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Recycled bacterial nanocellulose membranes as novel green gels for the cleaning of cultural heritage surfaces

Erica Sonaglia, Maria Paola Bracciale, Maria Laura Santarelli

Department of Chemical Engineering Materials and Environment, Sapienza University of Rome, via Eudossiana 18, 00184 Rome, Italy, marialaura.santarelli@uniroma1.it

***Abstract* – Gels allow the removal of extraneous materials from cultural heritage surfaces without changing artifacts' properties, thanks to solvent retention and its controlled release. In recent years, nanotechnologies and green chemical approaches created great interest in restoration and conservation field. The aim and innovation of this paper is to use bacterial nanocellulose (BNC) as a green gel in cleaning operations. Recycled BNC, a by-product from the fermented beverage Kombucha, is used. Purified by-products are investigated by means of different analytical techniques to characterize the chemical structure, crystallinity, microstructure, thermal and tensile behaviours, retention capacity of water (WRC) and organic solvents (OSRC). Materials show good mechanical behaviour, diffuse nanodimension and high WRC and OSRC. One gel is used in the removal of wax, a widespread deposit of dirt. It proved cleaning efficiency for removing beeswax after one hour and a weak removal of microcrystalline wax, showing BNC's potential as green gel.**

I. INTRODUCTION

The cleaning action of cultural heritage involves the removal of matter from the surface of artifacts, including aged coatings, degraded materials, graffiti, pollution deposits, dust, and grime. A specific approach in cleaning operations is the removal of extraneous materials without changing artifacts' original properties and aspect. Commonly, the cleaning is done using chemical solvents, but in the last decades, several gel formulations have been used as effective cleaning tools, presenting several advantages over free solvent employment. Indeed, gels are considered useful in solvent retention and for its controlled release on surfaces, preventing deep penetration and damage of the original layers or release of the dirt into the porosity of the original materials [1]. Furthermore, they allow the use of small quantities of solvent and better control of the application times. Consequently, gels guarantee better control of volatile and hazardous organic solvents (VOC), aiding to protect workers' health.

Gels can be obtained from natural polymers (e.g., xanthan and gellan gums, agar, and chitosan) [2] or synthetic polymers (e.g., acrylamide, bis-acrylamide, polyvinyl alcohol, and polyvinylpyrrolidone) [3,4] by physical or chemical interaction of organic chains, curing in network structures. In the first studies, synthetic polymer gels showed better performance [1,5,6]. However, in recent years, the nanotechnologies and green chemical approaches have created great interest in sustainable methodologies in restoration and conservation applications.

This paper aims to demonstrate the possibility to use bacterial nanocellulose (BNC), which is synthesized by various non-pathogenic gram-negative aerobic bacteria belonging to the group of acetic acid bacteria (AAB) [7]. BNC shows several important differences compared to the most common plant-derived nanocellulose, such as a higher purity and crystallinity, a significantly higher water adsorption capacity, and a different supramolecular structure [8]. The BNC can be produced by bacterial cultivation in laboratory-synthesized media, but also as a by-product from the fermented beverage known as Kombucha.

Kombucha is a large diffuse commercialized healthy drink, and during its brewing process, sweetened tea is fermented by a natural co-culture of symbiotic bacteria and yeasts, which always includes cellulose-producing AAB. During the fermentation process, a cellulosic biofilm is generated at the interface between the liquid phase and the air. This material is usually discharged as waste; however, it represents a promising low-cost source of BNC. The use of recycled Kombucha-derived BNC membranes as gel materials applied for cleaning of cultural heritage is an innovative application. Usually, BNC waste materials from Kombucha production derive from two types of cultures regularly employed by producers: the liquid product Kombucha commercialized as a drink; and the starter, a culture rich in nutrients that is fermented for longer time periods and used as inoculum to generate new Kombucha cultures. Several variables of the production process adopted by producers, which involve recipes -e.g., typology and concentration of tea, sugar, and microorganisms- fermentation times and tank sizes, are known to affect several BNC properties [9].

