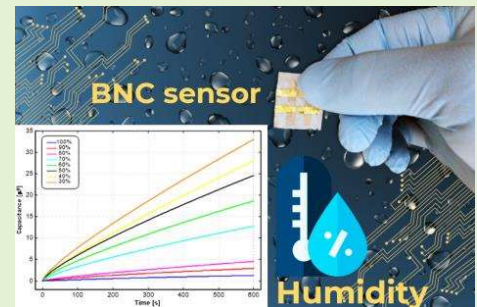


A Humidity Sensor Based on Bacterial Nanocellulose Membrane (BNC)

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Abstract—This paper presents the development of a humidity sensor based on bacterial nanocellulose membrane (BNC) produced from *Komagataeibacter xylinus*. BNC has a porous surface that absorbs water and therefore it changes the mechanical and electrical properties of the membrane. As the amount of water inside the membrane increases the capacitance of the membrane also increases. The capacitance of the BNC was measured in different values of temperature (from 30° to 100°) and relative humidity (from 30% to 100%). Chronoamperometry was used as a reproducibility test and the result was a linear and more precise variation for RH over 50% and a temperature of 30°. The measurements showed a combined sensitivity of $-4.13\text{nF}/^\circ\text{C}$ with relation to the temperature, and $+492\text{nF}/(\%\text{RH})$ and $+66.8\text{nA}/(\%\text{RH})$ with relation to the relative humidity.



Index Terms—Biomaterials, *Komagataeibacter xylinus*, Fermentation, Bacterial Nanocellulose (BNC).

I. Introduction

IN most applications the direct method of air humidity such as psychrometry is unsuitable. An alternative is to measure air humidity indirectly by electromechanical properties such as capacitance and resistance that change according to the air

humidity. Humidity is defined as the amount of water vapor in a certain gas [1] and air humidity is often used as a parameter of quality for foods, beverages, and environments. Arduino boards can interface with a DHT11 module, which contain a capacitive humidity sensor good enough for indoor applications. These modules have a single-bus data interface to communicate with the Arduino. This module measures relative air humidity from 20% to 80% with a 5% error [2]. Pravin et al. [3] used a micro-heater-based film to measure humidity. The film's impedance increased alongside air's humidity due to gas molecule absorption.

Biomaterials combine both efficiency and sustainability when they are used purely or combined with other sensors to measure humidity. The term biomaterial has been defined as "a material designed to take a form that can direct, through interactions with living systems, the course of any therapeutic or diagnostic procedure" [4]. Fiber Bragg grating combined with a moisture sensitive coating that expands with humidity thus deforming the Fiber Bragg grating's structure [5], [6]. The optical properties of the Fiber Bragg are directly related to the strain so the humidity can be measured indirectly by the wavelength that the fiber reflects.

Nanocellulose produced from bacteria, known as BNC (or bacterial nanocellulose), has gained a promising role as an alternative source compared to other cellulose classes. Due to its structure, this material shows several excellent properties such as high water-holding capacity [7], a high degree of polymerization [8], unique nanostructure [9], high crystallinity [10,11], and high mechanical strength [12-14]. Studies have shown that these particular features of BNC, paired with its biocompatibility, make this material an attractive candidate for a wide array of applications (e.g., biomedical, pharmaceutical,

Manuscript received Month xx, 2xxx; revised Month xx, xxxx; accepted Month x, xxxx. This work was supported in part by the infrastructure and financial funding from the Universidade Tecnológica Federal do Paraná, Campus Dois Vizinhos and by the Brazilian agencies CAPES (Grant 88882.333362/2019-01), FAPESP (Grants 2019/24354-2, 2019/05248-7, 2019/18656-6), and CNPq (Grant 304312/2020-7). The associate editor coordinating the review of this article and approving it for publication was Prof. yyyyyyyyyyy. (Corresponding author: Samara Silva de Souza.)

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biotechnology [15]; cosmetics [16], food [17,18], textile [19], and even in the electronics field [20-22]).

