



Boosting bacterial nanocellulose production from chemically recycled post-consumer polyethylene terephthalate

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ABSTRACT

The circular economy is emerging with new sustainable solutions to the ever-growing plastic waste challenge, garnering increasing attention. In this study, the possibility to modify expensive Hestrin–Schramm medium (HS) for bacterial nanocellulose (BNC) production and replace significant amounts of glucose with terephthalic acid (TPA) derived after reactive extrusion processing of mixed plastic waste yielding post consumer TPA (pcTPA), was evaluated from laboratory scale to fermentation at pilot scale. Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), Thermogravimetric Analysis (TGA) were used to assess the structural, thermal, and morphological properties of BNC and its generated derivatives. The study's findings highlight the positive impact of pcTPA on BNC yield, surpassing the performance of conventional TPA. The presence of pcTPA in the medium resulted in a BNC yield of 4.01 g/L in a scale-up step of 100 mL cultivation, while the positive control using glucose resulted in a yield of 3.57 g/L. The efficiency of glucose substitution with pcTPA increased with each scale-up step, ultimately reaching a 320% yield increase in comparison to the positive control. Additionally, the procedure that enhanced the materials' thermoplasticity in the form of derivatives has been established resulting in the production of BNC laurate and BNC octanoate derivatives with melting temperatures of 270 °C and 280 °C, respectively. Overall, this study investigates the potential of this approach as an important circular economic solution, enabling an increased sustainable perspective for polyethylene terephthalate (PET) circularity and significantly a much needed cost reduction for BNC production with enhanced thermoplasticity.

1. Introduction

Shortly after the widespread adoption of plastic production in the latter half of the 20th century, scientists began exploring the development of biomaterials designed to be compatible with the human body. These materials have since evolved for a range of applications spanning plastics value chain products to surgical tools such as tissue engineering scaffolds [1]. Fast forward five decades from their initial appearance in the literature, and biomaterials are now heralded as an imperative and clear-cut answer to impending challenges including the depletion of natural resources and plastic pollution [2]. Modern science, encouraged

by new advances in techniques for manufacturing, processing and materials functionalization, is focused on the development of biomaterials with wide range of applications in nanotechnology, 3D printing, tissue engineering, drug delivery, single-use materials, etc. [3,4]

Sustainability and circular economic lifecycles for plastics have become central themes in a significant amount of current global research projects, and they are becoming the key focus of scientific studies in fields ranging from material science and engineering, microbiology, environmental science, biotechnology and enzymatic engineering sustainable solutions to the ever-growing plastic waste issue, particularly from ubiquitous petroleum synthesized plastics [5,6]. For example,

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polyethylene terephthalate (PET), is a versatile thermoplastic which is widely employed across various industries for consumer products due to its excellent material properties, including high tensile strength, chemical resistance, and thermostability [1]. PET's high consumption, in conjunction with its slow degradation and considerable contribution to global plastic pollution is an urgent concern [2].

An approach to tackle this challenge is to decompose post-consumer PET back into its constituent building blocks, such as terephthalic acid (TPA), which can then be either remade as PET, equivalent to virgin PET, or in the case of mixed and contaminated PET, upcycling and bio-converting these monomer building blocks into high value biopolymers. Here, this is achieved by subjecting PET to both physical treatments and chemical exposure. This combination can break down PET into its constituent monomers or oligomers, offering a promising array of possibilities for recycling applications. Such an approach offers mitigation of the plastic waste problem and enables plastic waste to return as a resource rather than an environmental burden [3]. Another promising approach lies in the utilization of biomaterials as substitutes for synthetic plastics on suitable occasions. Currently commercially used biomaterials, such as polyhydroxyalkanoates, polylactic acid and bacterial nanocellulose (BNC), have the potential to rival conventional plastics due to their biobased nature and capacity for biodegradation, although their mechanical and thermal properties are still to be enhanced [7,8].

Bacterial nanocellulose (BNC) is considered the purest and most area-efficient form of cellulose, exhibiting excellent material properties such as biocompatibility [9], hemocompatibility [10], non-toxicity [11], mechanical stability, and high moisture content [12]. Furthermore, BNC is non-allergenic, semi-transparent, semi-crystalline [13] and possesses dynamic fiber forming capabilities, enabling versatile moldability for various applications [14,15]. The structure of BNC resembles ribbon-like cellulose fibers weaved into complex 3D network [16]. Unlike plant derived nanocellulose, this material is highly pure and crystalline, free of components such as pectin, lignin and hemicellulose [17]. The degree of polymerization is usually high, but it mostly depends on factors such as bacterial strain and conditions of production process including selected nutrients [18]. Besides, these factors are crucial for production yields of this material, especially carbon source. Various cultivation media have been used for BNC production, nevertheless, Hestrin-Schramm (HS) medium is still being reported as the most commonly used. However, due to its high concentration of glucose, this medium represents 30% of the overall production cost, which is one of the reasons this process cannot fulfill commercial production requirements [15]. In order to overcome this drawback, researchers have tried to use various agro-industrial wastes as potential carbon source alternatives for a more sustainable BNC production. Successful BNC production has been demonstrated using a variety of waste materials, such as industrial, confectionary, municipal residues, as well as unconventional sources like coconut water and fruit juices [19]. However, it still has not reached 15 g/L in 50 h which is estimated as an efficient production in comparison to the plant cellulose [18,20]. The limitation related to high production costs has been alleviated through the development of new production strategies, the design of innovative bioreactors [21], the utilization of genetically modified microbial strains [22]. Only a limited number of studies have been published on the successful conversion and reprogramming of bacterial strains that produce BNC due to the restricted availability of expression systems, such as plasmids, which are required for genes in biosynthetic pathways, as well as regulatory networks to enhance BNC production [23]. Additional efforts to create effective transformation methods and expression vectors would be valuable not only for achieving fast and high-yield BNC production but also for improving its characteristics and introducing new functionalities [24].

Taking into consideration the unsustainable and expensive techniques for BNC production, together with its thermal instability which does not allow its processing through common methods [25], the major challenges lie in the identification of low-cost cultivation media that

promotes BNC yield in shorter cultivation time and the modification of BNC's surface to improve its thermoplasticity [26].

In 2016, ResearchMoz reported that the BNC market is expected to reach and surpass \$700 million by 2026 [27]. These estimates were slightly affected by the COVID-19 pandemic, but nevertheless, new reports by MarketWatch imply that the global market expectancy will reach a readjusted size of \$439 million by 2030 [28]. These numbers, together with the plastic pollution issue on a rise have urged us to implement and evaluate post-consumer plastics and its derivatives as a potential medium compound for BNC production.

Recent studies have explored the addition of synthetic polymers such as ethylene glycol and silicone polyether surfactant to the fermentation media, demonstrating their potential as stimulating additives in the production of BNC [29,30]. In the study by Zhou et al. [31], a significant increase in the activity of α -UDP-glucose pyrophosphorylase and α -phosphoglucosyltransferase was detected in cultivation of *Taonella mepensis* after supplementation of HS medium with PET hydrolysate leading to higher yields and improved productivity of BNC. Against this backdrop, our study seeks to investigate the impact of terephthalic acid (TPA) derived from post-consumer PET on the production of BNC by *Komagataeibacter medellinensis* ID13488. We are focusing on decreasing the cost of commonly used HS medium by the partial substitution of glucose with TPA obtained from chemically recycled post-consumer polyethylene terephthalate (pcPET). Additionally, we tackle the surface modification of BNC through green chemical approaches to improve its thermoplastic properties and widen its applications in the packaging field. Overall, this study investigates the potential of this approach as a circular economy solution, presenting a sustainable perspective for polyethylene terephthalate (PET) circularity and cost reduction for BNC production with enhanced thermoplasticity.

