



Upcycling post-consumer household textiles into a biodegradable thermoplastic

Thomas Rodrigues^{a,b,1}, João Matias^{a,b,1}, Patrícia Moura^c, Mariana Abreu^c, Francisco Gírio^c, Adelaide Braga^d, Juliana C. Dias^d, Ana Rovisco^e, Elvira Fortunato^e, Cristiana A.V. Torres^{a,b}, Maria A.M. Reis^{a,b}, Susana Marques^c, Filomena Freitas^{a,b,*}, Carla J. Silva^d

^a Associate Laboratory i4HB—Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal

^b UCIBIO—Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal

^c Bioenergy and Biorefineries Unit, LNEG—National Laboratory of Energy and Geology, 1649-038 Lisboa, Portugal

^d CITEVE—Technological Centre for Textile and Clothing of Portugal, 4760-034 Vila Nova de Famalicão, Portugal

^e CENIMAT³i3N, Department of Materials Science, NOVA School of Science and Technology, NOVA University Lisbon and CEMOP/UNINOVA, 2829-516 Caparica, Portugal

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ABSTRACT

This study demonstrates the biological valorization of post-consumer cotton-based towels, a significant household textile waste stream, into poly(3-hydroxybutyrate) P(3HB), a biodegradable and biocompatible thermoplastic. The textile waste was mechanically defibrillated, and pre-treated by freezing in NaOH/urea, rendering the cellulose fibers more accessible to saccharification, which was achieved by hydrolysis with Cellic® CTec3 cellulase. The resulting hydrolysate, with a glucose concentration of 102.3 g/L, was used to supply glucose as the carbon source for the batch bioreactor cultivation of the bacterium *Burkholderia thailandensis* E264. With an initial glucose concentration of 40 g/L, a maximum P(3HB) production of 9.17 ± 1.01 g/L was observed after 53 h of cultivation, corresponding to an overall volumetric productivity of 0.165 g/(L h) and a product yield of 0.218 g/g. The P(3HB) exhibited an average molecular weight of 497 kDa and a polydispersity index of 2.6, with melting and thermal degradation temperatures of 170 and 291 °C, respectively, crystallinity index of 78.8%. Importantly, its tensile properties (Young's Modulus of 563 ± 62 MPa and tensile strength of 27 ± 2 MPa) were comparable to P(3HB) produced by other bacteria, including commercial products. The findings of this study demonstrate the feasibility of upcycling cotton-based household waste into a value-added sustainable and biodegradable material with properties matching those of commercial thermoplastics.

1. Introduction

The world faces a textile waste crisis, primarily fueled by the fast fashion industry, which promotes rapid consumption cycles and short garment lifespans, exacerbating the volume of waste generated globally [1,2]. Textile waste encompasses discarded clothing, production leftovers, and other fibrous remnants, and is estimated at approximately 5.8 million tons per year in the European Union (EU) [3]. In North-West Europe, despite ongoing efforts to improve recycling, less than 2% of textiles are recycled into new fabrics, and around 50% is either down-cycled, incinerated, or sent to landfills, with associated environmental

burden [3,4]. To deal with this, effective management strategies must be sought, not only for textile waste reuse and recycling, but also for its valorization through novel routes. Household textiles, including towels, bed linen and related products, constitute a substantial portion of cotton-rich post-consumer textile waste, with 100% cotton towels standing out as particularly abundant. The high cellulose content of such wastes, together with their frequently light or white coloration that minimizes the need for intensive chemical treatments to remove dyes and finishing agents [5], renders them an attractive alternative feed-stock for advanced biotechnological processes based on the sugar platform [6].

* Corresponding author at: Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal.
E-mail address: a4406@fct.unl.pt (F. Freitas).

¹ These authors contributed equally to this work.

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Enzymatic hydrolysis of cellulose into glucose is an environmentally friendly method to leverage cotton-rich textiles to produce high-value microbial bioproducts or biofuels. However, the efficiency of hydrolysis is strongly influenced by the pretreatment applied to disrupt the structural and chemical barriers in cotton fibers, increasing the surface area and enhancing susceptibility to cellulolytic enzymes. One of the most widely used strategies is alkaline treatment that induce fiber swelling, reduce cellulose crystallinity and break intermolecular hydrogen bonds, thereby increasing enzyme accessibility [7]. The efficiency of this pretreatment is heavily influenced by temperature, with sub-zero temperatures being more effective, as they facilitate the deeper hydration of the cellulose fiber [8]. Studies also showed that adding urea to an alkaline solution, while maintaining the temperature at -20°C , increased glucose yield [9].

Polyhydroxyalkanoates (PHA) are biobased bioplastics produced by algae, plants and bacteria, offering numerous advantages over fossil-based plastics, including biodegradability, non-cytotoxicity, biocompatibility, and bioresorbability [10]. The most widely studied PHA is the homopolymer poly(3-hydroxybutyrate), P(3HB), a rigid, highly crystalline thermoplastic, available at commercial scale and used for a broad range of applications, including biomedicine [11], packaging, and textiles [12]. Nonetheless, the production costs, greatly associated with the carbon sources that account for over 50% of overall bioprocess costs, still hinder the broader use of PHA. Thus, the use of low-cost, waste-based sustainable substrates is a valuable strategy to render PHA more cost-effective, while also tackling the environmental burden of such wastes [10].

This study aimed to establish an integrated valorization of post-consumer cotton-based towels, including mechanical defibrillation, NaOH/Urea freezing pretreatment, enzymatic hydrolysis, P(3HB) production, and polymer film processing. While the individual steps have been previously reported for lignocellulosic substrates, the present work specifically evaluates real post-consumer cotton towels as a feedstock for the bioreactor cultivation of *Burkholderia thailandensis* E264 and P(3HB) production. The produced polymer was characterized in terms of composition, molecular mass distribution, crystallinity, thermal and mechanical properties.

2. Experimental section

2.1. Feedstock processing

2.1.1. Mechanical pre-processing

Post-consumer home textiles, mainly terry towels (Supplementary Material, Fig. S1A), were used as the cellulose-rich feedstock. A qualitative identification of the fiber type was performed through microscopic evaluation according to AATCC TM20–2021, confirming that cotton was the predominate fiber in all samples. The textile waste underwent a mechanical defibrillation process comprising sequential cutting, shredding and gridding. An initial mass of 3.0 kg of textile waste was processed three times through the cutting machine (StarCut Cutting System, LAROCHE, France) (Fig. S2), with a target cut size of 50 mm, producing textile fragments (Fig. S1B). The material was further subjected to shredding and grinding using a multi-module shredding system (Cadette Waste Pulling Machine, LAROCHE, France) (Fig. S2) comprising five modules operating with progressively increasing feed belt speeds (1620–2199 rpm) and cylinder rotation speeds (0.80–1.35 rpm). The material was processed once through modules 1–3 and twice through modules 4–5, resulting in cellulose-rich fibers (Fig. S1C) that were collected and used in subsequent experiments.

2.1.2. Pretreatment

Two methods were tested to pretreat 5 g_{o.d.solids} (oven-dried basis) textile waste resulting from the mechanical pre-processing, at a solids' loading of 5% (w/v): (1) alkaline autoclaving [13], in which the textile waste was mixed with 15% (w/v) NaOH solution and autoclaved at

121 °C and 1 bar, for 15 min; and (2) freezing in NaOH/urea [9], wherein the waste was soaked in a solution of 7% (w/v) NaOH and 12% (w/v) urea, followed by freezing the mixture at -20°C and thawing after 6 h. The fibers treated by each method were washed by resuspension in 500 mL of distilled water (with magnetic stirring, for 5 min), followed by filtration through paper filter (F1004 grade, CHMLAB) until the filtrate reached pH 7. The washed samples were then dried overnight in an oven at 60 °C. The dry weight of the treated and untreated materials was determined by drying at 110 °C until a constant weight was reached. Their cellulose content was analyzed using the standardized NREL Laboratory Analytical Procedure [14].

The towel fibers' micromorphology was evaluated throughout the mechanical pre-processing and pretreatment methods. The images were observed by scanning electron microscopy (SEM; Hitachi, Regulus 8220) mounted on aluminum stubs using double-sided carbon tape and sputter-coated with a 20 nm thin Au/Pd (80/20 wt%) layer (150 T ES, Quorum, UK). The analysis was performed at an accelerating voltage of 5 kV and magnifications of 25× and 500 ×.