BNC's superior water absorption capacity can be useful in a variety of food production since it has a tasteless, hard texture, low calories, and high fiber quantities. Some examples of foods that use BNC are yogurts and pastries [23]. BNC also has applications as a green sustainable food package because it has a thin membrane as well as good mechanical properties and flexibility and has low-cost production [24]. However, pure BNC lacks antimicrobial and antioxidant properties [25]. Vilela et al. [26] created a film of polymerized sulfobetaine methacrylate with BNC (PSBMA/BNC) and compared it to pure BNC as a candidate material for intelligent food packaging. Both composites benefit from UV-light protection, moisture, and water absorption, as well as suitable thermal and mechanical properties. High thermal stability is often a requirement for food applications due to sterilization processes that use high temperatures at 150°C. Pure BNC films absorb roughly 79% of water after being immersed in water at 25°C for 48h. The PSBMA/BNC has 5.1 to 7.1 of the absorption capacity of pure BNC.

Nanocellulose has also been used as a biosensor in many applications. Biosensors consist of a biological recognition component and a physicochemical transduction device that can be employed as an analytical tool to detect an analyte in a wide range of environments [27]. Cellulose nanocrystals also have applications in the food industry. Cellulose nanocrystals combined with carbon nanotubes and embedded with polyaniline result in a conductive composite that absorbs methanol, acting as an effective detector of adulterated beverages [28]. Cellulose acetate nanofibers are used in the development of electrochemical paper-based analytical devices (EPADs). Ahmadi et al. [29] developed an EPAD based on cellulose nanofiber with Au electrodes to detect blood glucose levels by separating blood and plasma. Due to the unique characteristics of the BNC-based membrane, red blood cells and white blood cells were filtered and separated by the different size of the micropores.

BNC has also been modified and combined with other materials in order to create composites for supercapacitors [30-33]. For instance, BNC/graphene oxide is a flexible composite with high storage capability [30-33]. Jiang et al. [30] have developed a BNC with graphene oxide composite supercapacitor with a 373F.g⁻¹ at 1A.g⁻¹. Wang et al. [32] have demonstrated the feasibility to develop substrates made of surface modified nanocellulose fibers (NCFs) to synthesize supercapacitor with gravimetric capacitances of 127F.g⁻¹ and volumetric capacitances of 122F.cm⁻³ at current densities of 300mA.cm⁻² (or ≈33A.g⁻¹). Kang et al. [33] developed a solid-state flexible supercapacitor with BNC and carbon nanotube with a 50.5F.g⁻¹ capacitance and 15.5mWh.g⁻¹ storage power. In addition, BNC can be combined with polyaniline and used as a conductive electrode in supercapacitors. In this context, this paper presents the electrical characterization of a bacterial nanocellulose membrane as a biosensor for humidity measurement. As the humidity increases, the capacitance of the membrane also increases. The capacitance of the BNC was measured in different temperatures from 30°C to 100°C and relative air humidity from 30% to 100%.

II. EXPERIMENTAL

A. Nanocellulose membrane fabrication

The bacterial strain used was *Komagataeibacter xylinus* ATCC 53582. The strain was stored in an ultra-freezer, at -80°C (Nuair), in a culture medium containing 20% glycerol, reactivated in Hestrin–Schramm (HS) medium. HS medium contains 20.0g.L⁻¹ glucose, 5.0g.L⁻¹ bactopectone, 5g.L⁻¹ yeast extract, 2.7g.L⁻¹ sodium phosphate anhydrous and 1.15g.L⁻¹ citric acid monohydrate. The pH of the culture medium was previously adjusted to 6.5 and autoclaved for 20min at 121°C. Fig. 1 shows the steps of the fabrication of the BNC membrane.