The aim of the work is to identify the conditions to remove waxy deposits on marble using BNC from Kombucha loaded with organic solvent. The removal of hydrophobic waxy materials is particularly difficult due to their insoluble nature in most solvents. The application of BNC can allow the use of limited quantities of toxic solvents and guarantee their efficient loading, which is often impossible when using synthetic polymeric gels [10]. A BNC gel was selected for testing the efficiency of these materials as cleaner of dirt deposits on precious surfaces of cultural heritage. In particular, the cleaning performance was evaluated for the removal of waxes, one of the most widespread deposits of dirt on different surfaces.

II. MATERIALS AND METHODS

Four waste BNC membranes were gently supplied by three Italian Kombucha producers. Their total mass ranged between 1050.0 g and 3870.5 g. BNC membranes were labelled 1, 2, 3, and 4. From the obtained BNC materials, samples of comparable dimensions (approximately 15 cm x 15 cm) were taken, and each analysis was conducted on smaller samples of approximately 2 cm x 2 cm for an objective results comparison. A protocol for cellulose purification with sodium hydroxide solution (0.5 M) was developed and purification efficiency was evaluated by means of Attenuated Total Reflectance – Fourier Transform Infrared (ATR-FTIR) spectroscopy measurements.

Material characterization involved the employment of different analytical techniques. Lyophilization of samples was carried out to remove moisture without modifying network structure and to avoid fibrils interaction during drying.

ATR-FTIR with a single reflection Diamond ATR cell, a standard MIR source (HeNe) and a room temperature DTGS detector was carried out.

Wide Angle X-Rays Diffraction (WAXRD) was executed in Bragg-Brentano geometry with Mo-Ka radiation (λ =0.7093 Å, 40 keV, 35 mA), with angular scanning between 3.3° and 29.98° using a home-made "fork type" sample stage to minimize the background. X-ray diffraction patterns were converted in the CuKα1 (λ = 1.5418 Å) wavelength for an easier comparison with literature data.

Scanning Electron Microscope (SEM) analysis was conducted under high vacuum (10^{-5}-10^{-6} mbar) at variable kV on both surfaces and on cross-sectional area of each sample.

Swollen samples were analysed by Thermogravimetric Analysis with Differential Scanning Calorimetry (TGA-DSC) with a 10°C/min rate in an artificial air atmosphere with 100 ml/min flux until 1000 °C.

Tensile tests with a load cell of 1 kN and gravimetric measurements were carried out on swollen samples. Furthermore, organic solvent loading (pure ethanol, pure acetone and pure ethyl acetate) was evaluated by means of UV-VIS spectrophotometry.

Cleaning efficiency was evaluated by removal tests of synthetic wax (microcrystalline wax) and a natural wax (beeswax) applied by brush (after wax liquefying at 50°C) on Carrara marble mock-ups (5 cm x 5 cm x 2 cm in dimensions). The cleaning action was determined by treatment for 30, 60 and 120 minutes with membrane swelled by pure ethyl acetate as solvent. The remaining presence of the waxes on the samples was measured by Micro-Raman spectroscopy. Moreover, ATR-FTIR spectra of the membranes after treatment were also acquired.

III. RESULTS

The Kombucha cellulosic by-products appeared as compact and uniform membranes with variable thickness between different products, from approximately 0.3 cm to 2.0 cm. The samples collected from the whole membranes were efficiently purified as detected by FTIR analysis shown in Figure 1.

The WAXRD results presented in Figure 2. identified the cellulose crystallinity as a type I crystal structure in all samples, with a high ratio between crystalline and amorphous regions of the polymer.

All samples, observed by SEM on both surfaces and in the cross-sectional area, showed similar characteristics of the microstructure. SEM micro-images are shown in Figure 3. BNC networks consist of interconnected cellulose fibrils, which form aggregates (bundles). Furthermore, one membrane surface showed a compact appearance with a very tight network and low intra-fibril porosity, while the opposite surface showed an enlarged network with high and open porosity. Fibrils and bundles resulted randomly oriented. In cross-section, a large porosity was detected, in which the pores were mostly oriented parallel to the membrane surface. Fibril layers reflect cellulose deposition by bacteria during membrane growth in thickness. The SEM analysis showed that the dimension of the BNC fibrils was mainly 25-30 nm in diameter.

The measure of the WRC, which represents grams of water content per gram of cellulose, demonstrated the capacity to retain water from 67.0 g/g to 156.4 g/g.