2. Results and discussion

2.1. Bacterial nanocellulose production

The industrial production of BNC nowadays is mostly focused on the search of alternative carbon sources, which is understandable taking into consideration the high cost of glucose. In the case of nata de coco, coconut water is employed as a carbon source, while other developed industrial systems such as Kusano Sakko Inc. employ molasses or other agro-industrial wastes. These products are mostly commercially exploited as food/food additives, thickeners or suspending agents [19,32,33]. In material science, BNC has been used as reinforcement compound or filler to improve the required surface properties [34]. However, due to its temperature profile, potential application of this material is still underexplored. In this study, we examined the possibility to modify expensive HS medium and to replace significant amount of glucose with TPA derived after reactive extrusion (REX) process of mixed plastic waste in several consecutive steps involving scale-up set of experiments (Fig. 1). In this way, we are tackling productivity of this material, but also looking into options for PET circularity. Additionally, we have established a procedure that enhanced the produced materials' thermoplasticity in the form of derivatives.

Using the methodology described in the Experimental section, large concentrations of post-consumer TPA (pcTPA) have been obtained, making this study easily scalable. This straight-forward process secures high conversion of post-consumer PET into TPA in several simple steps. In economic terms, this solution offers swift, and straight-forward post-consumer PET recycling followed up with elegant monomer extraction, not involving additional reactors or solvents. For all the performed experiments, the amount of TPA present in the medium reached a maximum concentration of 2% (w/v), including the media where TPA and glucose were used together. Our initial idea was to assess the ability of our BNC producer to consume TPA as a carbon and energy source which was further refuted by HPLC results presented later in the manuscript, in the case of both pcTPA and commercial TPA (cTPA).

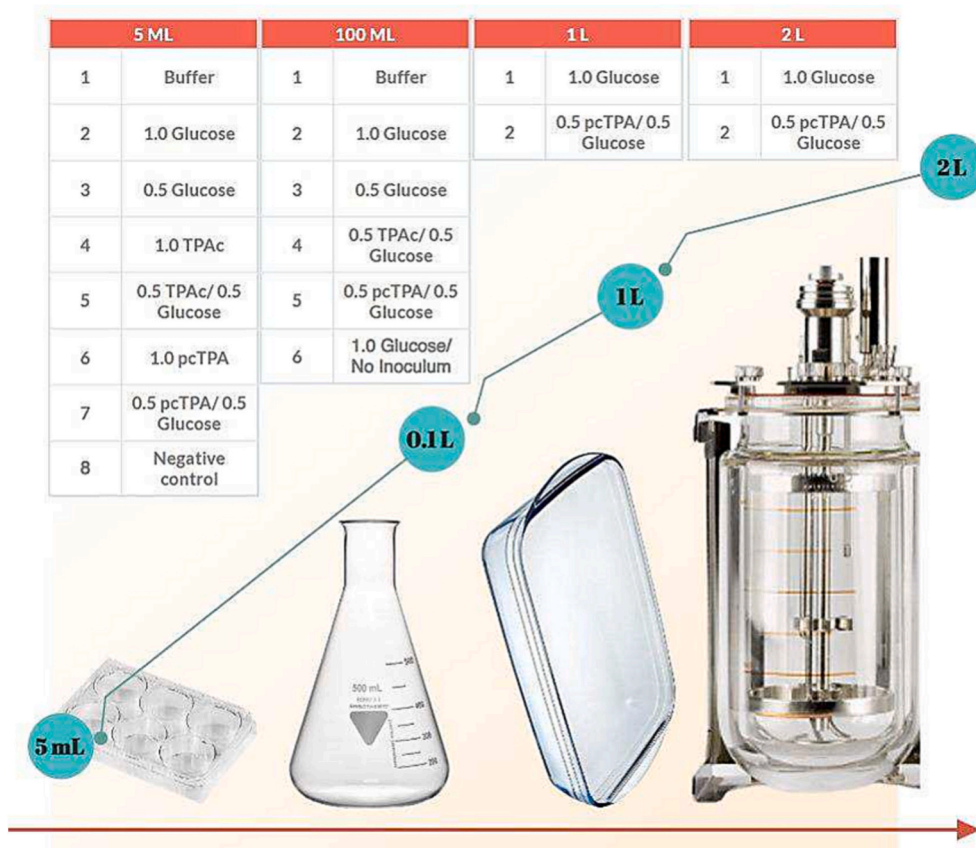


Fig. 1. Schematic diagram of bacterial nanocellulose (BNC) production in four different volumes: 6 well plate (5 mL), Erlenmeyer flask (100 mL), borosilicate glass tray (1 L), fermenter (2 L).

After cultivation in a 6 well plate, the BNC membranes were washed and weighted. Analyzing the means confidence intervals from all samples on a pooled standard deviation comparison, the BNC production from 0.5 pcTPA medium achieved the closest dry mass yield (4.52 g/L) when compared with the positive control, 1.0 Glucose (5.65 g/L) (Fig. 2A). According to the results, the pcTPA impact on *K. medellinensis* BNC production seemed significantly higher in comparison to the commercial TPA, suggesting that post-consumer products used in this experiment have undetectable traces of REX process and/or previous treatments in production and consumption chain. These results were confirmed in the first scale-up step (100 mL cultivation) where the presence of pcTPA in the medium resulted in a BNC yield of 4.01 g/L. For comparison, a positive control experiment (1.0 Glucose) resulted in a yield of 3.57 g/L. On the other hand, TPAC had no effect on BNC production (Fig. 2B). The average amount of produced BNC with TPAC was very similar to the yield achieved with 0.5 Glucose (2.73 g/L, 2.86 g/L, respectively).

In this second experiment, the yield output analysis on a pooled standard deviation comparison of the actual mean (CI 95%) displays a failure to reject the null hypothesis, since no significant difference is perceived between the observations (Fig. S1). Furthermore, 1.0 Glucose and 0.5 pcTPA displayed slightly higher dry yield means, supported by Fisher pairwise comparisons, which ingroup both conditions together (Table S1).

When submitted to an even bigger cultivation volume (1 L), BNC from the pcTPA-supplemented culture medium showed the highest dry yield, around 40% more dry mass than the BNC from the positive control and a minor effect of chain collapse after drying process, resulting in a wider film (Fig. 3A, B). This could be related to a better contact surface allowing better oxygen incorporation, which plays a critical role in *Komagataeibacter* BNC production [35]. Yield data analyses, especially

concerning the cultivation volume and TPA supplementation, raise a question about how those physiochemical factors interact synergistically for yield enhancement, suggesting further investigations.

The last scale up step was performed in a bioreactor vessel, where two batches ran simultaneously (0.5 pcTPA and 1.0 Glucose) in a volume of 2 L. Static fermentation has been successfully used for industrial production of BNC. These products are commercially exploited mostly as a thickener and/or suspending agent especially for suspension of particles due to their unique structure with 3D reticulated network [36,37]. Resulting BNC yield after cultivation supplemented with pcTPA has reached almost 160% of the increase in comparison to the non-modified HS medium (1.48 g/L, 0.93 g/L, respectively). However, in the case of agitated fermentation, the yield of 0.5 pcTPA has reached up to 2.35 g/L as presented in Fig. 3C.