Komagataeibacter xylinus was inoculated on HS agar plates and incubated at 28°C for 7 days. Bacteria colonies were randomly selected and suspended in the selected media. For the synthesis of BNC membranes, 10% v/v of bacterial inoculum was added to HS medium, transferred to petri dishes, and incubated for 7, 10, 15 and 30 days under static conditions at 28°C. The fermentation time changes the thickness of the membranes. The membranes formed at the liquid/air interface were then removed and transferred to a flask containing a 0.1M sodium hydroxide solution and maintained for 24 hours at 50°C to remove bacteria and/or residues from the culture medium. The BNC membranes were then subjected to successive washes with distilled water, or until the pH of the rinse water was equivalent to that of the distilled water used in the wash. The membranes were autoclaved for 20 minutes at 121°C and kept refrigerated until use. Before the electrode deposition the BNC membranes were dried at 40°C on a surface for 48 hours, until constant weight.

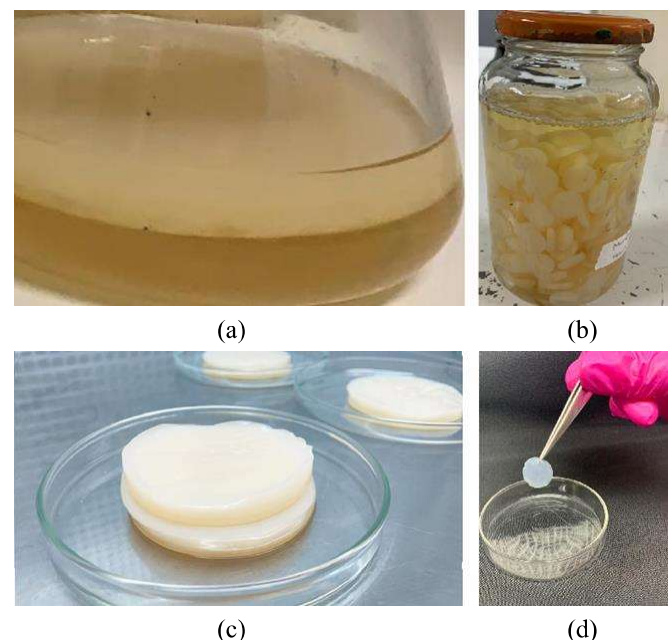


Fig. 1: Photographs showing the (a) BNC production in static cell culture, (b) BNC membranes in NaOH solution prepared for the purification process, (c) BNC membrane before purification, and (d) BNC membrane after purification and sterilization in an autoclave.

B. Electrodes deposition

The electrodes were fabricated on both sides of the

nanomembranes by DC sputtering (BALZERS BAE 370, Switzerland) in a 1000 class cleanroom environment. This process is reproducible. The deposition was performed using a copper shadow mask fabricated by lithography followed by etching in ferric chloride (FeCl_3). The metallic thin-films were 5nm 80:20wt% Ni:Cr deposited at a ratio of 1.1Å/s for 45s and 95 nm Au at 2.8Å/s for 5 minutes and 40 seconds. The base pressure and temperature pre-deposition were approx. 4×10^{-6} mbar and 30°C, respectively. After injecting argon into the chamber, the pressure increased to 1×10^{-3} mbar. Nichrome was used to improve the adhesion of gold to dielectric surfaces. The overlapping area that defined the active area of the capacitive sensors was 3mm×3mm (ca. 9mm²). Fig. 2 provides schematics related to the configuration of the electrodes.

C. Measurement setup

The study and characterization of nanocellulose membranes in a reproducible way was carried out in a hermetically sealed acrylic chamber developed in our group, consisting of a heating and a humidity control system. The heating system consists of an XH-W1315 digital thermostat with a temperature control range of -99°C to 999°C and a resolution of 1°C. The output of the module is connected to a 50W PTC aluminum ceramic heating element with type K thermocouple for temperature sensing.

