soluble layers was attempted with this treatment at different concentrations of protons; however, there might be some water-soluble cuticle layers that may be altering the proper opening of stomata leading to a decreased ion mobility. Therefore, the bio-membrane lifetime did not increase significantly [35,36].

A similar behavior was observed for the clean-up treatment with the organic solvents. This process, with the exception of the methanol treatment, removes waxes that promote the opening of stomata, and causes conductivity values to increase in comparison with the ones obtained with the non-treatment bio-membrane. Clean-up treatments cause the bio-membrane dehydration and its easy fracture in a single use, which explains its shorter useful life [37].

Polymeric coating with Nafion increases the electrical resistance compared to the non-treated bio-membrane, due to the obstruction of the bio-membrane channels, which generates a temperature increment leading to a separation of the polymeric phase from the bio-membrane. However, with these two phases, the life time increases.

Mechanical treatment is the simplest and fastest; it significantly decreases the required potential and its electrical resistance, placing itself as a good treatment option. Nevertheless, the associated lifetime does not increase significantly compared to the non-treated bio-membrane, since for this treatment the bio-membrane is submitted to mechanical.

The thermal treatment bio-membrane was the best option among all evaluated treatments. The results obtained are competitive to these obtained with commercial membranes. This membrane minimizes the potential (2.0 volts) required to separate the elements from water, and it also reduces the electrical resistance, promoting a higher mobility of the ions (conductivity of 10 mS cm<sup>-1</sup>) which is similar to other already reported membranes such as: Alginate bio-membrane and Nafion membrane [11,31,34]. It is also worth mentioning that the half-life of the thermally-treated bio-membrane remained in good condition even after two weeks of continuous use. Considering that ion mobility is the main performance factor, the best performing bio-membrane is the thermally-treated one, which reached the highest ion mobility values

### 3.2. Bio-Membrane Characterization Coupled Fuel Cell

Once the bio-membrane was evaluated in a water electrolysis system, it was coupled to a fuel cell. Considering the results obtained from the electrolyzer analysis, thermal treatment was regarded as the most adequate alternative. The performance of this bio-membrane was compared to a commercial reference (Nafion membrane) in the fuel cell.

Electric parameters were recorded during the performance assessment of the bio-membrane in the hydrogen and methanol fuel cell. Resistance between plates was measured in order to determine the membrane ionic conductivity. For all experiments a contact area of 4.0 cm<sup>2</sup> between the plate and the membrane was set with a membrane thickness of 0.08 mm.

All the experiments were run for a week, and the potential obtained did not change in this time for both the *Agave salmiana* bio-membrane and the Nafion membrane. Table 2 shows the potential (V) obtained in the fuel cell using hydrogen and methanol as fuel. When using hydrogen, the potential value was greater than that obtained with the Nafion membrane. The bio-membrane was not completely hydrated, and its efficiency was affected in comparison to the Nafion membrane. Nonetheless, the obtained potential is deemed to be competitive. On the other hand, when using methanol, the potential was greater for the *Agave salmiana* bio-membrane compared to the reference Nafion membrane; the bio-membrane was observed to be completely hydrated, which led to an increased ionic conductivity. The results obtained using the thermally-treatment bio-membrane in the methanol fuel cell are similar to those reported in methanol fuel cells with Alginate and Nafion membranes [11,38]. Results demonstrate that the proposed bio-membrane is a competitive option to replace Nafion in hydrogen and methanol fuel cells.

**Table 2.** Electric data of the bio-membrane performance in a methanol fuel cell and hydrogen fuel cell at room temperature (298 K).