2.1.3. Enzymatic hydrolysis

The pretreated textile waste samples obtained by the alkaline autoclaving and freezing in NaOH/urea methods were subjected to enzymatic hydrolysis, using commercial cellulase (Cellic® CTec3, exhibiting a FPase activity of 151 FPU/mL; Novonesis A/S, Bagsværd, Denmark) at an enzyme dosage of 10% (w/w, oven-dried fibers). The hydrolysis was performed in 0.05 M citrate buffer pH 5 (1 mL in 2 mL flasks), at 50 °C, in an orbital shaker (150 rpm), in the presence of 0.04% (w/v) sodium azide to prevent biological contamination. Two substrate loadings of 30 and 100 g solids_{o.d.solids}/L were tested, in triplicates, for each chemically pre-treated sample, which were incubated for different time periods (6, 24, 48 and 72 h). Crude textile waste, without pretreatment, was used as a control, at both substrate loadings. After hydrolysis, the remaining solids were removed by centrifugation (15 min, 13700 ×g) and the collected supernatant was analyzed for glucose by high-performance liquid chromatography (HPLC), as described below. The hydrolysis yield (Y_H , %) was determined using the following equation, by applying the 1.11 hydration factor for glucan conversion into glucose:

$$Y_H = [\text{Glc}_h / (1.11 \times \text{Gn}_{in})] \times 100 \quad (1)$$

where Glc_h (g/L) corresponds to glucose quantified in the hydrolysate subtracted by glucose contribution from the enzyme cocktail, and Gn_{in} (g/L) is the initial glucan (cellulose) concentration in the substrate.

2.2. Media preparation for P(3HB) production

The enzymatic hydrolysate of the post-consumer towels was used as the sole carbon source for the bioreactor cultivation runs. For hydrolysate preparation, the cellulose-rich fibers (400 g) obtained by the mechanical defibrillation procedure were subjected to pretreatment by freezing in NaOH/urea (7% and 12% (w/v), respectively) and thawing (for this upscaled assay, the freezing period at -20°C was prolonged to 20 h), followed by enzymatic hydrolysis with Cellic® CTec3, for 48 h, at 100 g o.d.solids/L (at an enzyme dosage of 10%, w/w), in six 1-L Erlenmeyer flasks. The feedstock was sterilized by autoclaving (at 121 °C, for 15 min) and the enzyme solution was filter-sterilized (through polyethersulfone membrane filter 0.22 μm pore size, Millipore Express® PLUS). The hydrolysate was centrifuged (13700 ×g, 15 min, 4 °C) and stored at -20°C until further use.

For the bioreactor cultivation experiments, Medium E* [15] was supplemented with the hydrolysate prepared as described above, to reach an initial glucose concentration of 40 g/L. The pH of the medium was adjusted to 7.0 by the addition of 5 M NaOH before autoclaving at 121 °C and 1 bar, for 20 min.

2.3. P(3HB) production

2.3.1. Inoculum preparation

Burkholderia thailandensis E264 was reactivated from the cryo-preserved vial by plating on CHROMagar™ Orientation agar plates (Paris, France) and incubating at 30 °C for 48 h. The pre-inocula were prepared by collecting a single colony from the agar plate and inoculating it into 20 mL of Luria-Bertani (LB) broth [16] in a 100 mL Erlenmeyer flask with a cotton stopper. After 24 h incubation in an orbital shaker (200 rpm) at 30 °C, 20 mL of the culture were transferred into 200 mL of Medium E* supplemented with the enzymatic hydrolysate (at a glucose concentration of 40 g/L), in a 500 mL baffled shake flask, and further cultivated for 24 h under identical conditions, to obtain the inoculum for the bioreactor cultivation.

2.3.2. Bioreactor assays

B. thailandensis E264 was grown in a 3 L bioreactor (Jupiter, Solaris, Italy), under batch-mode, with a 2 L working volume comprising 10% (v/v) inoculum (200 mL). The temperature was controlled at 30 ± 0.1 °C, and the pH was set at 7 ± 0.1 by automatic addition of 5 M NaOH and 2 M HCl. The airflow rate (2 SLPM, standard liter per minute) was kept constant throughout the cultivation run, and the dissolved oxygen (DO) concentration was maintained at 30% of the air saturation through the automatic adjustment of the stirrer speed between 200 and 1800 rpm. Foam formation was prevented by the automatic addition of Antifoam A (Sigma-Aldrich). Samples (12 mL) were collected from the bioreactor and centrifuged (9000 ×g, 15 min, 4 °C). The cell-free supernatant was preserved at −20 °C for glucose and ammonium quantification, while the pellet was washed with deionized water and freeze-dried for cell dry weight (CDW) and PHA quantification.

2.3.3. P(3HB) extraction

At the end of the cultivation run, the broth was centrifuged (9000 ×g, 20 min, 4 °C) and the cell pellet was washed with deionized water. Then, the pellet was freeze-dried, and the PHA was recovered from the bacterial cells through Soxhlet extraction with chloroform (Honeywell, 99.8%) (10 g of biomass in 250 mL chloroform), at 90 °C, for 48 h [17]. The collected solution was filtered, and the polymer was precipitated drop-by-drop into ethanol (1:10, v/v), under vigorous stirring.

2.3.4. Calculations

The maximum specific cell growth rate was determined from the linear regression slope of the exponential phase of $\ln X_t$, versus time, where X_t (g/L) represents the active biomass (cells without P(3HB)), at time t (h). The active biomass was determined as follows:

$$X_t = \text{CDW}_t - \text{P(3HB)}_t \quad (2)$$

where CDW_t (g/L) and P(3HB)_t (g/L) are the CDW and polymer concentrations, respectively, at time (t). The specific cell growth rate (μ_x , h^{-1}) and the specific P(3HB) production rate (q_p , $\text{gP(3HB)}/(\text{gX h})$) were determined as follows:

$$\mu_x = \frac{dX}{dt} \frac{1}{X} \quad (3)$$

$$q_p = \frac{d\text{P(3HB)}}{dt} \frac{1}{X} \quad (4)$$

where X (g/L) corresponds to the arithmetic mean of the active biomass values between the points associated with dX/dt and $d\text{P(3HB)}/dt$. The polymer ($Y_{p/s}$, $\text{gP(3HB)}/\text{g}_s$) and growth ($Y_{x/s}$, gX/g_s) yields on a substrate basis were calculated as follows:

$$Y_{p/s} = \Delta P / \Delta S \quad (5)$$

$$Y_{x/s} = \Delta X / \Delta S \quad (6)$$

where ΔP (g/L) is the produced P(3HB), ΔX (g/L) is the active biomass and ΔS (g/L) is the glucose consumed over the cultivation run. The volumetric productivity of P(3HB) (r_p , $\text{g}/(\text{L h})$) and biomass (r_x , $\text{g}/(\text{L h})$) was calculated as follows:

$$r_p = \frac{d\text{P(3HB)}}{dt} \quad (7)$$

$$r_x = \frac{dX}{dt} \quad (8)$$

2.3.5. Analytical techniques

The CDW was gravimetrically determined by weighing the freeze-dried cell pellets. The P(3HB) content within the cells was determined by gas chromatography (GC), after methanolysis as described by Rebocho et al. [17]. The ammonium concentration was determined colorimetrically using a flow-segmented analyzer (Skalar 5100, Skalar Analytical) [16]. Glucose concentration was determined using a VWR Hitachi Chromaster HPLC, equipped with a Micro-Guard Cation H Refill Cartridge (Bio-Rad) precolumn and a Aminex HPX-87H (300 × 7.8 mm) (Biorad) column coupled to a refractive index (RI) detector (Hitachi 5450). The analysis was conducted at 30 °C in isocratic mode with a mobile phase of 0.01 N sulfuric acid, at a flow rate of 0.5 mL/min and a 99 µL injection volume. Glucose (Scharlau, 97.5–100%) was used as standards at concentrations between 0.01 and 1 g/L.

Furfural, 5-hydroxymethylfurfural (5-HMF), formic acid, and acetic acid were quantified by HPLC using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) coupled to an Ultraviolet – visible detector [15] at 210 nm (for acetic and formic acids) and at 280 nm (for furfural and 5-HMF). Calibration curves were obtained using standard solutions of glacial acetic acid (Fisher Chemical, 99.7%) and formic acid (Sigma-Aldrich, ≥95%) at concentrations between 0.01 and 1 g/L, furfural (Sigma-Aldrich, 99%) at concentrations of 0.0196–0.392 g/L, and 5-HMF (Sigma-Aldrich, ≥99%) between 0.00925 and 0.185 g/L.

Replicate measurements were performed for all analyses.