While the production of BNC requires significant capital investment, scientists and manufacturers have been dedicated to innovating approaches that lower production expenses. These strategies include isolating high-yield strains, optimizing the fermentation processes, and employing low-cost nutrients [19]. In this study we presented the potential of BNC production using PET recycling leftovers in a form of TPA as a stimulant that would secure replacement of the critical and the most expensive component – glucose, up to 50%. The concentration of derived materials in different scale-up batches did not significantly change, by the contrary, it decreased, especially when it comes to the HS medium. However, efficiency of using pcTPA has increased in every step of the scale-up in comparison to the positive control (Fig. 4). The fact that agitation employment improved concentration of derived material in comparison to static cultivation indicates that higher BNC yield can be achieved by further optimization of the fermentation process which will be implemented in our future studies. Another perk of this process is the possibility of recycling the fermentation medium for the next batch by

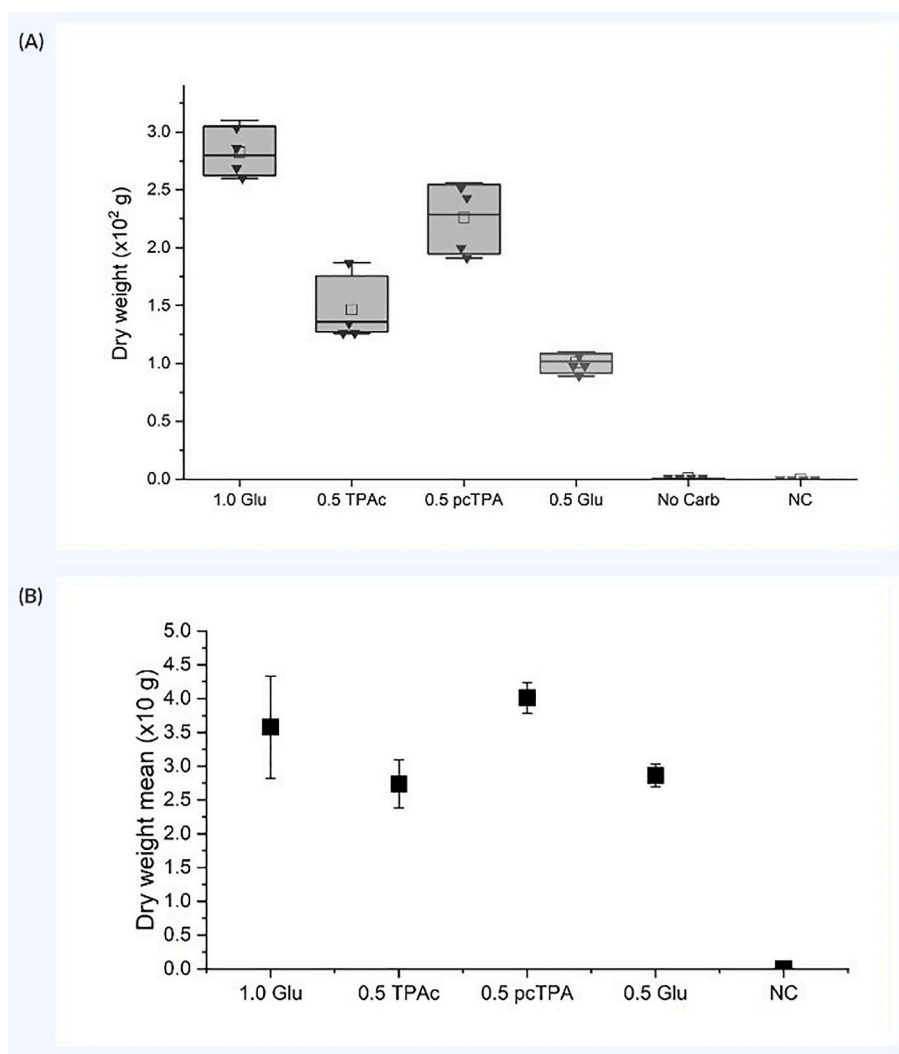


Fig. 2. Bacterial nanocellulose (BNC) dry weights after incubation in 5 mL (A) and 100 mL (B) of medium.

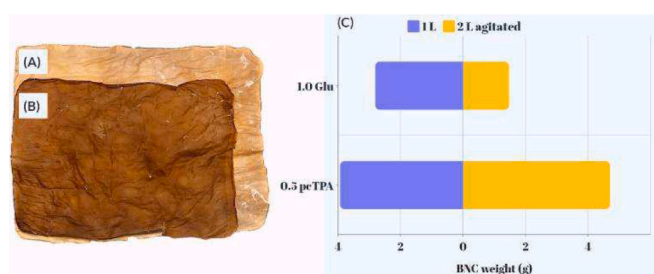


Fig. 3. (A) Bacterial nanocellulose (BNC) membrane, positive control (1.0 Glucose); (B) BNC membrane from the (post-consumer terephthalic acid) pcTPA-supplemented culture medium (0.5 pcTPA); (C) BNC yields after incubation in 1 L and 2 L of media.

addition of used nutrients (carbon and nitrogen sources) or simple pcTPA extraction for PET repolymerization [38].

2.2. Properties of derived BNC membranes

In order to assess the morphological differences between derived BNC membranes, thickness evaluation and SEM were performed. In terms of thickness, when the means of all available samples were submitted to One-way ANOVA, the Dunnett simultaneous analysis (95% CI)

displayed the control group significantly differ from the other preparations output, even though the control group dispersion data overlap at some point (<0.05 mm) with the other sample upper measurements (Fig. S2). Moreover, the BNC thickness from 0.5 pcTPA (0.062 ± 0.00957 mm) displayed the smallest data dispersion in terms of SD (Fig. S3), possibly indicating a better homogeneity through the film, based on its data set measurements [39], in comparison to the positive control (0.122 ± 0.0544 mm). Similar statistical results have been obtained after the 100 mL cultivations. When compared with the positive control (0.358 ± 0.0757 mm), differences remain to reject the null hypothesis, with thinner films produced from cTPA (0.2738 ± 0.0358 mm). BNC thickness from 0.5 pcTPA (0.4011 ± 0.0231 mm) displayed the smallest data dispersion in terms of SD (Fig. S4), supporting more morphological regularity as stated above.

As presented in Fig. 5, the surface of BNC membranes remained the same after substituting 50% of the glucose amount with commercial or post-consumer TPA. No significant changes have been detected, in each tested sample the surface was smooth, indicating compact cellulose network structure [40].

In this study FT-IR analysis was employed in order to assess the structural characteristics of the BNC membranes produced under different nutritional conditions. Fig. 6A illustrates the shifts and shoulders at the 3261 cm^{-1} regions (H-bond) observed in the FT-IR graph, indicating changes in hydrogen bonding of BNC with the addition of TPA from both commercial and post-consumer sources [41,42]. The

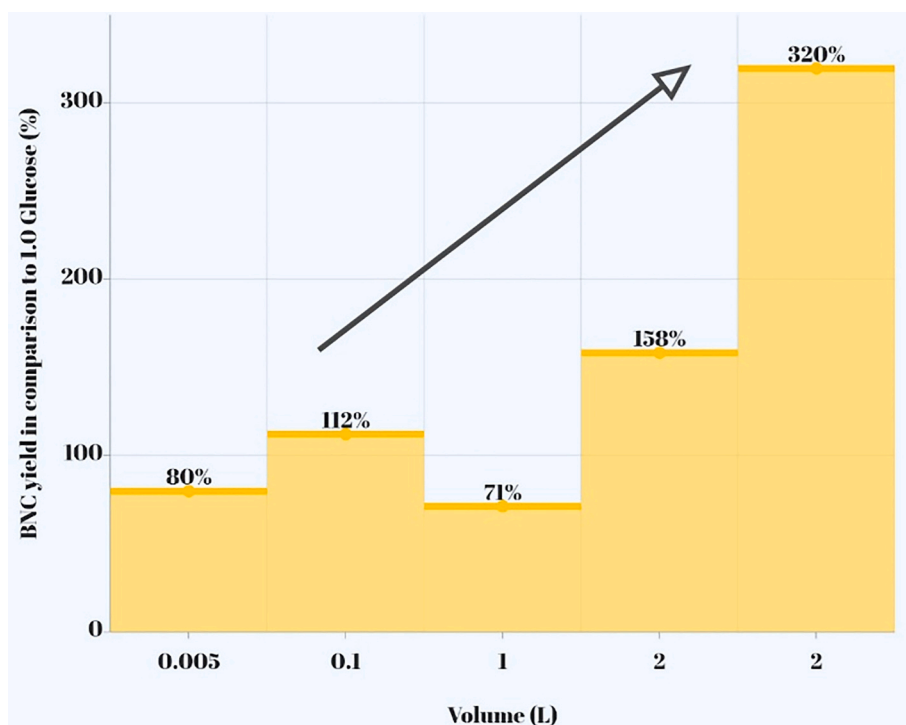


Fig. 4. Efficiency of the process involving post-consumer terephthalic acid (pcTPA) in comparison to positive control vs. BNC yields in scale-up experiments.