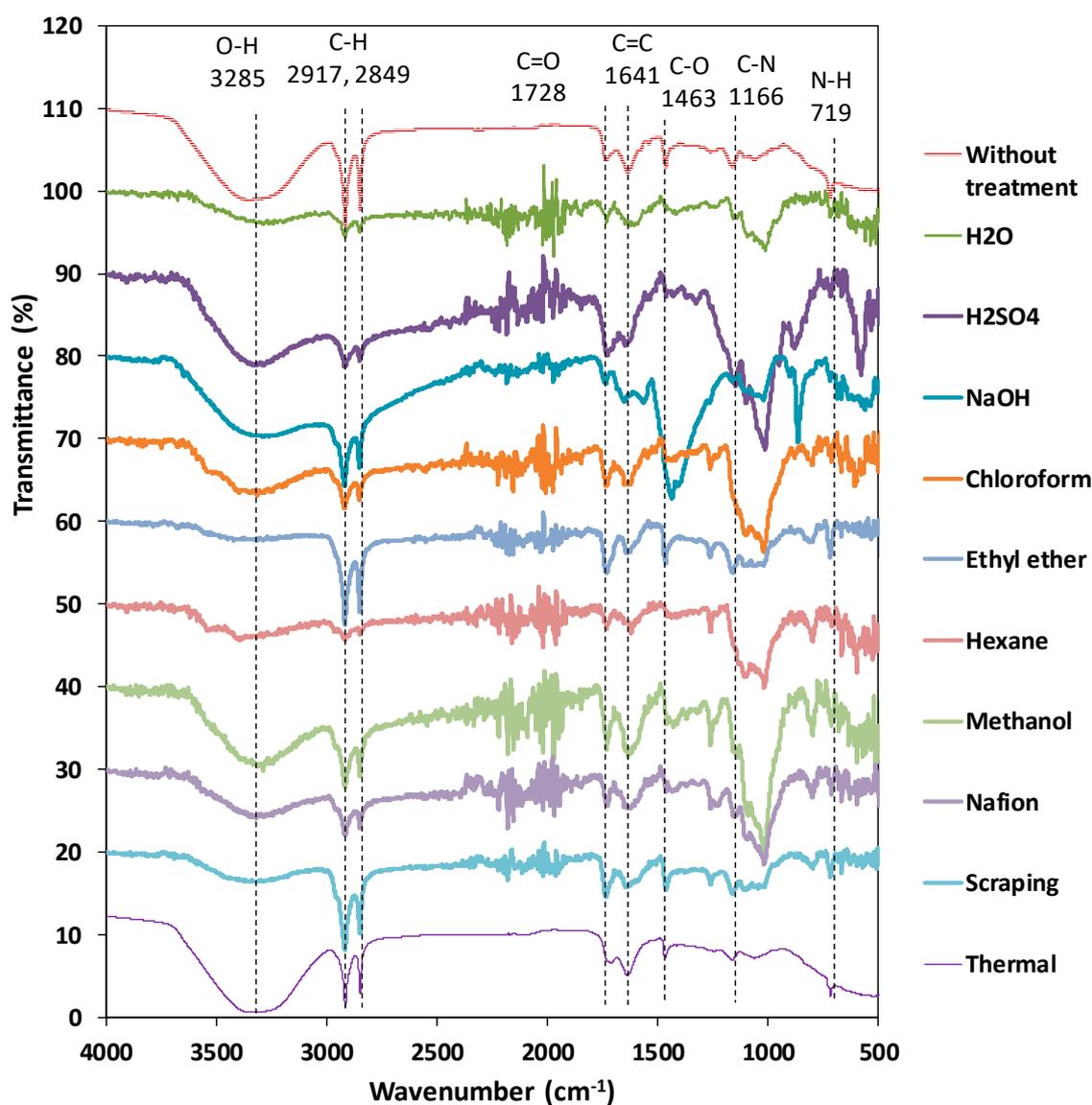
	E (volts)	i (mA)	Conductivity mS cm <sup>-2</sup>
Hydrogen fuel cell with Nafion membrane	0.81 ± 0.01	1.00 ± 0.05	4.29
Hydrogen fuel cell with thermal bio-membrane	0.70 ± 0.03	0.09 ± 0.01	1.06
Methanol fuel cell with Nafion membrane	0.46 ± 0.02	0.30 ± 0.02	1.11
Methanol fuel cell with thermal bio-membrane	0.50 ± 0.01	0.50 ± 0.02	7.14