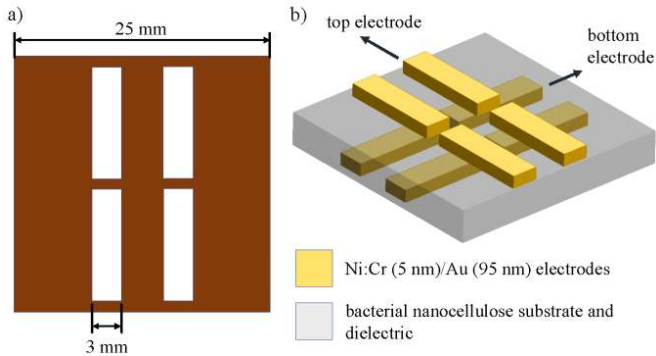


Fig. 2: Electrical contacts in the BNC: (a) top view of the shadow mask; and (b) 3D representation illustrating the electrodes overlapping area.

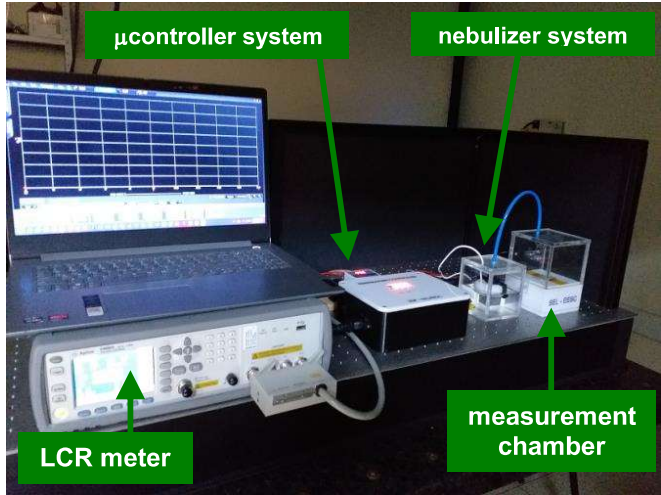
The current applied to the heating element was set using an XL4015 step-down converter DC/DC. This converter has a fixed frequency of 180 kHz and can drive a 5A load with high efficiency, low ripple, and excellent line and load regulation, allowing better performance and control in the membrane heating experiment.

The humidity in the chamber was controlled using a commercial hygrometer, model XH W3005, with a humidity range of 00% RH to 99% RH and an accuracy of 0.1% RH. The output of the module was connected to an ultrasonic nebulizer system, which was responsible for increasing the % RH level in the chamber. However, when it was necessary to lower the % RH level, dry air was supplied to the chamber. The inlet and outlet of humid and dry air in the chamber are automatically controlled. Fig. 3 shows the experimental setup developed in this work.

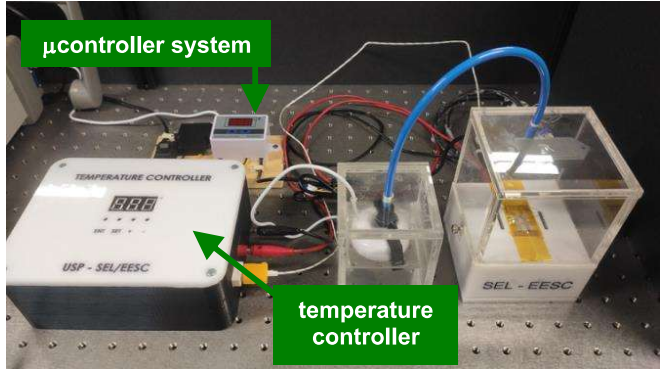
D. Characterization techniques

The structure and morphology of the developed

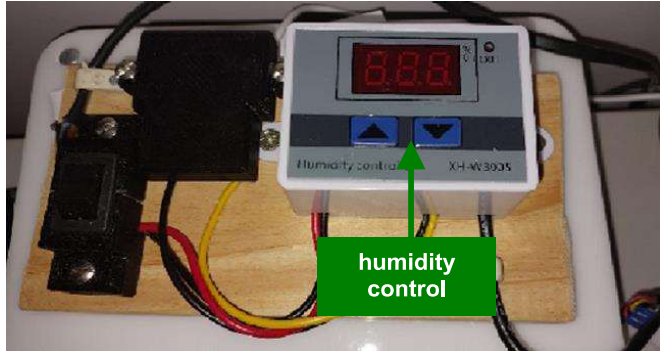
nanocellulose membrane was investigated with a field emission scanning electron microscope (FE-SEM) JEOL JSM-6390LV operating at 10kV. X-ray diffraction (XRD) measurements were carried out using a $\text{CuK}\alpha$ radiation (Phillips diffractometer, model X'Pert) in the range of 5-40° 2 θ at a scan rate of 1°min⁻¹.