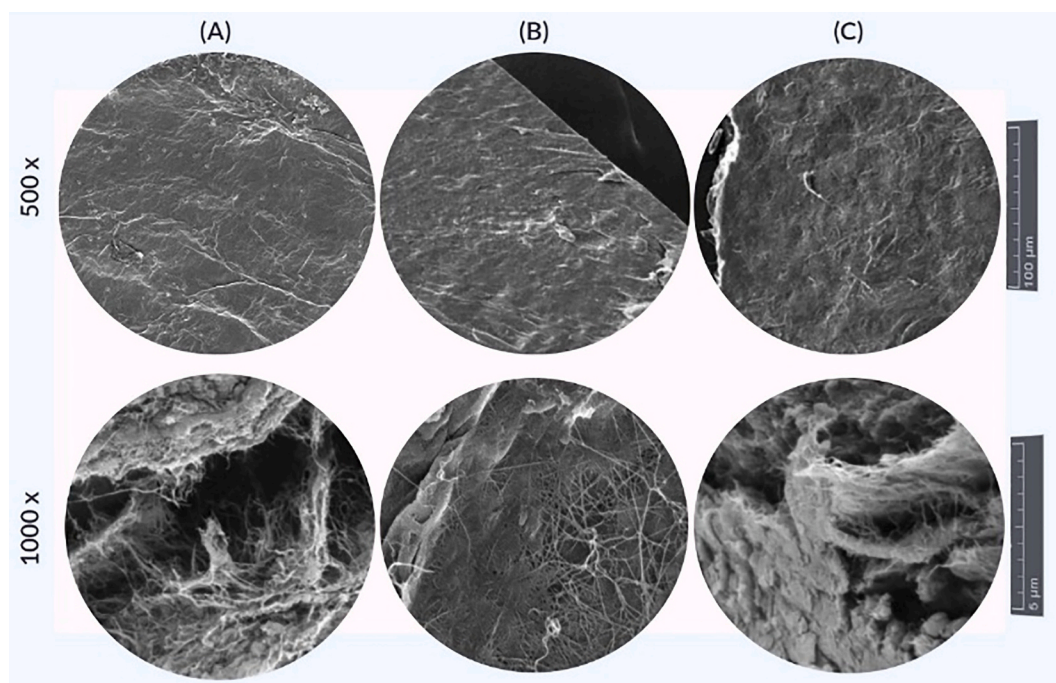


Fig. 5. SEM micrographs of bacterial nanocellulose (BNC) membranes produced by *Komagataeibacter medellinensis* ID13488 using HS medium and its varieties at 500 \times and 1000 \times magnification: 0.5 post-consumer terephthalic acid (pcTPA) (A); 0.5 commercial terephthalic acid (cTPA) (B); positive control (1.0 Glucose) (C).

overall structure of BNC remained largely unaffected with both types of TPA, with no indication of TPA impregnation. The main characteristic peaks at 3350 cm^{-1} (OH stretching), 2922 cm^{-1} (CH stretching of CH₂ and CH₃ groups), 1625 cm^{-1} (OH deformation), and 1056 cm^{-1} (anti-symmetric out-of-phase bending) were detected in all samples [43]. Notably, changes with impact on the mechanical properties of BNC were observed with the use of commercial TPA, where the observed increase in hydrogen bonding was more pronounced, resulting in a brighter

characteristic, while with the pcTPA showed hydrogen bonding profile more similar to the control (1.0 Glucose). These findings suggest that pcTPA has the potential to be a cost-effective addition for BNC production stimulation, as it can enhance the BNC quality without introducing major structural changes. This has important implications for the development of novel BNC-based materials for potential biomedical and industrial applications.

The thermal stability of BNC membranes was assessed as it is an

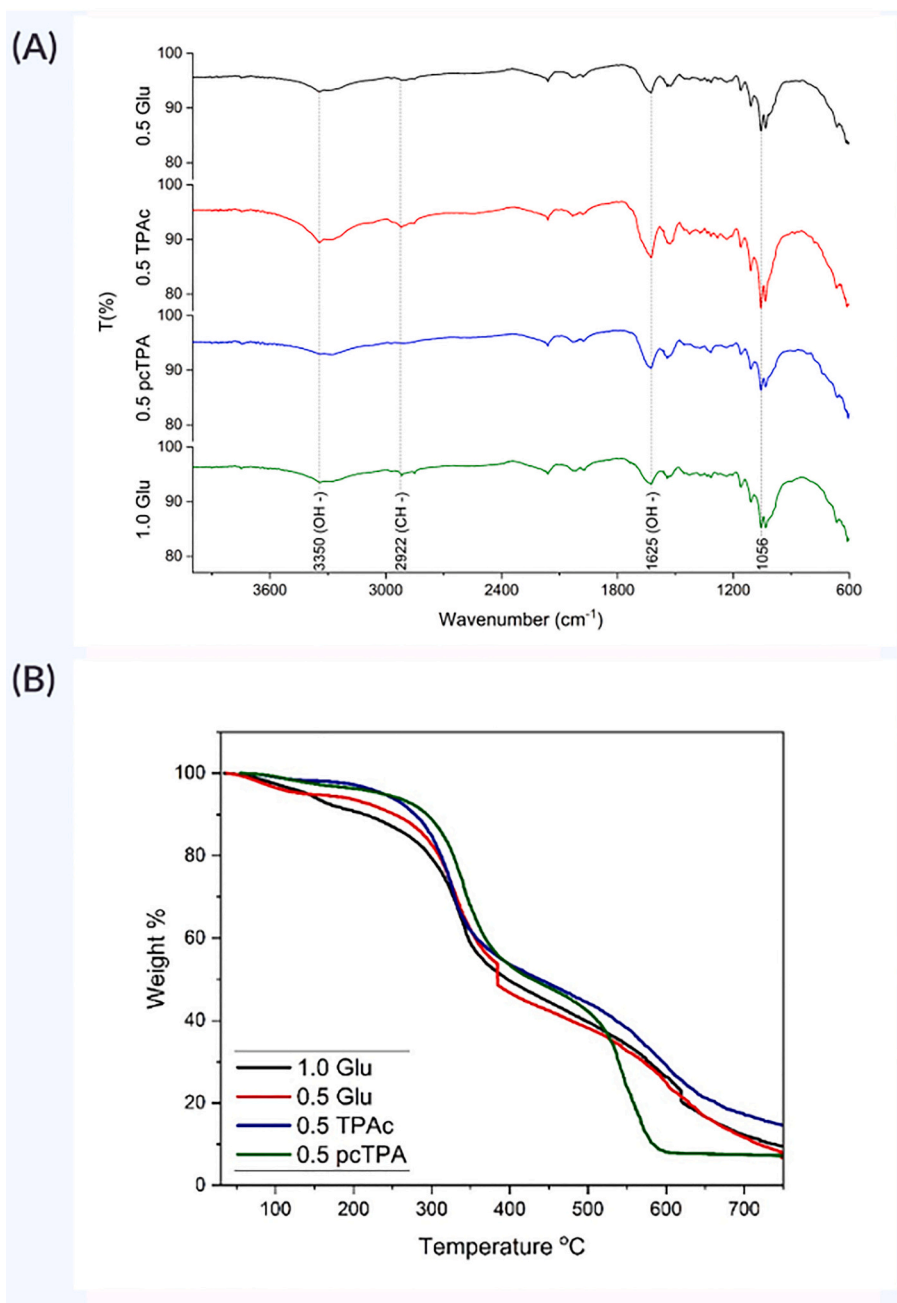


Fig. 6. FT-IR (A) and TGA (B) analysis of bacterial nanocellulose (BNC) membranes produced by *Komagataeibacter medellinensis* ID13488 using HS medium and its modifications.