Instead, the OSRC (cm^3/g) showed a retention in the ranges 67.2 - 122.5 cm^3/g, 69.0 - 113.7 cm^3/g and 60.4 - 132.7 cm^3/g for pure ethanol, pure acetone and pure ethyl acetate solvents, respectively. Moreover, UV-VIS results showed membrane volume can be efficiently swollen by organic solvents.

The membranes showed a viscoelastic behaviour in tensile analysis, presenting a linear trait followed by a yield point and a plastic region, before failure. Calculated parameters such as Young's modulus, yield stress, maximum tensile stress, strain at yield and strain at maximum stress reported in Table 1. indicate differences in the tensile behaviour of samples, which can be attributed to differences in thickness and WRC.

Thermal behaviour is comparable between different samples, showing a loss of total water dehydration until a temperature of 200°C, followed by degradation at a temperature of approximately 350°C, due to degradation reactions, including dehydration, decomposition and depolymerization of glycoside units. A third thermal event was evidenced at 450°C–470°C which reflected the oxidation and breakdown of carbonaceous residues, producing low-molecular-weight gaseous products. Samples were fully degraded at 550°C.

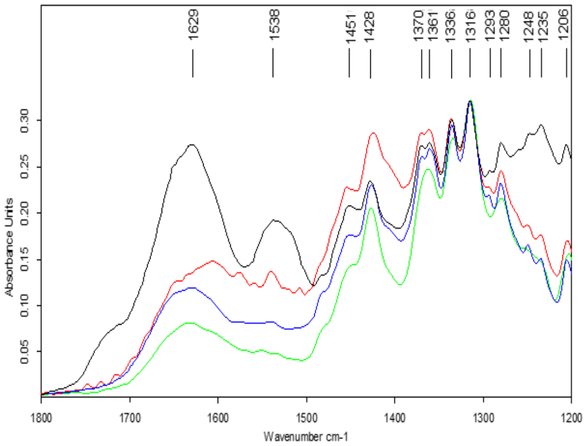

Fig. 1. ATR-FTIR spectra of representative dried Kombucha-derived BNC membrane obtained after purification and neutralization. In black: no purification; in red: one purification cycle; in blue: two purification cycles; in green: three purification cycles.

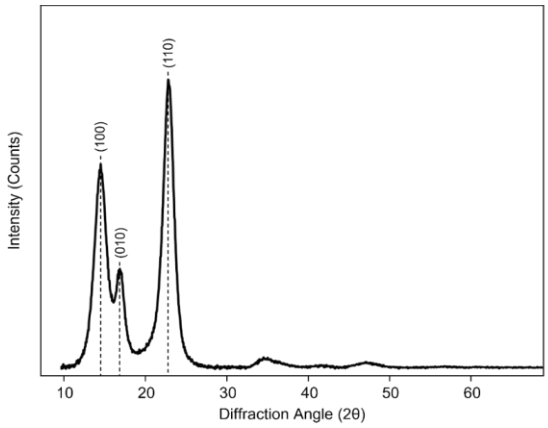

Fig. 2. X-ray diffraction pattern of representative freeze-dried Kombucha-derived BNC membrane.