2.4. P(3HB) characterization

Elemental analysis was conducted using a Flash EA 1112 CHNS analyzer (Thermo Scientific, Waltham, MA, USA).

Number (Mn) and weight (Mw) average molecular weights and the polydispersity index ($\text{PDI} = \text{Mw}/\text{Mn}$) of the P(3HB) samples were determined by size exclusion high performance liquid chromatography (SE-HPLC), as described by Esmail et al. [11] in a System (KNAUER Smartline, Germany) with support Phenomenex Phenogel Linear Liquid Chromatographic Column 300 × 7.8 mm (Phenomenex, USA), at an equilibration temperature of 30 °C, using chloroform as the mobile phase at a flow rate of 1 mL/min. Monodisperse polystyrene standards (Sigma-Aldrich), with Mw in the range 800 kDa to 504 kDa, were used to calculate the relative Mw according to the universal calibration method, using Millennium SEC software.

Fourier transform infrared spectroscopy (FTIR) was carried out using a Perkin-Elmer Spectrum Two spectrometer. The spectra were recorded with 10 scans between 400 and 4000 cm^{-1} resolution, at room temperature. X-ray diffraction was done in an X-ray diffractometer (PANalytical X'Pert PRO MRD) with a monochromatic radiation source of Cu K α (45 kV and 40 mA) to scan the samples, over a 2θ range from 10° to 90°, at a scan rate of 10°/min in continuous scanning mode.

Differential scanning calorimetry (DSC) was performed in a differential scanning calorimeter Discovery Series DSC25 (TA Instruments, New Castle, DE, USA) coupled to a cooling system 90 (TA Instruments Refrigerated cooling system 90, USA), in the temperature range from −90 to 200 °C, as described by Rebocho et al. [17]. The melting temperature (T_m , °C) was taken as the endothermic peak's minimum.

Thermogravimetric analysis (TGA) was performed in a thermogravimetric equipment Labsys EVO (Setaram, France), over a temperature

range from 25 to 800 °C, as described by Matias et al. [16]. The degradation temperature ($T_{\text{deg},5\%}$) was determined as the temperature at which the sample exhibited a 5% mass loss, indicating the onset of significant thermal degradation. The maximum degradation temperature ($T_{\text{deg,max}}$) was determined as the temperature corresponding to the maximum degradation rate throughout the main mass-loss event.

2.5. P(3HB) films

P(3HB) was molded into films using a manual hot press (TMAXCN, RYJ-600E2, China) with double heating plates. The plates were heated to 170 °C. Then, the mold (200 × 200 mm) containing the polymer (1 g) was placed between the plates and pressed for 2 min, applying a 1-ton (2.9 MPa) pressure. The film was cooled from 170 °C to approximately 50 °C over 25 min using the water-cooling system of the press, corresponding to an average cooling rate of approximately 4.8 °C min^{-1} , and carefully peeled from the plates. The films' micromorphology was observed by SEM as described in Section 2.1.2. For the cross-sectional view, the films were immersed in liquid nitrogen and fractured.

The films were cut into rectangular-shaped test pieces (30 × 10 mm), with an average thickness of $87 \pm 8.6\text{ }\mu\text{m}$, and submitted to tensile tests using a texture analyzer (Food Technology Corporation, Wales, England), applying a 10 mm/min tensile rate until break, with a 50 N cell [11]. The Young's Modulus (E, MPa) was determined from the curve's initial slope, while the tensile strength (σ , MPa) was calculated from the highest point of the curve, just before rupture. The elongation at break (ϵ , %) corresponded to the ratio of the test piece length at rupture to its initial length. Several replicas were analyzed.

3. Results and discussion

3.1. Feedstock processing: pretreatment of post-consumer towels

3.1.1. Mechanical defibrillation

A total of 11 post-consumer towels were used as the cotton-rich textile waste raw material for this study. The set comprised different sizes and designs of typical household towels, representative of post-consumer cotton-rich textiles. The highly ordered crystalline structure of cotton fibers requires the application of pretreatments, including physical (e.g., milling, thermal) and chemical treatments, to render them more prone to saccharification by enzymatic hydrolysis [18]. Therefore, 3 kg of the post-consumer towels were initially subjected to mechanical processing to obtain a fibrous material suitable for subsequent hydrolysis steps. After three passes through a cutting machine, with a target cut size of 50 mm, the towels were converted into smaller pieces that facilitated feeding into the defibrillation system. Mechanical defibrillation was performed using five modules, each operated at progressively increasing feed-belt and cylinder rotation speeds, with one to two passes per module. This stepwise processing resulted in 2.3 kg of loose and homogeneous cellulose-rich fibers (Fig. 1B). The observed mass loss (~0.7 kg) is consistent with typical processing losses during mechanical opening and shredding, where short fibers and dust are removed or retained within the equipment, reducing the recoverable fiber yield [19].

The structural changes induced in the textile waste by mechanical processing were confirmed by SEM imaging. The raw towels (Fig. 1A) exhibited compact and highly entangled fiber bundles, forming dense aggregates with limited inter-fiber spacing (Fig. 1A.1 and A.2). In contrast, the mechanically defibrillated material (Fig. 1B) showed a clear increase in fiber individualization, resulting in a looser fibrous network (Fig. 1B.1 and B.2). The reduction in fiber entanglement and the concomitant greater exposure of cellulose surfaces is expected to improve enzyme accessibility during subsequent hydrolysis, potentially

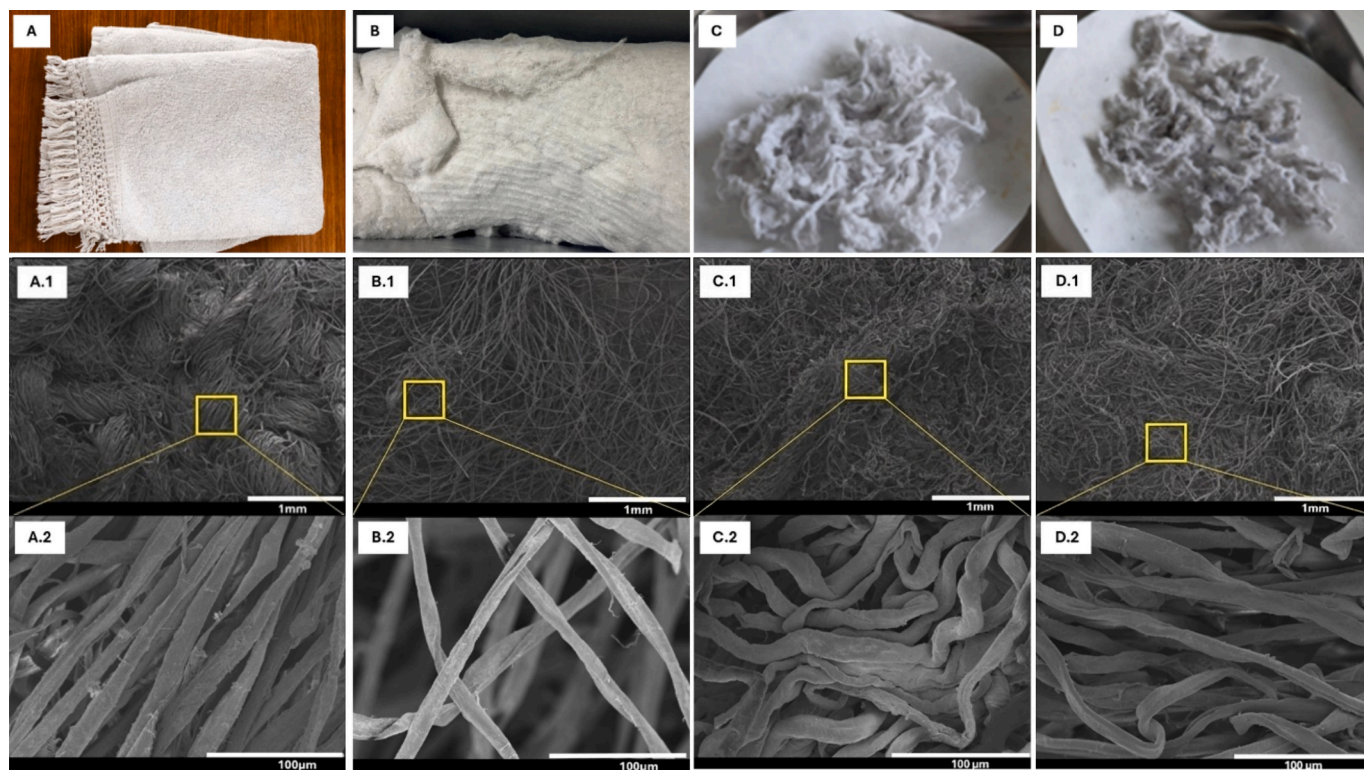


Fig. 1. Images of the post-consumer towels (A), the cellulose-rich fibers after the mechanical defibrillation processing (B) and further treated by alkaline autoclaving (C) or freezing in NaOH/urea (D), and the corresponding SEM micrographs (lower images).

enhancing saccharification efficiency.