important indicator for potential applications, but also to provide some insight on BNC fibers interactions [44]. In general, bacterial cellulose presents three main mass loss steps, which are: the release of water molecules, ranging from room temperature up to around 200 °C, undergoing partial oxidative decomposition of the carbohydrate segment from the polymer chain, and dissociation of the remaining chain with another oxidative step. BNC membranes showed a first-step weight loss at around 250 °C for all examined samples as presented in Fig. 6B. These results are consistent with previously reported studies [40]. The thermal stability of the samples was compared with each other by using the values “ $T_{d,10}$ ” and “ $T_{d,25}$ ” which correspond to the temperature at the weight loss of %10 and %25, respectively. In the case of 0.5 pcTPA, the BNC membrane showed higher stability (294 °C, @ $T_{d,10}$ and 334 °C, @ $T_{d,25}$), in comparison to the positive control (0.1 Glucose) (215 °C, @ $T_{d,10}$ and 313 °C, @ $T_{d,25}$). A similar result was obtained in the 0.5

TPAc experiment (278 °C, @ $T_{d,10}$ and 322 °C, @ $T_{d,25}$). These results are strong evidence that BNC produced with pcTPA had greater degradation temperature than the other BNC products. These differences might be explained by fibril size variation, crosslinking arrangement and compactness, which impute to higher crystallinity and higher thermal stability [45,46].

2.3. Analysis of post-cultivation media

As previously mentioned, our initial idea was to assess the ability of *K. medellinensis* to use TPA as a sole carbon source. It has been reported in the literature that some of BNC producing strains possess the ability to utilize PET related monomers such as TPA or EG as a carbon source [29]. However, after the 6 well plate assay where we had a trial with pcTPA and TPAc without glucose present, using negative controls as a

parameter, we could conclude that TPA is not adequate substitute for carbon source in HS medium. This was further confirmed after evaluation of TPA and glucose concentrations in the post-cultivation media (Fig. 7). The amount of TPA remained almost the same prior and after cultivation, while the amount of glucose decreased to minimum values. These results imply that the cultivation period used for this strain might be shorter under examined conditions. In the previously mentioned study by Zhou et al., it was clear that the presence of PET hydrolysates affects enzymatic toolbox of the examined strain resulting in an enhanced BNC production [31]. The effect of additives such as acetic acid, ethanol [47], sodium citrate [48], and lignosulfonate [49] on BNC production are extensively studied and reported as directly connected to cell proliferation and BNC synthesis. All these studies implied that having increased α -phosphoglucomutase and α -UDP-glucose pyrophosphorylase activities is favorable for boosting the BNC synthesis rate. There is no data on the effect of TPA in the BNC production medium to our knowledge, it can be presumed that this effect is similar to previously mentioned additives. However, to provide definitive confirmation, additional studies will be conducted in that direction.

2.4. Characterization of BNC and its derivatives for enhanced thermoplasticity

The FT-IR spectra of BNC and its derivatives with different bulky groups are shown in Fig. 8A. Compared with that of BNC, the intensity of the wide peak at 3348 cm^{-1} corresponding to the stretching vibration of -OH group in the spectra of BNC and its derivatives, is considerably greater in intensity for BNC 2-naphthoate. The absorption bands at 2953 , 2866 cm^{-1} , and that at 1440 cm^{-1} assigned to C-H stretching and C-H bending, respectively, became stronger and sharper in the spectra of BNC derivatives. This indicates that bulky groups have been successfully introduced into the backbone of BNC moieties. Comparing the FT-IR spectra of BNC derivatives with unmodified BNC, it could be clearly observed that a new ester carbonyl band at $1748\text{--}1720\text{ cm}^{-1}$ appeared, which confirmed a successful bulky groups substitution [50]. Moreover, the FT-IR analysis of BNC benzoate and 2-naphthoate also indicated the appearance of transmittance peaks at 1645 and 901 cm^{-1} confirming the presence of C=C stretch and bend of a phenyl group and the conformation of C-OH groups between 1230 cm^{-1} and 1400 cm^{-1} confirms the synthesis of a modified BNC [51,52].

The thermal behaviors of BNC and its derivatives with different chain length and bulkiness were characterized since the degradation temperatures are key parameters to determine the processing window of the polymer material. The thermogravimetric curves are shown in Fig. 8B, and the analysis results are listed in Fig. 8C. According to Fig. 8B, a small weight loss ranging between 7 and 9% was observed at $80\text{--}100\text{ }^{\circ}\text{C}$ in all

the tested samples. This could be related to the loss of moisture content on the surface or interlayer trapped water molecules [53]. The decomposition of BNC and its derivatives occurred on two major decomposition stages that are clearly demonstrated in the DTG curves as (T_{dm1} and T_{dm2} ; the temperatures at the maximum rate of the 1st and 2nd weight

losses) proceeding at T_{dm1} of 286 , 301 , 302 , 322 and $346\text{ }^{\circ}\text{C}$ and T_{dm2} of 340 , 387 , 375 , 385 and $524\text{ }^{\circ}\text{C}$ for BNC and its derivatives; BNC benzoate, BNC 2-naphthoate, BNC octanoate and BNC laurate, respectively as shown in Fig. 8D., the percentage weight losses at T_{dm1} were ranging between 22 and 43% while a total weight loss% of 45 to 89% was observed at T_{dm2} for the tested samples. BNC laurate and BNC 2-naphthoate showed the highest and lowest percentage weight loss, respectively. Thus, indicating that BNC 2-naphthoate had the highest thermal stability in all the synthesized BNC derivatives which could be attributed to its bulkier structure. The final decomposition temperature (T_{df}), and the temperatures at the maximum rate of weight loss (T_{dm1} and T_{dm2}) of the BNC derivatives were higher than that of BNC. Such behavior could be attributed to the introduction of the proposed bulky terminal moieties to the BNC side chains. Moreover, all the prepared BNC derivatives were thermostable at $200\text{ }^{\circ}\text{C}$, which can be considered as the lower critical temperature for the processing of these products.

Fig. 9 shows the degree of substitution (DS) of the different BNC samples determined by titration. The maximum DS was observed by BNC laurate having a DS of 3.75 followed by BNC benzoate with a DS of 1.56 then BNC 2-naphthoate and BNC octanoate with DSs at 1.32 and 0.82, respectively. Negligible level of substitution was observed for BNC recorded as 0.04. Technically, the substitution of the hydroxyl groups by ester groups destroys the hydrogen bonding interactions, increases the distance between the molecular chains, and improves the free volume and mobility of the macromolecular chains. The glass transition in non-crystalline polymers is considered an indicator of the macromolecular chains' movement becoming more feasible; thus, the glass transition temperature (T_g) significantly depends on the intermolecular interactions between the polymer's macromolecular chains. By conventional DSC, unmodified BNC has no glass transition prior to its decomposition due to the existence of strong hydrogen bonding interactions (Fig. S5). As shown in Fig. 9, obvious T_{gs} of BNC derivatives appear. Modified BNC with the studied bulky moieties demonstrated T_{gs} ranging between 133 and $136\text{ }^{\circ}\text{C}$. This could be attributed to the distortion of the crystalline structure of the BNC after the acylation reactions. The decrease of T_g value was also dependent on the bulkiness of the modifying moiety where BNC laurate and BNC 2-naphthoate showed lower T_g than that of BNC octanoate and BNC benzoate, respectively.