(a)



(b)



(c)

Fig. 3: Photographs of the (a) experimental setup developed in this work, (b) power supply, control, and nebulizer system, and (c) Microcontroller used to control temperature and humidity.

The electrical characterization was performed on a square BNC sample (2.5cm×2.5cm) where changes in capacitance were observed as a function of temperature and humidity. Measurements were done with an Agilent LCR model

E4980A with Keysight BenchVue software. For all experiments, the parameters were settled to 2V and a frequency of 2kHz.

III. RESULTS AND DISCUSSION

A. Structure and morphology characterization

Fig. 4(a) and (b) show, the SEM images of the top and bottom side of the BNC and its XRD, respectively. As it is possible to observe, the membranes have a tangled structure with pores randomly dispersed throughout the matrix. This porous structure facilitates the trapping and adsorption processes of H₂O due to its highly tangled network of fine fibers. As can be seen, all XRD patterns are well in agreement with cellulose I structures as defined by ICDD (International Centre for Diffraction Data). A well-defined peak indicates a high degree of crystallinity. The peaks are located at 14.46°, 16.66° and 22.53° and they correspond to the cellulose I polymorph structure [34].

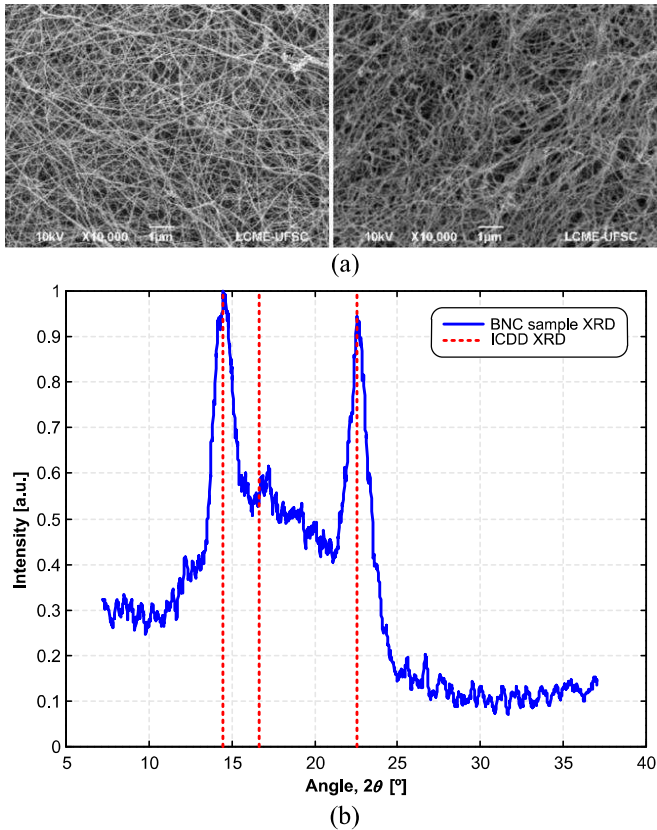


Fig. 4: (a) SEM images of the top (left) and bottom (right) side of the BC nanocellulose membrane, and the respective (b) XRD.