Chemical analysis of the mechanically defibrillated fibers showed that they were predominantly made of cotton ($91.3 \pm 1.5\%$ of cellulose) (Table 1), with minor synthetic components, mostly decorative features. These results align with previous reports in which cellulose usually represents up to 99% of the cotton textile fibers content [9]. Such high cellulose contents, much higher than those of lignocellulosic materials often used as feedstocks for bioprocesses, which typically exhibit cellulose contents in the range of 30–55% [20], make cotton-based textiles very attractive, low-cost renewable sources of fermentable sugars for microbial cultivation.

3.1.2. Chemical pretreatments

Treatment with NaOH, which is known to reduce cellulose crystallinity (Jeihanipour and Taherzadeh, 2009) and to act as a swelling agent for cotton [21], was used to treat the mechanically defibrillated cotton-based towels to render the material more prone to the subsequent enzymatic hydrolysis step. Therefore, soaking the cellulose-rich fibers in a 15% NaOH solution, followed by mild hydrothermal treatment in the autoclave (121°C , 1 bar, for 15 min) was attempted. In addition, considering NaOH has a higher impact on the structure and morphology of cellulose fibers at low temperatures, the procedure reported by Boondaeng et al. [13] consisting of freezing the fibers in a NaOH/urea solution, was also tested.

Solids' recovery yield values of 86.7% and 91.1% (dry weight basis) were obtained for alkaline autoclaving and the freezing NaOH/urea treatment, respectively, with cellulose contents exceeding 95% (Table 1). Moreover, there was limited solubilization of cellulose, especially with the freezing NaOH/urea method, wherein a 5% cellulose loss was observed. SEM analysis (Fig. 1C and D) confirmed the impact of each chemical treatment on the structure of the cotton-rich fibers. Overall, both chemical pretreatments preserved the fibrous nature of the material while inducing visible morphological changes. Alkaline autoclaving resulted in a more compact and collapsed fiber network (Fig. 1C), whereas the freezing NaOH/urea treatment (Fig. 1D) yielded a slightly more open and individualized fiber arrangement, which may be favorable for the subsequent enzymatic hydrolysis step.

3.2. Feedstock preparation: sugars recovery from the post-consumer towels

3.2.1. Optimization studies

To select the best conditions for cellulose conversion into glucose, the chemically pretreated samples, as well as the initial mechanically defibrillated textile waste fibers, were subjected to enzymatic saccharification trials (in triplicate) under two substrate loadings: 30 and 100 $\text{g}_{\text{o.d.solids}}/\text{L}$, with commercial Cellic® CTec3. As shown in Fig. 2 and Table 2, limited saccharification was achieved with the control samples, which were not subjected to prior chemical pretreatment. At the 100 $\text{g}_{\text{o.d.solids}}/\text{L}$

Table 1

Cellulose contents (expressed in mass percentage of the oven-dried material) of the post-consumer textile waste, initial fibers (obtained by mechanical defibrillation) and after alkaline autoclaving or freezing NaOH/urea pretreatments, together with the corresponding yields (o.d., oven-dried basis).

Sample	Solid Yield (%)	Cellulose	
		Content ($\text{g}/100 \text{ g}_{\text{o.d.solids}}$)	Yield (%)
Mechanically defibrillated fibers	100	91.3 ± 1.5	...
After pretreatment			
Alkaline autoclaving	86.7	95.5 ± 0.4	91
Freezing NaOH/urea	91.1	95.5 ± 0.2	95
After freezing NaOH/urea – Scale-up for media preparation	87.3	93.4 ± 1.4	89

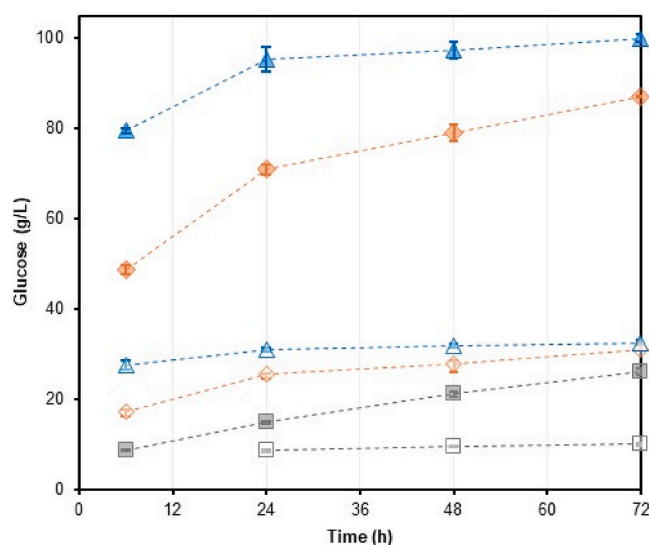


Fig. 2. Kinetic profiles of glucose release upon enzymatic hydrolysis of the cotton textile waste samples with Cellic® CTec3 (10%, w/w), at 50°C during 72 h: control, initial mechanically defibrillated fibers (grey squares), and the samples pretreated by alkaline autoclaving (orange diamonds) or by freezing NaOH/urea (blue triangles), at initial solids concentrations of 30 $\text{g}_{\text{o.d.solids}}/\text{L}$ (open symbols) or 100 $\text{g}_{\text{o.d.solids}}/\text{L}$ (closed symbols). The assays were carried out in triplicate (presented as average, with standard deviations as error bars).

Table 2

Extent of enzymatic hydrolysis of cellulose into glucose, achieved after a 72 h-incubation at 50°C with Cellic® CTec3 (10%, w/w), of the cotton textile waste without pretreatment (mechanically defibrillated fiber, as control) and the samples pretreated by alkaline autoclaving or by freezing in NaOH/urea, at initial solids concentrations of 30 or 100 $\text{g}_{\text{o.d.solids}}/\text{L}$ (Y_H , hydrolysis yield). All assays (except the one with freezing in NaOH/urea for scaling up) were carried out in triplicate (results presented as average, with standard deviations).

Sample	Solids loading ($\text{g}_{\text{o.d.solids}}/\text{L}$)	Glucose (g/L)	Y_H (%)	Overall Yield ($\text{g}_{\text{Glc}}/\text{g}_{\text{o.d.waste}}$)
Mechanically defibrillated fibers	30	10.0 ± 0.4	31.1 ± 0.5	0.33
	100	26.1 ± 0.7	24.6 ± 0.6	0.26
Alkaline autoclaving	30	30.9 ± 1.7	95.7 ± 5.3	0.94
	100	87.1 ± 0.1	81.0 ± 0.1	0.79
Freezing in NaOH/urea	30	32.5 ± 0.6	100.7 ± 1.6	0.94
	100	100.0 ± 0.9	93.2 ± 0.9	0.87
Freezing in NaOH/urea - Scale-up for media preparation	100	102.3	97.5	0.89

$\text{g}_{\text{o.d.solids}}/\text{L}$ substrate loading, there was a progressive release of glucose over the 72-h period, reaching a final concentration of $26.1 \pm 0.7 \text{ g/L}$, while for the 30 $\text{g}_{\text{o.d.solids}}/\text{L}$ substrate loading no significant release was observed after 6 h of reaction, with the glucose concentration remaining at $10.0 \pm 0.4 \text{ g/L}$ (Fig. 2). These results correspond to hydrolysis yields of $31.1 \pm 0.5\%$ and $24.6 \pm 0.6\%$, for the 30 or 100 $\text{g}_{\text{o.d.solids}}/\text{L}$ substrate loadings, respectively, with overall yields of 0.33 and 0.26 $\text{g}_{\text{Glc}}/\text{g}_{\text{o.d.waste}}$ (Table 2).