The optical micrographs in Fig. 9 show the thermal flow behaviors of BNC and its derivatives at different temperatures. The pure BNC is difficult to melt, and it does not even soften at $330\text{ }^{\circ}\text{C}$.

The introduction of bulky groups destroyed the hydrogen bonding networks and increased the distance between the macromolecular chains and as a result, the free volume and mobility of the molecular chains is improved. Therefore, the obtained BNC laurate and BNC octanoate softened and started melting at $270\text{ }^{\circ}\text{C}$ and $280\text{ }^{\circ}\text{C}$, respectively. Assumingly, the added bulky straight chain moieties acted as internal plasticizers reducing the intermolecular interactions of BNC chains and improving its thermoplastic properties. On the other hand, BNC benzoate and 2-naphthoate showed some softening of the material at temperature $270\text{--}280\text{ }^{\circ}\text{C}$ but did not melt. Such finding indicate that the addition of bulky phenyl groups could lead to some disruptions in the internal chains of the BNC but not as significant as that of the straight chains of octanoate and laurate.

3. Experimental

3.1. Materials

Benzoyl chloride, octanoyl chloride, Lauroyl chloride, 2-Naphthoyl chloride, 1-N-butyl-3-methylimidazolium chloride ($[\text{C4mim}] + \text{Cl-}$),

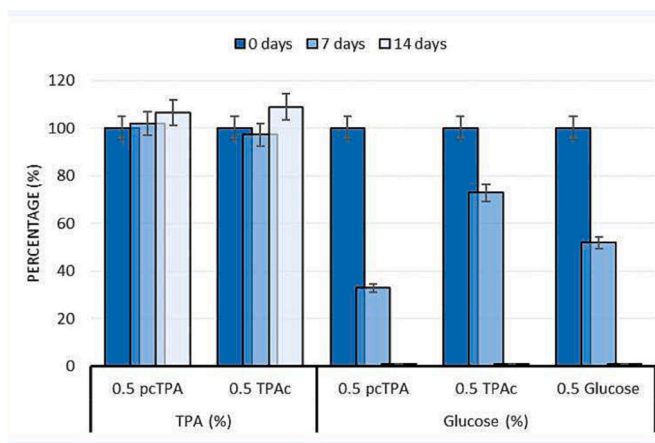


Fig. 7. Analysis of post-cultivation broth: The amount of leftover terephthalic acid and glucose.

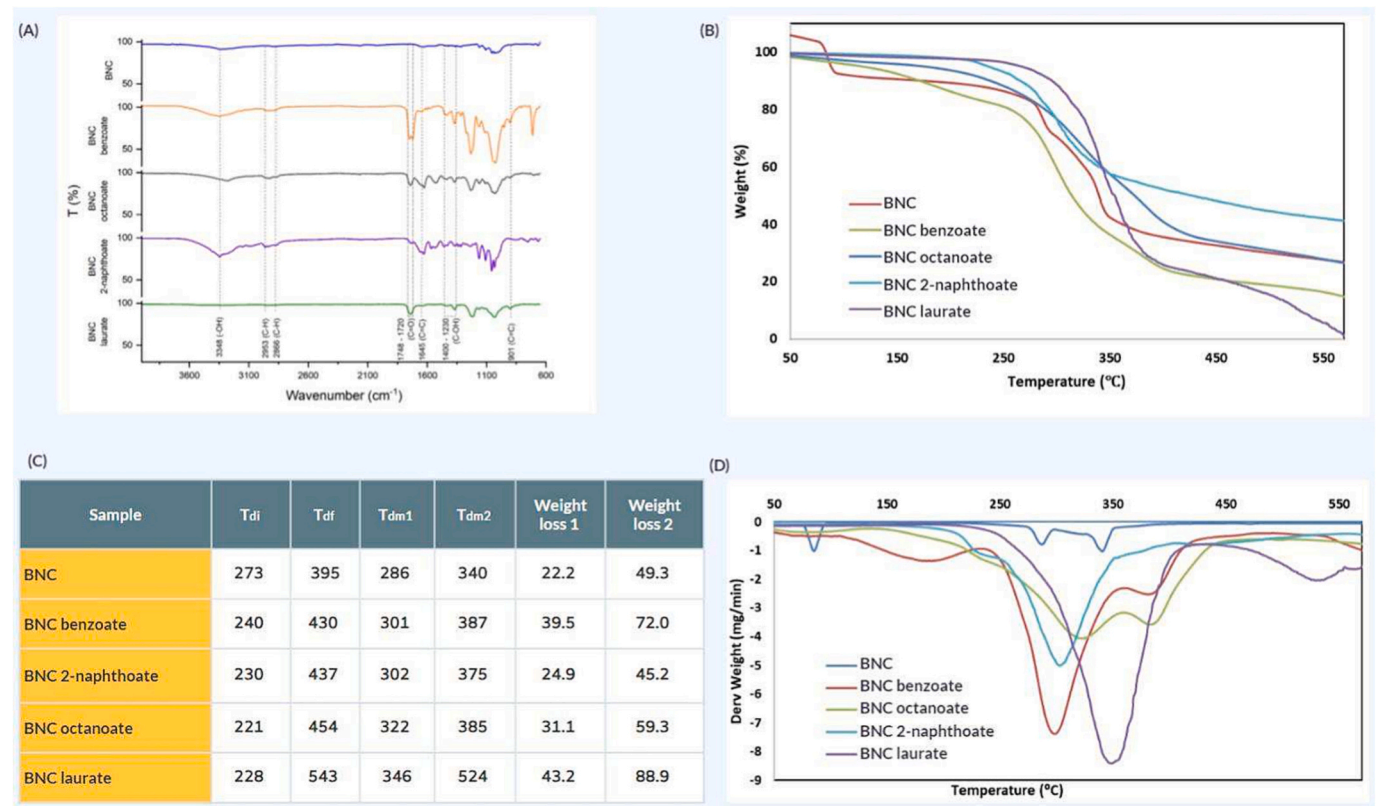


Fig. 8. Properties of bacterial nanocellulose (BNC) derivatives: FT-IR spectra (A); Thermogravimetric curves (B); Thermogravimetric analysis results (C); Derivative Thermogravimetry analysis (D).