B. Electrical characterization of the membrane as a function of temperature and humidity

Fig. 5(a) shows how the capacitance response varies over time as a function of temperature applied to the membrane. The capacitance was monitored for 10 minutes in order to evaluate if the capacitance value would be stable over time. For temperature values from 70°C to 100°C, the capacitance values were almost the same (around 10nF to 20nF) and for lower temperatures the capacitance value varied from 80nF to 275nF. Fig. 5(b) shows the capacitance threshold versus

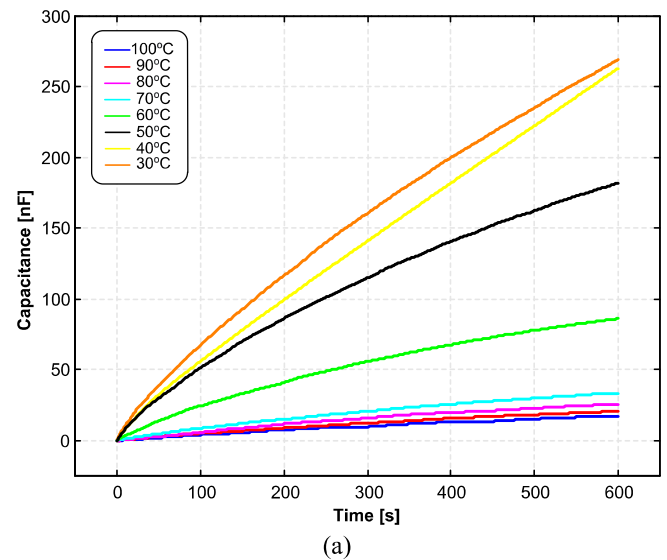
temperature. The capacitance threshold was greater for 30°C than for temperatures higher than 60°C. Therefore, the humidity experiments were executed at a temperature of 30°C.

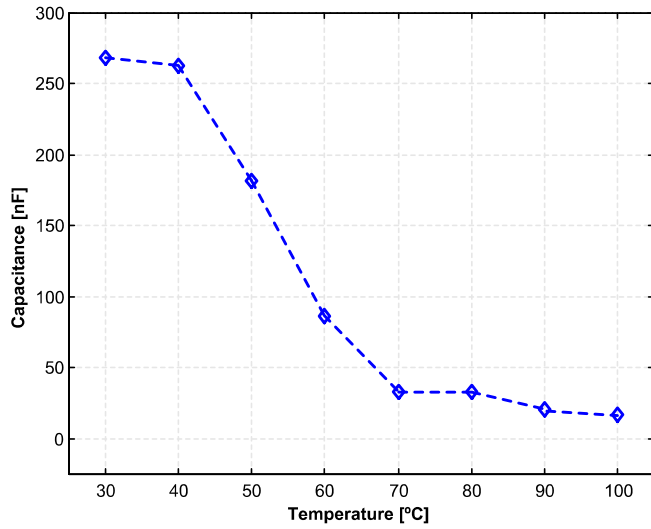
As it can be observed in Fig. 5(c) and (d), the membrane capacitance value changes when exposed to different percentages of relative humidity in the chamber. The significant increase in the capacitance value may be related to the porosity and roughness of the membrane. Therefore, rough, and porous surfaces allow a greater availability of absorption for water molecules. On the other hand, this increase in capacitance can also be attributed to the difference in dielectric values of the water and the bacterial membrane. In this way, the BNC works by attracting water molecules with their hydrophilic functional groups, resulting in an increase in its capacitance and the dielectric constant. For values of %RH over 50% the capacitance threshold (Fig. 5(c)) over time is significantly higher than the capacitance for lower values of %RH. The capacitance variation versus %RH (Fig. 5(d)) for 50% to 100% is about 27μF whereas the variation for 30% until 50% is less than 5μF. Consequently, the membrane is more accurate to measure %RH values over 50%. A reproducibility experiment was performed with the nanocellulose membranes as a function of %RH over time using chronoamperometry. Fig. 5(e) shows a constant current response of the membrane after 150 seconds exposed to a set humidity level. Finally, Fig. 5(f) shows the current versus %RH. The results show that when the humidity level is above 50% RH there is a greater difference in current values of about 0.72μA. However, at humidity levels of 30%, 40% and 50% RH the difference in response current was about 0.28μA. Therefore, the membrane has better resolution in detecting humidity above 50% RH over time.

It must be noted that the interpolations on (b), (d) and (f) are only for a better visualization purpose.