Significantly higher hydrolysis yield values (above 90%) were observed for the samples treated by alkaline autoclaving or freezing in NaOH/urea, at both solids' loadings tested (Table 2). For the 30 $\text{g}_{\text{o.d.solids}}/\text{L}$

solids/L substrate loading, glucose liberation was more pronounced within the first 6 h of hydrolysis (Fig. 2), yielding concentrations of 17.3 ± 0.3 g/L and 27.5 ± 1.0 g/L, respectively, reaching final concentrations of 30.9 ± 1.7 g/L and 32.5 ± 0.6 g/L after 72 h, corresponding to overall yields of 0.94 g_{Glc}/g_{o.d.waste} (Table 2). Similar trends were observed for the 100 g_{o.d.solids}/L substrate loading assays, wherein 48.7 ± 0.5 g/L and 79.5 ± 1.0 g/L of glucose were liberated after the same 6 h-period for the fibers treated by alkaline autoclaving or freezing in NaOH/urea, respectively (Fig. 2). Glucose titers further increased to 70.9 ± 1.1 g/L and 95.3 ± 2.8 g/L at 24 h, respectively, reaching maximum values of 87.1 ± 0.1 g/L and 100.0 ± 0.9 g/L at the end of the 72-h incubation, corresponding to overall yields of 0.79 and 0.87 g_{Glc}/g_{o.d.waste} (Table 2). Nevertheless, freezing in NaOH/urea appears to be the most effective chemical pretreatment, allowing for higher hydrolysis yields for both the 30 g_{o.d.solids}/L ($100.7 \pm 1.6\%$) and the 100 g_{o.d.solids}/L ($93.2 \pm 0.9\%$) substrate loadings, compared to the alkaline autoclaving pretreatment ($95.7 \pm 5.3\%$ and $81.0 \pm 0.1\%$, respectively). The yield of 100.7 above 100% achieved for 30 g solids_{o.d.}/L comes from the analytical uncertainty on the determination both of glucose titer in hydrolysate and cellulose-content. Indeed, the standard deviation associated to the value of 100.7 (determined as an average of the yields achieved for the triplicate assays) is 1.6, which is higher than its increment to 100%.

Lower hydrolysis yields were achieved at the highest substrate loading tested, probably due to mass-transfer limitations, associated to higher slurry viscosity, affecting enzyme accessibility, as previously tackled by Liu et al. [22], it resulted in a significantly higher glucose concentration in the final hydrolysate, which is crucial for sustainable enzyme-based biorefineries. Similar results were observed by Li et al. [23] on the enzymatic hydrolysis of a textile waste mixture (with an average cellulose content of 84.8%) by applying Celluclast 1.5 L at a dosage of 20 FPU/g substrate (higher than the dosage of 15.1 FPU/g hereby applied). Still, a much lower hydrolysis effectiveness was achieved by these authors (probably due to the presence of colored fabrics). The authors reported a decrease in hydrolysis yield from 52.3% to 36.7%, corresponding to glucose concentrations of 14.8 and 31.1 g/L, respectively, upon increasing the substrate loading from 3 to 9% (w/v). Similarly to the results achieved in this study, Li et al. [9] reported that a 60/40 cotton-polyester textile waste blend, pretreated using the same freezing methodology and processed at a substrate loading of 3% (w/v), provided a glucose recovery of 98.3% when hydrolyzed with Celluclast 1.5 L at 20 FPU/g substrate, supplemented with β -glucosidase (10 IU/g). Boondaeng et al. [13], also using textile waste blending of cotton and polyester 60/40, pretreated by the same autoclaving methodology hereby applied, attained a maximum hydrolysis yield of 89.7% when applying 30 FPU/g of Cellic® CTec2 to 7% of substrate loading. Jeiha-nipour and Taherzadeh [7] reported yields above 99% after 4-days enzymatic hydrolysis of cotton-based materials subjected to alkali pretreatment by 12% NaOH (3 h at 0 °C), with a substrate loading of 3% (w/v), using commercial cellulase (20 FPU/g) and β -glucosidase (30 IU/g) from SIGMA.

3.2.2. Preparation of textile waste hydrolysate for use as feedstock

Given the higher glucose concentration obtained from the fibers treated by the freezing NaOH/urea method in the preliminary enzymatic assays at a 100 g_{o.d.solids}/L loading, this procedure was selected for preparing the feedstock for the bioreactor experiments, balancing higher glucose availability with economic benefits and upscaling feasibility. The treatment was applied to 400 g of the cellulose-rich mechanically defibrillated fibers, and the freezing time was increased to 20 h (instead of 6 h), reaching a cellulose recovery yield of 0.89 g_{Glc}/g_{o.d.waste} and a hydrolysate with 102.3 g/L of glucose (Table 2). This slight increase in the concentration of glucose in the hydrolysate (102.3 relatively to 100.0 g/L), corresponding to a higher yield of enzymatic hydrolysis (97.5% relatively to 93.2%), observed when scaling up the pretreatment from 5 g to 400 g, may arise from the difference in freezing time that

could have affected the structural properties of the pretreated textile waste, namely in terms of cellulose swelling, which consequently might have increased its susceptibility to enzymatic hydrolysis. Despite the micromorphology of these fibers was not analyzed, this scaled-up pretreatment promoted a higher cellulose loss of 11% (instead of 5%). Given the differences observed for these bench-scale experiments at different scales, future process optimization of pretreatment and enzymatic hydrolysis, to produce glucose solutions from textile waste, could be accomplished by applying machine learning tools targeting industrial scale-up, as highlighted by Al Azad et al. [24]. No inhibitory compounds, namely furfurals, 5-hydroxymethylfurfural (5-HMF) or short-chain organic acids were detected in the hydrolysate, supporting the suitability of the hydrolysate for bacterial cultivation without the need for detoxification steps [25]. Soluble protein, which derives mainly from the enzyme cocktail applied for the fibers' hydrolysis, was detected at a concentration of 3.3 ± 0.2 g/L.

3.3. Production of P(3HB) from the textile waste hydrolysate

The glucose-rich hydrolysate obtained from the post-consumer cotton-based towels was used as the carbon source for P(3HB) production by the bacterium *B. thailandensis* E264 that was cultured under batch conditions. The culture followed a two-stage pattern, with an initial cell growth phase that lasted around 42 h, followed by enhanced intracellular polymer storage under nitrogen limiting conditions (Fig. 3). The active biomass fraction increased from 2.66 ± 0.18 g/L at 0 h to a maximum of 6.91 ± 0.85 g/L at 42 h of cultivation, remaining nearly constant thereafter (6.72 ± 1.03 g/L, at 53 h; Table 3; Fig. 3), concomitant with depletion of the nitrogen source. Glucose was continuously consumed, and it was exhausted after 53 h (Fig. 3). The temporal decoupling of carbon and nitrogen depletion established a carbon-availability/nitrogen-limited regime, which coincided with maximal P(3HB) synthesis. The protein concentration remained essentially constant (0.70 ± 0.13 g/L) throughout the assay, indicating that it was not used as a nitrogen source.

The CDW reached a value of 15.89 ± 0.21 g/L at 53 h of cultivation, with a P(3HB) content in the cells of 58 ± 6 wt%, yielding a final polymer titer of 9.17 ± 1.01 g/L (Table 3; Fig. 3). The stabilization of X while the CDW continued to increase confirms that late-stage gains were mainly driven by the intracellular polymer accumulation rather than net biomass formation. Polymer content exceeded 50 wt% of the CDW after 42 h of cultivation (Fig. 3), when P(3HB) reached 7.43 ± 0.85 g/L with a cell content of 52 ± 6 wt%, corresponding to $\sim 81\%$ of the final polymer titer. This indicates that most polymer synthesis occurred within a defined storage-dominated window, supporting the operational relevance of harvesting at peak productivity rather than at full carbon exhaustion.

The maximum specific growth rate ($\mu_{\max}$) was 0.042 h⁻¹ (estimated from the slope of ln X versus time over the 18–29 h interval). Maximum polymer synthesis occurred at 42 h, with a specific production rate (q_p) of 0.058 g/(g h) (Fig. 4) and a batch-averaged volumetric productivity (r_p) of 0.165 g/(L h) (Table 3). After peaking at 43 h (0.352 g/(L h)), the r_p decreased at 48 h (Fig. 4), reflecting the late-stage carbon limitation (glucose concentration < 5 g/L). Global growth and product yields were $Y_{X/S} = 0.099$ g_x/g_s and $Y_{P/S} = 0.218$ g_p/g_s (Table 3). From an operational standpoint, the co-occurrence of maximum q_p and r_p at 42 h supports a harvest-at-peak productivity approach: 7.43 ± 0.85 g/L P(3HB) and polymer contents in the cells of 52 ± 6 wt% can be recovered at ~ 42 h ($\sim 81\%$ of the final polymer titer), reducing batch residence time and improving reactor turnover (space-time yield).