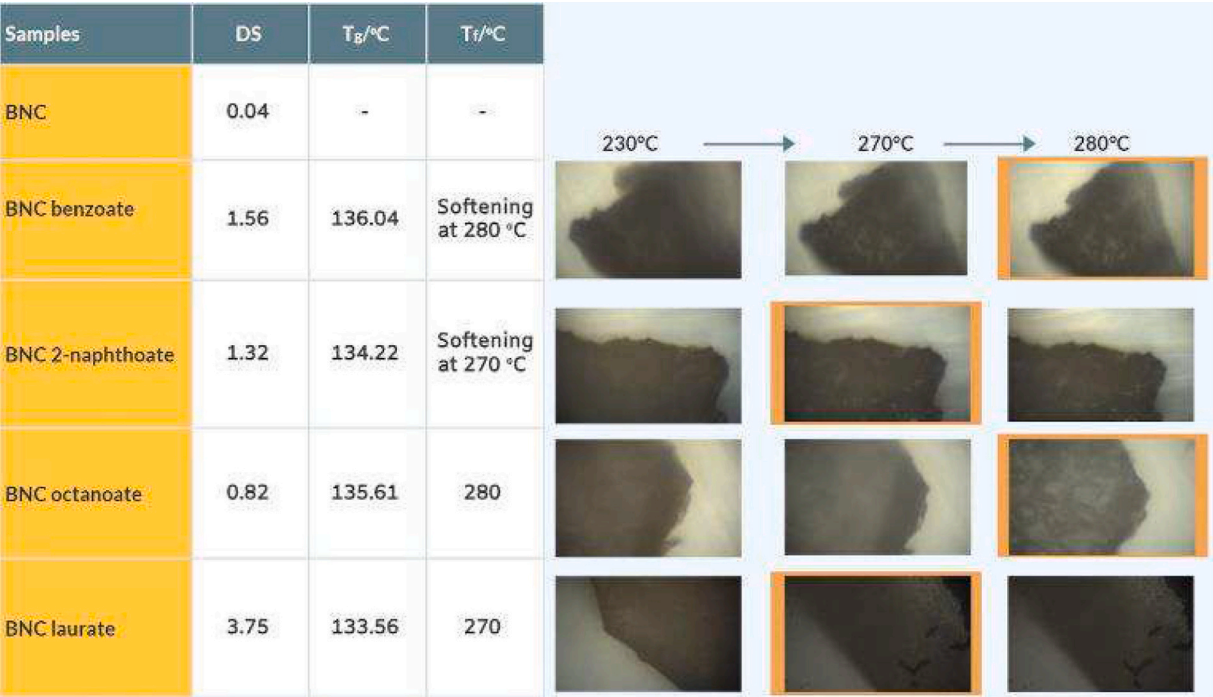


Fig. 9. Degree of substitution for bacterial nanocellulose (BNC) derivatives and their morphology under examined temperatures.

pyridine, and methanol were purchased from Sigma Aldrich (UK). Commercial TPA (TPAc) (Terephthalic acid 98%, Sigma-Aldrich, USA).

3.2. Chemical recycling of postconsumer PET via reactive extrusion

Green pigment-mixed PET pellets (PET/green) were supplied by Novelpast (Ireland) and named as postconsumer PET. The material is used as the standard substrate without further processing. The depolymerization experiment via reactive extrusion (REX) was conducted on a bench-top Prism™ twin-screw extruder (Thermo Electron GmbH, Karlsruhe, Germany) using a modified procedure from Fournet et al. [55]. The PET/green material was mixed with solid NaOH in the ratio (2:1) in a sealed plastic bag and the mixture was dispensed through the main shaft into the barrel. The temperature of the barrel was kept constant at 250 °C and the rotational speed of the screws was adjusted at 20 rpm. The resulting REX product was further processed to extract and separate its TPA content named as pcTPA. The identity and purity of TPA were then confirmed using Fourier-transform infrared spectroscopy (FT-IR), High-performance liquid chromatography (HPLC) and Nuclear magnetic resonance (NMR) analysis are demonstrated in Fig. S6, Fig. S7 and Fig. S8, respectively.

3.3. Preparation of pcTPA solution from chemically recycling post-consumer PET

Solution of pcTPA was obtained by dissolving 1 g of REX product (obtained in Section 2.2) in 30 mL of distilled water proportion, followed by homogenization on a hotplate at 600 rpm for 1 h. Further, the solution was filtered on a cellulose filter followed by pH 5.5 normalization by HCl adding. The final solution consisted of 15.8 mg/mL of recovered pcTPA and distilled water.

3.4. Bacterial nanocellulose production

BNC production was performed in several cultivation volumes (5 mL, 100 mL, 1 L and 2 L) as presented in Fig. 1. Preculture of *K. medelinensis* ID13488 was prepared in HS medium (0.5% peptone, 0.5% yeast extract, 0.27% Na₂HPO₄, 0.15% citric acid, 2% glucose; w/v) for four days at 30 °C, 180 rpm [56]. This strain was previously described as BNC producer by Castro et al. [57]. After incubation, preculture was rinsed with 0.9% saline solution, followed up with centrifugation of the cells (13,000 rpm, 15 min). In order to secure clean inoculum without glucose residuals, the rinsing process was performed twice per inoculum. For all performed experiments, HS medium was used as a positive control (1.0 Glucose).

The experiment in a 6-well plate (Sarstedt, Nümbrecht, Germany) was conducted to evaluate the effect of terephthalic acid (TPAc) from commercial and recovered pcTPA on BNC production. Glucose amount in HS medium was substituted up to 50 and 100% with the same amount of the TPAc (0.5 TPAc/0.5 Glucose; 1.0 TPAc) and pcTPA (0.5 pcTPA/0.5 Glucose; 1.0 pcTPA). Overall percentage of glucose and TPA combined was 2% in the media. Besides positive control used in all experiments (1.0 Glucose), this experiment required addition of another positive control containing 50% of glucose amount in HS medium (0.5 Glucose). Citrate-Phosphate 0.15 M buffer was used as a negative control in this experiment. Experiment was performed in 4 replicates, with 500 µL of inoculum and 4.5 mL medium solution (10% inoculum), and incubated at 30 °C, under static conditions for 14 days.

In order to analyze the effect of cultivation volume increment on yield and material properties, the same experiment was performed in 500 mL Erlenmeyer flask with 100 mL working volume. This experiment was performed in duplicates with non-inoculated HS medium as a negative to investigate the possibility of TPA repolymerization in medium itself.

According to the results in previous experiments, substitution of glucose with pcTPA up to 50% was chosen to be further assessed. For

further volume increase borosilicate glass tray (35 × 23 × 6 cm; Pyrex, Corning Inc., New York, USA), covered with a sterilized foil lid was used. Cultivation was performed in 1 L of medium with 10% inoculum for 3 weeks at 30 °C, statically. In order to keep the process conditions as similar as in previous experiments, additional oxygen was not supplied but it certainly will be part of our further process optimization studies.

Furthermore, working volume has been raised to 2 L using Bionet F2–3 twin bioreactor system (Bionet, Fuente Alamo, Spain) equipped with a double-wall tank which allows control over the inner chamber temperature and with an airflow rate control. Fermentation vessels were sterilized according to manufacturer's guidelines and cultivation started with the addition of previously prepared and cleaned inoculum (10%). Two batches have been running simultaneously: 1.0 Glucose and 0.5 pcTPA/0.5 Glucose. Temperature has been kept constant at 30 ± 1, pH 5.5 ± 1 and agitation at up to 300 rpm. Processes were monitored using ROSITA software [58]. Same experiment was conducted with the agitation of 80 rpm.

After cultivation BNC membranes were taken from the containers and washed with 2 M sodium hydroxide (20 min, 80 °C), neutralized with distilled water and left for a room-temperature drying process for 48 h to 72 h followed by the measurement of the dried mass [40].

3.5. Estimation of TPA content in cultivation media by the HPLC analysis

After the separation of BNC membranes, the cultivation broth was centrifuged (13,000 rpm, 15 min) and the supernatants were filtered (0.2 µm, VWR) and diluted with 30 mM NaOH in order to assess the amount of TPA. HPLC method was applied using anion exchange column (Ionpac AS11-HC 4.6 × 250 mm equipped with a pre-column) coupled to a conductivity detector. The analysis was performed at 30 °C, 30 mM NaOH was used as an eluent at a 1.5 mL/min flow rate. TPA standards in concentration range from 0.006 to 1.0 g/L were used for calibration curve construction.

3.6. Estimation of glucose consumption by the Dinitro Salicylic Acid (DNS) method

The total amount of reducing sugars was measured with dinitrosalicylic acid (DNS) colorimetric method, which was performed according to traditional method modified to reduced reaction volumes [59]. Set of solutions with known glucose concentrations (2 mL) were mixed with DNS reagent (Sigma Aldrich) and boiled for 5 min. Samples were allowed to cool down in cold water and absorbance was measured using spectrophotometer at 540 nm (CM-3610 A, Tokyo, Japan). The same procedure was performed with medium before and after cultivation in order to assess glucose consumption over time.