### 3.3. Structural Characteristics of the Bio-Membrane with Thermal Treatment

The several treatments of the bio-membrane were analyzed by Fourier-transform infrared spectroscopy (FTIR) in order to identify its functional groups contained. Characteristic peaks of N–H at 719 cm<sup>-1</sup>, C=O at 1,728 cm<sup>-1</sup>, C–O at 1,463 cm<sup>-1</sup>, C=C at 1,641 cm<sup>-1</sup>, C–H at 2,849 and 2,917 cm<sup>-1</sup> and O–H at 3,285 cm<sup>-1</sup> were observed (Figure 3).

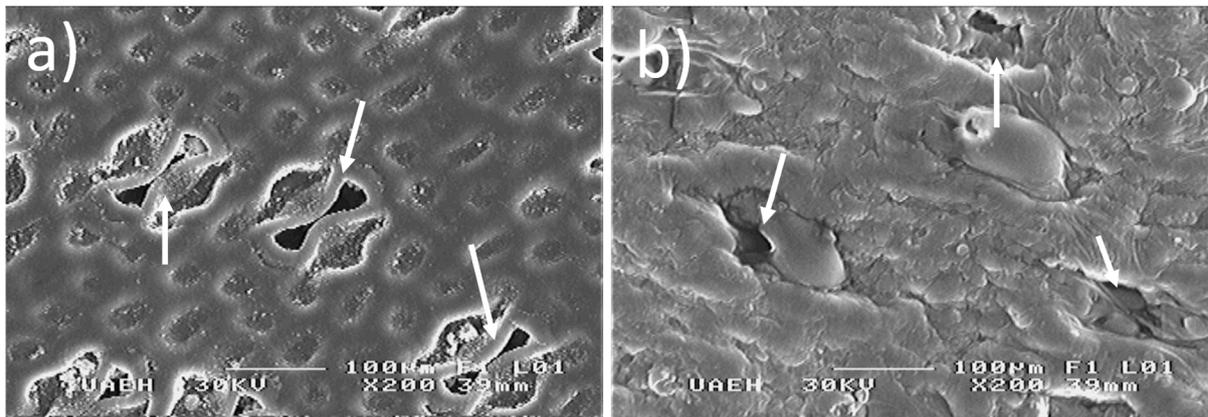
As shown in Figure 3, the FTIR profile of the bio-membranes is similar, which indicates that the chemical structure is maintained despite the thermal treatment undergone by the propose bio-membrane, and further, this membrane compared to the others that support a solvent (clean up and immersion), or a mechanical stress undergoes a modification minor at bands. The clean-up treatment with solvents (Chloroform, ethyl ether, Hexane, methanol) shows that the OH band decreases, indicating a dehydration. The functional groups found are consistent with the extracellular lipid membrane, composed by amorphous polyester (–CO–NH–) which is formed from the condensation of hydroxy-acids of 16 and 18 carbon atoms [23]. The thermal treatment applied to the bio-membrane generates a stable film useful to separate the anode from the cathode, preventing a short circuit.

Morphology is another important aspect to evaluate in membranes because their channels or clusters have a positive impact upon their ionic conductivity by allowing mass transport (mainly ion conductivity and water transport) [39].