Matias et al. [16] reported the cultivation of the same strain on eucalyptus bark hydrolysate using lower initial substrate concentrations (20 g/L glucose and 0.3 g/L ammonia, compared to 41.16 ± 2.58 g/L and 0.59 g/L in the present study). Although the C/N ratio was maintained, the lower carbon loading inherently limited achievable biomass (CDW = 7.67 ± 0.16 g/L), as well as the polymer titers (P(3HB) = 4.60

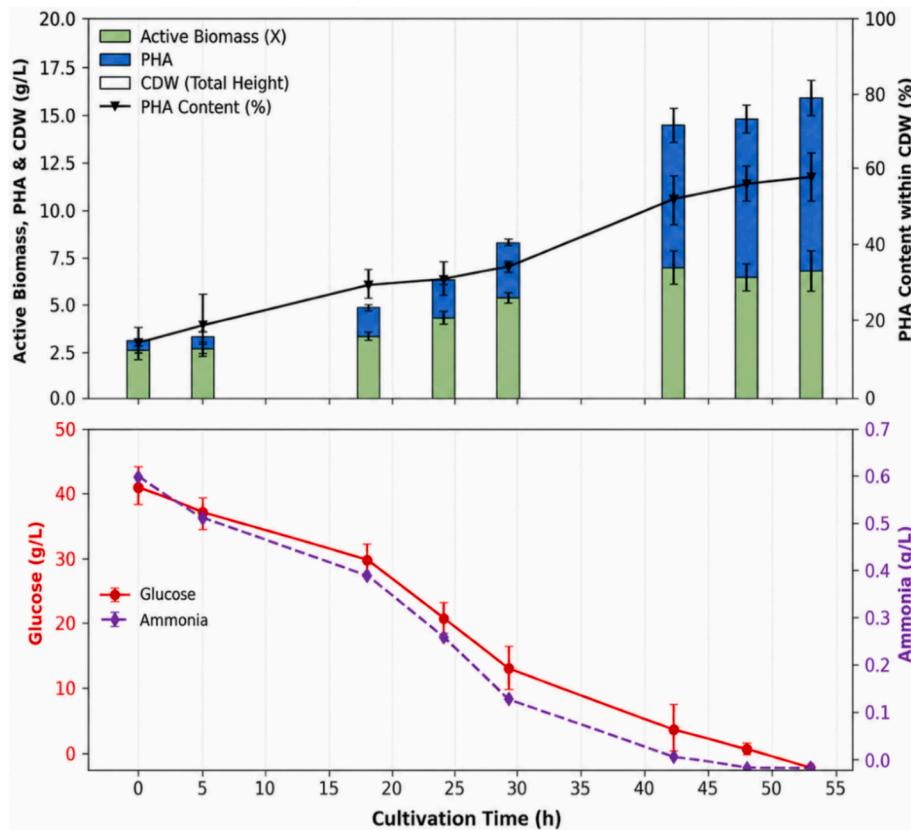


Fig. 3. Kinetic profiles of *B. thailandensis* E264 batch cultivation for P(3HB) production. (Top image) Evolution of the cell dry weight (CDW), P(3HB) concentration (blue), active biomass (X, non-PHA cell mass, green) and P(3HB) content. (Bottom image) Nutrients concentration, glucose (red circles) and ammonia (purple diamonds). Error bars represent standard deviation from triplicate measurements ($n = 3$).

Table 3

Benchmarking of P(3HB) production performance using post-consumer cotton towels hydrolysate as feedstock against reported values for *Burkholderia thailandensis* E264 and other PHA-producing strains.

Bacterium	Scale / mode	Carbon source	Cultivation time (h)	$\mu_{\max}$ (h^{-1})	CDW (g/L)	P(3HB) (%CDW)	P(3HB) (g/L)	r_p (g/(L h))	$Y_{P/S}$ (gP/gS)	References
<i>Burkholderia thailandensis</i> E264	Bioreactor / batch	Post-consumer towels hydrolysate	53	0.042	15.89 ± 0.21	58 ± 6	9.17 ± 1.01	0.165	0.218	This study
		Eucalyptus bark hydrolysate	72	0.11	7.67 ± 0.16	60.0 ± 2.19	4.60 ± 0.17	1.36	0.19	16
		Used cooking oil	120	0.031	12.6	60	7.5	0.063†	0.35	26
	Shake flask / batch	Glucose and xylose	192	n.a	5.99	64	3.84	0.0200	0.19	28
		Glycerol	144	n.a	8.4 ± 0.16	50	4.22 ± 0.24	0.03 ± 0.002	n.a	29
<i>Paraburkholderia sacchari</i> DSM 17165	Shake flask / batch	Corn stover hydrolysate	n.a	n.a	14.6	52	7.6	n.a.	0.33	27
		Glucose and xylose	n.a	n.a.	9.2	56	5.2	n.a.	0.23	27
	Bioreactor / Fed-batch	Glucose, xylose and arabinose	41	n.a	87.2	77	67.5	1.65	0.33	30
<i>Burkholderia sacchari</i> DSM 17165	Bioreactor / Fed-batch	Sucrose	27.5	0.41	70.0	53	36.8	1.29	n.a	31

± 0.17 g/L) and content in the cells ($60.0 \pm 2.19\%$) [16]. Under the higher carbon availability of the present study, biomass formation (15.89 ± 0.21 g/L) and the polymer titer (9.17 ± 1.01 g/L) were nearly doubled, while maintaining comparable intracellular polymer accumulation ($\sim 58 \pm 6$ wt%). Thus, differences in volumetric productivity appear to be primarily driven by substrate loading and overall biomass formation rather than intrinsic differences in strain performance. In batch bioreactor cultivation on used cooking oil, *B. thailandensis* E264 reached ~ 60 wt% polymer and ~ 7.5 g/L P(3HB) only after ~ 120 h,

with $\mu_{\max} \approx 0.031 \text{ h}^{-1}$ [26]. Batch cultivations of *Paraburkholderia sacchari* on defined glucose–xylose mixtures (simulating lignocellulose hydrolysates) reached P(3HB) titers on the order of ~ 5 g/L at ~ 56 wt% polymer content in the cells [27]. Although direct comparisons are inherently limited by differences in strain physiology, reactor configuration, and operating conditions (e.g., pH/DO control and nitrogen availability), the present cotton hydrolysate-based batch achieved a higher polymer titer (9.17 ± 1.01 g/L) while maintaining a comparable polymer content (58 wt%). Nonetheless, the productivity obtained in this study (0.165 g/(L h)) remains quite below values reported for high-

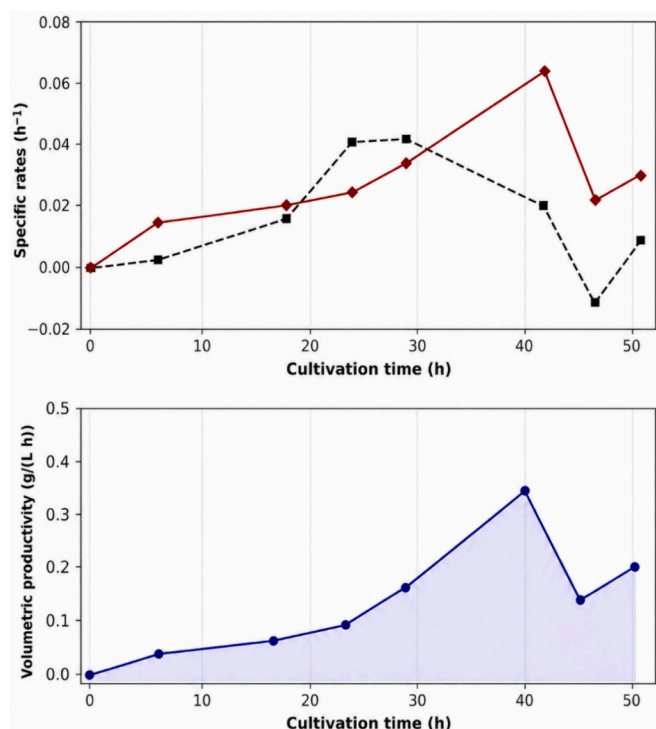


Fig. 4. Kinetic parameters of P(3HB) production during the batch cultivation assay: specific growth rate (μ , dashed black line) and specific polymer production rate (q_p , solid red line) (top image), and volumetric productivity (r_p) (bottom image). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cell-density fed-batch systems, such as the 1.65 g/(L h) reported for *P. sacchari* (Table 3). Thus, the present results demonstrate the suitability of lignocellulosic wastes like cotton towels as an efficient energy waste-to-bioenergy residue for P(3HB) production³². Further process intensification, including fed-batch operation, optimization of nutrient feeding, and life-cycle assessment studies would be required to approach industrial productivities.