3.7. Material characterization

3.7.1. BNC membrane thickness

Dry membranes from 6-well micro-cultivation were shuffled to create a random selection of samples. For the 100 mL cultivation, the BNC membranes were cut in a rectangular shape (1 cm × 0.5 cm), forming a random pool of observations. Then the samples were measured randomly by a digital micrometer resulting in 4 observations per each sample.

3.7.2. Scanning electron microscopy (SEM) of BNC films

Scanning electron microscopy (SEM) was used to analyze the surface of the samples. Specifically, the Mira XMU SEM (Tescan™, Brno, Czech Republic) was employed to capture back-scattered electron mode images with the accelerating voltage of 10 kV. Samples were prepared on an aluminium stub and coated with a thin layer of gold using Baltec SCD 005 (Schalksmühle, Germany). The sputtering process lasted 110 s and was performed under a vacuum pressure of 0.1 mbar.

3.7.3. Thermogravimetric analysis (TGA) of BNC films

Thermogravimetric analysis of dried BNC samples was performed on a Pyris 1 TGA (PerkinElmer, Waltham, MA, USA) to evaluate their thermal properties. Each film (10 mg) was submitted to a heating process from 25 to 800 °C with a temperature increment speed of 10 °C/per min in order to obtain thermogravimetric curves.

3.8. Preparation of BNC derivatives for enhanced thermoplasticity

Obtained BNC membranes were soaked in 0.5 N sodium hydroxide at 80 °C for 2 h to remove bacterial cells and other ingredients, then washed several times with water till pH was neutralized. The resultant BNC was dried at 60 °C for 48 h before use. BNC derivatives were synthesized according to Chen et al., 2018 [54] with slight modification. In brief, one gram of BNC was dispersed in 19.0 g of 1-N-butyl-3-methylimidazolium chloride ([C4mim] + Cl⁻). After stirring at 80 °C for 1 h, a transparent BNC/[C4mim] + Cl⁻ solution was obtained. Pyridine (0.73 g) and then benzoyl, 2-naphthoyl, octanoyl and lauroyl chlorides were added separately at a mole-to-mole ratio (3:1) of bulky moiety to anhydroglucose repeat unit (AGU) of BNC and left stirring for 3 h. Subsequently, the products were precipitated in methanol, and the white floccules were collected by filtration. After washing three times with methanol, the product was re-dissolved in dimethyl sulfoxide (DMSO), precipitated again in methanol and washed another three times. Finally, the products were filtered and dried under vacuum at 60 °C for 24 h before characterization.

3.9. Characterization of synthesized BNC derivatives

FT-IR analyses were performed using Perkin-Elmer Spectrum One FT-IR spectrometer (Perkin Elmer Inc., Washington, DC, USA) fitted with a universal ATR sampling accessory and Perkin Elmer software, which was used to record the spectra of dried BNC films, applying 4 cm⁻¹ and 20 scans resolution on a 4000–650 cm⁻¹ spectrum.

TGA analysis of the derivatives was performed using previously described equipment and temperatures in the range from 50 to 650 °C, at a heating rate of 20 °C/min under nitrogen flow.

Degrees of substitution (DS) was determined by volumetric method (Eq. 1, 2) [60,61]. Dry BNC and its derivatives (0.1 g) were put into 30 mL of 75% ethanol in a glass bottle. The bottle, loosely stoppered, was heated to 50–60 °C for 30 min for better swelling of the material. Then 30 mL of 0.25 N NaOH solution, accurately measured, was added to the sample and the mixture was heated to 50–60 °C for 15 min. The bottle was stoppered tightly and allowed to stand at room temperature for about 48 h. The excess alkali was then titrated with 0.1 N HCl using phenolphthalein as an indicator.

The DS was calculated by the following equations:

$$n = \frac{(V_0 - V_1) \times c_{HCl}}{1000} \quad (1)$$

$$DS = \frac{162 \times n}{w - [(M_{wt} - 1) \times n]} \quad (2)$$

Where: w is the weight of BNC and its thermoplastic derivatives, cHCl is the molar concentration of hydrochloric acid solution, V₀ and V₁ are the volumes for neutralization of NaOH solution in the presence and absence of BNC, respectively. M_{wt} is the BNC derivative molecular weight (M_{wt} of benzoyl group is 105.12 g, M_{wt} of 2-naphthoyl group is 155.18 g, M_{wt} of octanoyl group is 127.20 g and M_{wt} of lauroyl group is 183.31 g).

Differential scanning calorimetry (DSC) measurements were conducted on a differential scanning calorimeter (TA Instruments, Q20, USA) under nitrogen flow (50 mL/min). To achieve the same thermal history before measurements, each sample was first heated at a scanning rate of 50 °C/min to 200 °C and was maintained at 200 °C for 5 min and then immediately quenched to 5 °C at the same scanning rate and

maintained at 5 °C for 5 min. The second heating scan was run from 5 to 250 °C at a scanning rate of 5 °C/min to record the glass-transition temperature (T_g).

Thermal flow behaviors of samples were observed by an Olympus BX5.1 optical microscope (Massachusetts, US) equipped with a Linkam THMS 600 hot-stage device. A small piece of the sample was sandwiched between two cover glasses and heated from 20 to 330 °C at a rate of 10 °C/min. The whole process was recorded by taking photos at the desired temperature intervals. The temperatures, at which the BNC and its derivatives were softened or melted, was recorded as the melt flow temperature (T_f).

4. Conclusions

The shifting landscape of the BNC market highlights the need for innovative strategies to ensure sustainability and growth. Despite the revised market expectations, BNC's substantial commercial potential persists. This study underscores the significance of post-consumer PET as a valuable resource in advancing bacterial cellulose production. The integration of pcTPA into BNC production processes offers a promising solution to the challenges posed by plastic pollution and resource depletion. By optimizing fermentation processes and harnessing pcTPA from mixed plastic waste, this research demonstrates the capacity to enhance BNC yield and contribute to the circular economy by utilizing waste materials. Our findings illuminate the positive impact of terephthalic acid derived from post-consumer PET on BNC yield, surpassing the performance of conventional terephthalic acid. Additionally, the exploration of improved thermoplasticity and modified BNC derivatives reveals potential applications in diverse fields, including biomedicine and industry.

This research not only underscores the feasibility of sustainable BNC production but also underscores the adaptability of BNC for diverse material science applications. By embracing pcTPA and innovative modifications, BNC production can potentially contribute to addressing environmental challenges while fostering new opportunities for advanced materials.

CRediT authorship contribution statement

Everton Henrique Da Silva Pereira: Conceptualization, Investigation, Data curation, Formal analysis, Methodology, Writing – original draft. **Olivia A. Attallah:** Investigation, Data curation, Formal analysis, Methodology, Supervision, Validation, Writing – original draft. **Cuneyt Erdinc Tas:** Investigation, Data curation, Formal analysis, Methodology, Writing – original draft. **Bor Shin Chee:** Investigation, Data curation, Formal analysis, Methodology. **Filomena Freitas:** Supervision, Validation, Writing – review & editing, Funding acquisition. **Eduardo Lanzagorta Garcia:** Investigation, Data curation, Formal analysis, Methodology, Writing – original draft. **Michael A.P. Mc Auliffe:** Investigation, Data curation, Formal analysis, Methodology. **Marija Mojicevic:** Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing. **Maria N. Batista:** Investigation, Data curation, Formal analysis, Methodology. **Maria A.M. Reis:** Writing – review & editing, Funding acquisition. **Margaret Brennan Fournet:** Supervision, Validation, Funding acquisition.

Declaration of Competing Interest

There are no conflicts to declare.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.susmat.2023.e00784>.

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