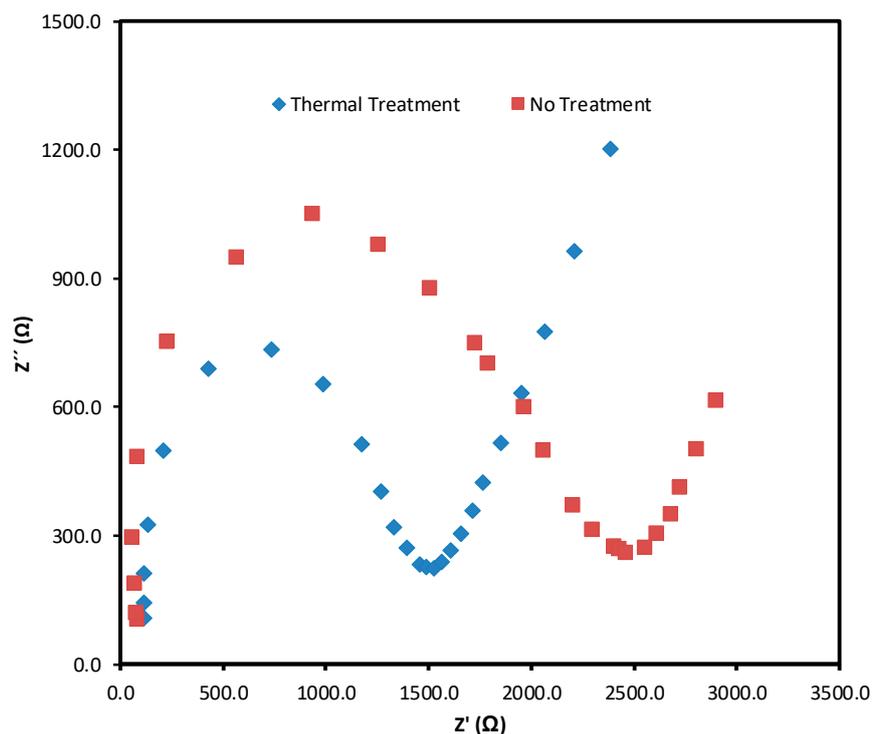
**Figure 3.** Fourier-transform infrared spectroscopy (FTIR) spectrum of the several treatments of the bio-membrane: Without Treatment, Immersion ( $\text{H}_2\text{O}$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{NaOH}$ ), Clean-up (Chloroform, ethyl ether, Hexane, methanol), Polymeric Coating (Nafion), Scraping and Thermal.

Bio-membrane morphology was studied through scanning electron microscopy (SEM). The surface stomata of the non-treated *Agave salmiana* bio-membrane (Figure 4a) are observed to be closed, limiting ion transfer and reducing its life time in an electrochemical system. The surface of the thermally-activated membrane (Figure 4b) displays stimulated stomata, which is desirable for ion (proton) transfer. From these observations, it is also possible to deduce an increased contact area and decreased electrical resistance.



**Figure 4.** (a) SEM image of the bio-membrane without activation; (b) SEM image of the bio-membrane with thermal activation.

One way of corroborating that there is a conductive improvement (efficiency) in a modified bio-membrane is to record an impedance spectrum using four electrodes at 0.2 V. Figure 5 shows a decrease in the electrical resistance of the thermally-treated bio-membrane, which indicates an increase in ionic conductivity, and therefore in its efficiency, which makes it an option as a bio-membrane in an electrolyzer or fuel cell [21,22].



**Figure 5.** Impedance spectra (Nyquist plots) of the bio-membranes without treatment and with thermal treatment.

#### 4. Conclusions

An *Agave salmiana* cuticle incorporated into electrolytic cells or electrolyzer and the fuel cells with hydrogen and methanol as fuel, is a sustainable alternative to replace Nafion membranes. Thermal treatment is an adequate process to improve ion exchange (achieved conductivity of  $10 \text{ mS cm}^{-1}$ ), since it poses the advantage of maintaining the functionality groups while stimulating the cuticle stomata. Another favorable point to consider is the reusability and biodegradability of this membrane of natural

origin. Results presented in this work demonstrate that the proposed bio-membrane is a competitive option to replace Nafion in hydrogen and methanol fuel cells.

**Author Contributions:** C.H.A. and P.H. performed the experiments and write the manuscript; G.A.-L. and I.P.-S. analyzed the FITIR and SEM; J.A.R. and G.I. reviewed and edited the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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