3.4. PHA extraction and characterization

3.4.1. P(3HB) purity

The solvent extraction and purification protocol used to recover P(3HB) from the bacterial cells enabled efficient recovery of the polymer,

yielding an extraction efficiency of 98%. The recovered material presented the characteristic white, brittle appearance typically associated with purified P(3HB) (Fig. 5). Elemental analysis confirmed the high purity of the recovered polymer (C = 56.24 wt%, H = 7.47 wt%, N = 0.01 wt%, S = 0.00 wt%), indicating minimal protein carryover after extraction. These values are consistent with literature-reported CHN contents for purified P(3HB) (C $\approx$ 55.0 wt% and H $\approx$ 7.4 wt%, with no nitrogen detected) [28] and are also in close agreement with the theoretical elemental composition of P(3HB) (C = 55.81%, H = 7.04%).

Although the chloroform-based procedure enabled high polymer recovery efficiency and purity, its environmental performance represents a limitation of the present downstream process. In this context, Matias et al. [16] demonstrated that PHA can be recovered using an enzymatic extraction strategy with high efficiency (96%), providing a promising alternative to solvent-based extraction and reducing the environmental burden associated with chlorinated solvents.

3.4.2. Chemical structure

The functional groups of the recovered polymer were examined by Fourier transform infrared (FTIR) spectroscopy in the range of 4000–600 cm⁻¹ (Fig. 6). The spectrum showed the characteristic absorption bands of P(3HB). A strong and sharp band at 1722 cm⁻¹, assigned to ester carbonyl stretching (C=O), was observed and is widely reported as a diagnostic marker of P(3HB). The band at 2932 cm⁻¹ corresponds to aliphatic C–H stretching, while peaks at 1453 cm⁻¹ and 1380 cm⁻¹ are attributed to CH₂ bending and CH₃ deformation, respectively. In addition, the fingerprint region exhibited bands at 1279, 1183 and 1057 cm⁻¹, corresponding to ester C–O–C and C–O stretching vibrations, confirm the polyester structure of P(3HB). Overall, the presence of the characteristic alkyl (–CH₂/–CH₃), carbonyl (C=O) and ester (–COOR) bands confirms the recovered polymer as P(3HB), with a spectral profile consistent with previously reported FTIR spectra [29,30].

3.4.3. Molecular mass distribution

The produced P(3HB) was characterized by a weight-average molecular weight (M_w) of 497 kDa and a polydispersity index (PDI) of 2.6 (Table 4). These values fall within the range reported in the literature for bacterial P(3HB) produced from renewable feedstocks. For example, Etxabide et al. [31] reported a M_w of 380 kDa and a PDI of 2.9 for the P(3HB) produced by *Cupriavidus necator* DSM 454 grown on saccharified grape pomace. Kourmentza et al. [26] reported the production of P(3HB) with a M_w of 511 kDa and a PDI of 2.86 by *B. thailandensis* E264 grown on UCO, while Matias et al. [16] described a lower M_w (386 kDa) but a narrower M_w distribution (PDI = 1.78) for P(3HB) produced by

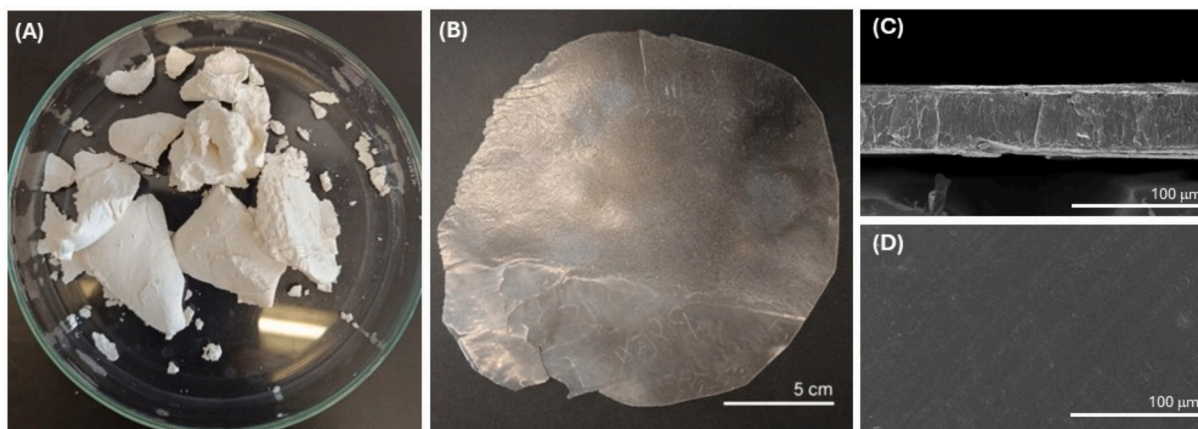


Fig. 5. P(3HB) produced by *Burkholderia thailandensis* E264 cells cultivated on the post-consumer cotton-based towels enzymatic hydrolysate: image of the polymer obtained by solvent extraction with chloroform and purification with ethanol (A); P(3HB) film prepared by hot pressing at 170 °C for 2 min under 1 ton (B); Scanning Electron Microscopy (SEM) images of the film's cross-section (C) and surface (D).

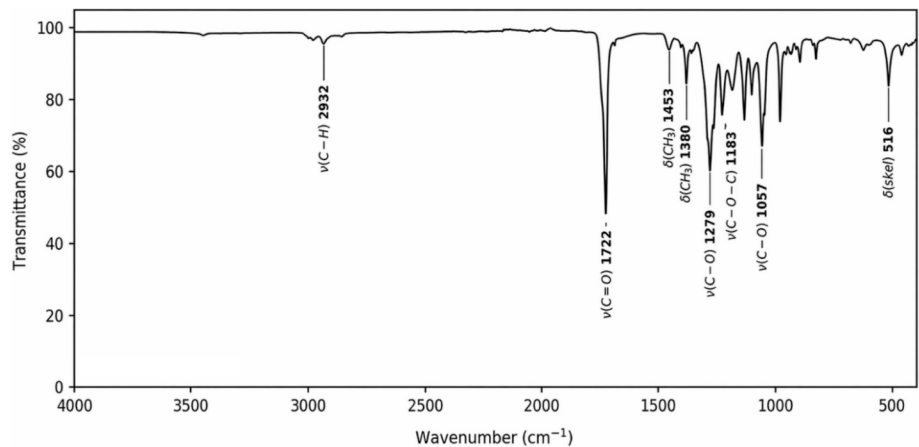


Fig. 6. FTIR spectrum for the P(3HB) produced by *Burkholderia thailandensis* E264 from the post-consumer cotton-based hydrolysate and recovered from the cells by solvent extraction with chloroform and purification with ethanol.

Table 4
Molecular weight distribution and thermal properties of P(3HB) produced by *B. thailandensis* and other microorganisms (M_w , weight-average molecular weight; M_n , number-average molecular weight; PDI, polydispersity index; T_m , melting temperature; T_{deg} , maximum thermal degradation temperature; χ_c , degree of crystallinity; n. a., data not available).

Bacteria	Carbon source	M_w (kDa)	M_n (kDa)	PDI	T_m (°C)	T_{deg} (°C)	χ_c (%)	Reference
<i>Burkholderia thailandensis</i> E264	Cotton-based towels hydrolysate	497	191	2.6	170	291	78.8	This study
	Glucose and xylose	983	417.8	2.61	171	278.3	n.a.	28
	Eucalyptus bark hydrolysate	386	n.a.	1.78	175	294	90	16
	Used cooking oil	511	179	2.86	166	279	54.7	26
<i>Cupriavidus necator</i> DSM 454	Red grape pomace	380	n.a.	2.9	175	297	20.7	36
<i>Bacillus subtilis</i> MTCC 1789	<i>Aegle marmelos</i> shell waste	n.a	n.a.	n.a.	168	251	76.2	34

the same strain from eucalyptus bark hydrolysate, indicating variability in polymer chain length and dispersity depending on cultivation conditions. The polymer obtained in this study also exhibits molecular characteristics comparable to industrial benchmarks. Commercial P (3HB) from Biomer, P(3HB) T19, has a M_w of 254 kDa and PDI of 2.4 [32], while GoodFellow P(3HB) (BU396311) presents a M_w of 550 kDa and a PDI of 2.2 [33].