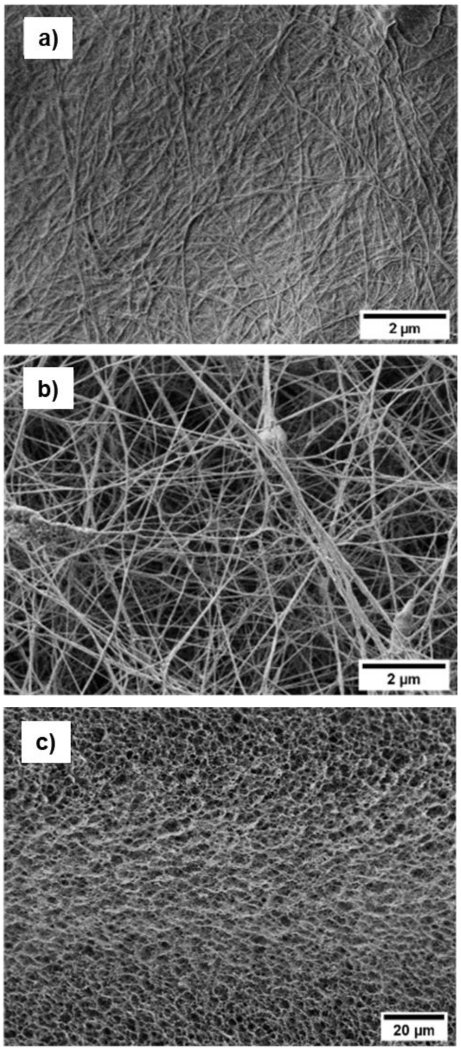

Fig. 3. SEM micro-images of representative freeze-dried Kombucha-derived BNC membrane. a) surface 1; b) surface 2; c) cross section.

Table 1. Calculated values from tensile curves of Kombucha-derived BNC membranes. Calculated Young's moduli (MPa), yield stress (MPa), maximum stress (MPa), strain at yield (%) and strain at maximum stress (%).

Sample	Young's modulus (Mpa)	Yield stress (Mpa)	Max stress (Mpa)	Strain at yield (%)	Strain at max stress (%)
BNC_1	6.9	42.5	46.6	7.3	9.0
BNC_2	0.3	3.3	3.4	14.0	14.7
BNC_3	1.2	25.7	26.7	25.4	28.5
BNC_4	1.5	18.3	18.4	15.5	15.6

The cleaning action was evaluated by micro-Raman investigation before and after the removal of a layer of the synthetic and natural waxes applied on Carrara marble samples. Selected Raman spectra are shown in Figure 4. The investigation showed lower effects related to the presence of the waxes on the marble samples after 120 minutes of cleaning of both waxes. The natural wax was much more removable, as the obtained FTIR on the membranes after treatment demonstrated. Indeed, the presence of effects due to the beeswax was identified on the FTIR spectra already after 30 minutes and it resulted in the maximum removal after 120 minutes.

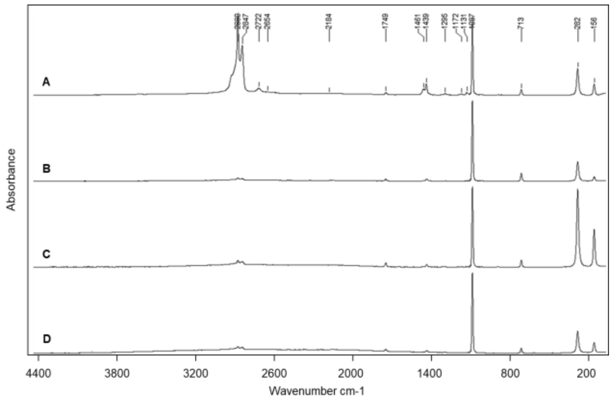

Fig. 4. Selected Raman spectra obtained on marble mock-up surface covered in beeswax. Spectrum obtained before cleaning (A.) and spectra obtained in spot 1 (B.), spot 2 (C.) and spot 3 (D.) after 60 minutes cleaning.

IV. CONCLUSION

The waste BNC membranes produced as a by-product of Kombucha beverage production showed good mechanical behaviour and diffuse nanodimension. Moreover, the application of BNC gels as solvent-supporting materials in cleaning operations of cultural heritage demonstrated the possibility to develop and explore the topic further. The gel network is composed of cellulose nanofibrils forming a high porose structure, which shows good solvent absorption capacity. Indeed, very high water and organic solvent retention capacities were measured. Differences in tensile behaviour could be attributed to the diversity in thickness and WRC of samples, but generally tensile tests showed a viscoelastic behaviour with a small maximum deformation for all samples.

Cleaning action was evaluated in the removal of waxes, one of the most diffuse dirt with low solubility. Indeed, organic solvents are necessary to remove layers of wax deposits, usually in high quantities, which raises issues regarding safety and pollution. The experiment showed cleaning efficiency for removing beeswax already after one hour and a weak removal of microcrystalline wax was highlighted. A different solvent will be considered to increase its removal efficiency. Waste BNC membranes can be considered a promising alternative to synthetic polymer membranes thanks to their bio-based, biodegradable, and renewable properties and behaviours.

V. REFERENCES

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