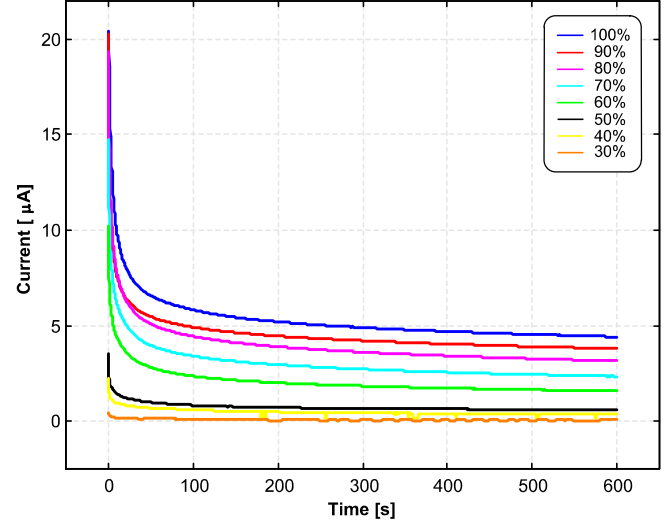
The sensitivity of this sensor applied to the variation of the capacitance in relation to the temperature is given by:

$$S_{C,T} = \frac{\partial C}{\partial T} \text{ [nF/°C]} \quad (1)$$

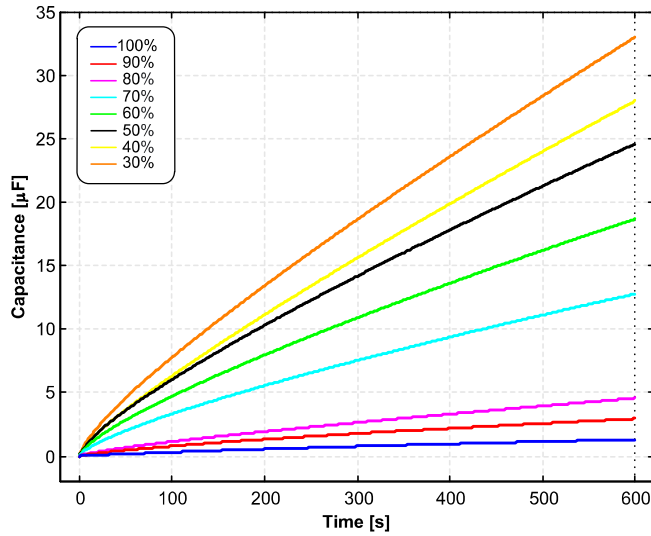




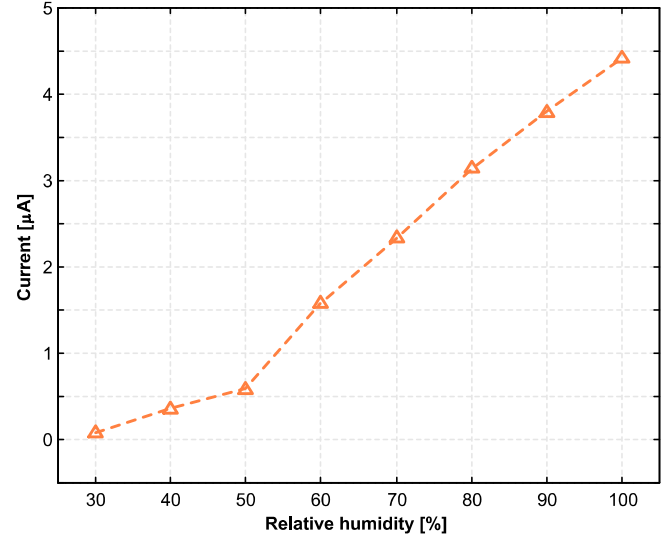
(b)



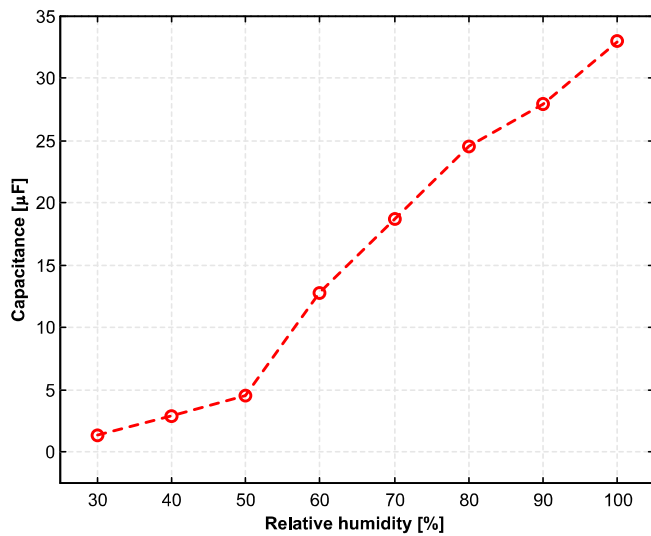
(e)



(c)



(f)



(d)

Fig. 5: (a,b) Capacitance response of the nanocellulose membrane as a function of temperature, (c,d) humidity; and (e,f) reproducibility experiment as a function of the humidity applied over time. It must be noted that the interpolations on (b), (d) and (f) are only for a better visualization purpose.

It was done a linear regression to the sensor datapoints in Fig. 5(b) (obtained from the current after 600s in Fig. 5(a)) to obtain an estimation of the sensitivity. The estimated value of this sensitivity resulted on $S \approx -4.13 \text{ nF}/^\circ\text{C}$, e.g., the capacitance of the BNC sensor decreases 4.13 nF for an increase of 1°C in the temperature with a high correlation coefficient of 93.2%.

The same procedure was applied to the capacitance in terms of the relative humidity:

$$S_{C,RH} = \frac{\partial C}{\partial RH} \text{ [nF/(% RH)]} \quad (2)$$

It was done a linear regression to the sensor datapoints in Fig. 5(d) (obtained from the current after 600s in Fig. 5(c)) to obtain an estimation of the sensitivity. Thus, the slope of this polynomial function corresponds to the estimated value of sensitivity, resulting on $S \approx 492 \text{ nF}/(\% \text{ RH})$. This result indicates

that for an increase of 1% in the relative humidity, the capacitance of the BNC sensor increases 492nF. This gives a high correlation coefficient of 98.8%.

The third sensitivity is the variation of the electrical current in relation to the relative humidity, which is given by:

$$S_{I,RH} = \frac{\partial I}{\partial RH} \text{ [nA/(% RH)]} \quad (3)$$

It was done a linear regression to the sensor datapoints in Fig. 5(f) (obtained from the current after 600s in Fig. 5(e)) to obtain an estimation of the sensitivity. The estimated value of this sensitivity resulted on $S \approx 66.8 \text{ nA/(% RH)}$ with a high correlation coefficient of 99%.

IV. CONCLUSIONS

In this study, the excellent electromechanical properties of the bacterial nanocellulose membrane were applied to moisture detection. The membrane capacitance varies as a function of the applied temperature and exposure time. For temperature values above 70°C, the limit of detection by the membrane was observed, which was around 10nF. The sensitivity of the membrane exposed to different temperatures resulted in 4.13nF for each temperature increment. In the presence of humidity, the membrane sensor was able to discriminate significant capacitance values from 50% RH, with a sensitivity threshold of 492nF for a 1% increase in humidity. Chronoamperometry also improved the resolution and discrimination of the moisture levels detected by the membrane, as well as the stability analysis. In conclusion, this work presents the possibility of developing highly sensitive and stable humidity sensors with low manufacturing costs based on a bacterial nanocellulose membrane combined with signal processing techniques. Figure 6 shows a photograph of a manufactured device containing electrical contacts.



Fig. 6: Photograph of a selected sample of a complete fabricated device, already with the electrical contacts deposited on both sides of the BNC.

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