3.4.4. Crystallinity
The diffractogram of the extracted polymer (Fig. 7) exhibited the characteristic crystalline reflections of P(3HB), with intense peaks at $2\theta \approx 13\text{--}14^\circ$ and $16\text{--}17^\circ$ corresponding to the (020) and (110) planes, together with additional reflections in the $20\text{--}30^\circ$ region assigned to the (101), (111), (121) and (040) planes. These features are consistent with the typical orthorhombic α -form P(3HB) crystal structure commonly

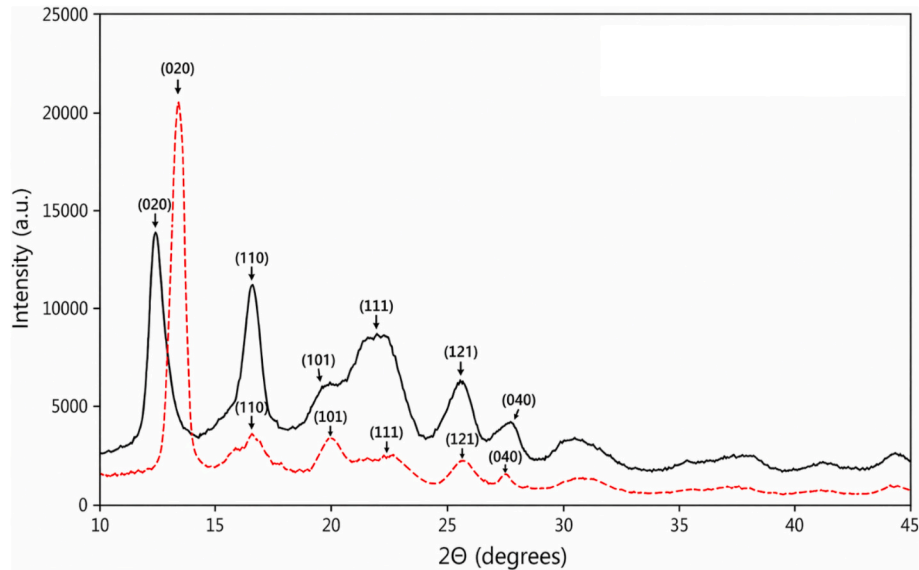


Fig. 7. X-ray diffraction (XRD) patterns of the P(3HB) produced by *Burkholderia thailandensis* E264 from cotton towel hydrolysate: chloroform-recovered P(3HB) (black full line) and hot-pressed P(3HB) film (red dashed line).

reported for microbial P(3HB) [29,31].

The crystallinity index (χ_c) of the extracted P(3HB) was 78.8% (Table 4), confirming the highly semicrystalline nature of the polymer. This value lies within the upper range (55–80%) typically reported for P(3HB) homopolymers [34] and lies between those reported by Kourmentza et al. [26] (54.7%) for the P(3HB) produced by *B. thailandensis* from UCO, and by Matias et al. [16] (90%) for the polymer produced from eucalyptus bark hydrolysate, which is close to that reported for commercial Biomer P309 (91.52%) [35]. Notably, the crystallinity achieved here exceeds the values reported for commercial grades such as Biomer P(3HB) T19 (~58.2%) and GoodFellow P(3HB) BU396311 (~44.1%) [32,36].

3.4.5. Thermal properties

The P(3HB) showed a melting temperature (T_m) of 170 °C (Table 4) (Fig. S3), which is consistent with typical values (173–180 °C) reported for P(3HB) homopolymers obtained from microbial sources [37]. Similar T_m values were reported for P(3HB) produced by *B. thailandensis* E264, namely 166 °C when grown on UCO [26] and 171 °C when cultivated on glucose and xylose [38]. Matias et al. [16] reported a slightly higher T_m (175 °C) for P(3HB) produced by *B. thailandensis* E264 grown on eucalyptus bark hydrolysate, concomitant with a higher crystallinity (90%). The slightly elevated T_m observed by Matias et al. [16] is consistent with increased crystalline ordering, as higher crystallinity generally correlates with marginal increases in melting temperature.

Thermal degradation occurred in a single main step, with a maximum degradation temperature (T_{deg} , dTG peak) of 291 °C (Fig. S3), indicating good thermal stability. This results in a processing window ($T_{deg} - T_m$) of approximately 120 °C, which is comparable to or slightly broader than values reported for the same strain grown on alternative substrates. For example, degradation maxima of 279.3 °C and 278.3 °C were reported for UCO- and glucose-xylose-derived P(3HB), respectively [26,38]. Matias et al. [16] reported $T_{deg} = 294$ °C, which is in close agreement with the present findings. A relatively high degradation temperature is advantageous for melt processing, as it increases the gap between melting and decomposition, facilitating thermoplastic operations such as extrusion and injection molding [39]. Importantly, despite the high crystallinity observed here ($\chi_c = 78.8\%$), the thermal degradation profile remained comparable to commercial P(3HB) grades, confirming that increased structural ordering did not compromise thermal stability.

3.5. P(3HB) films

3.5.1. Morphology

The recovered P(3HB) (Fig. 5A) was processed by compression molding at 170 °C for 2 min under a load of 1 ton, using a 200 × 200 mm mold to obtain a stand-alone film (Fig. 5B). The selected temperature was at the onset of melting (≈ 170 °C), enabling polymer fusion while remaining well below the degradation temperature (≈ 291 °C), thereby preserving thermal stability and material integrity during processing. The resulting film exhibited a continuous, self-supporting structure with uniform thickness and no visible macroscopic defects such as bubbles, cracks, or phase separation. The film displayed a translucent-to-matte appearance, typical of semicrystalline PHB materials, resulting from light scattering by crystalline domains. The smooth surface and coherent morphology indicate adequate melt flow and interchain diffusion during compression molding, confirming that the recovered polymer retains suitable thermoplastic processability despite its high crystallinity.

After hot pressing, the crystallinity index increased to 89% (Fig. 7), suggesting that thermal processing promoted additional crystalline ordering and lamellar reorganization during melting and subsequent cooling. Such behavior is consistent with literature reports indicating that compression molding or hot pressing can enhance crystalline perfection in PHB by allowing chain relaxation, recrystallization, and

thickening of lamellae, often reflected in sharper XRD reflections and increased crystallinity following film formation [40]. Overall, the morphological transition from brittle crystalline flakes to a cohesive molded film, together with the increase in crystallinity after processing, demonstrates that the extracted P(3HB) maintains appropriate melt behavior while allowing structural reorganization under controlled thermal conditions, which is relevant for thermoplastic shaping applications.

SEM analysis was used to evaluate the morphology of the hot-pressed P(3HB) film. The cross-sectional image (Fig. 5C) revealed a dense and continuous structure, with no evidence of large voids or macroporosity, indicating effective polymer coalescence during thermal compression. The fracture surface displayed a rough lamellar-like texture, which is consistent with the typical fracture morphology of semicrystalline P(3HB) materials. The surface micrograph (Fig. 5D) shows a relatively smooth, homogeneous topography, with no visible pores or phase-separated domains at the analyzed magnifications. This compact morphology is consistent with literature descriptions of P(3HB) films, which are commonly reported as smooth and uniform in the absence of plasticizers or secondary phases [41].

3.5.2. Mechanical properties

The film exhibited a Young's modulus of 563.3 ± 62.3 MPa, tensile strength of 26.7 ± 2.1 MPa, and elongation at break of $14.6 \pm 2.6\%$, indicating a mechanically robust P(3HB) film with enhanced deformation relative to many neat P(3HB) materials reported in the literature. The stress-strain curve (Fig. 8) shows an initial elastic region followed by a progressive increase in stress without a clearly defined yield plateau. After reaching the maximum stress, a short post-peak softening region was observed, followed by an abrupt decrease in stress associated with film fracture. This behavior indicates that, although the material exhibits some deformation capacity, the final failure remains relatively abrupt. The relatively high elongation at break ($14.6 \pm 2.6\%$) should be interpreted considering both the high XRD-estimated crystallinity ($\sim 89\%$) and the thermal history of the film. Although high crystallinity generally limits chain mobility, the mechanical response of P(3HB) may also be affected by molecular weight, crystalline morphology, residual stresses, and processing conditions. In the present study, hot pressing at 170 °C followed by controlled cooling may have influenced the final polymer morphology and mechanical response.

Indeed, Anbukarasu et al. [42] demonstrated that the mechanical response of P(3HB) films is strongly influenced by processing conditions and that crystallinity alone does not fully describe their deformation behavior, since deviations from the general inverse relationship between crystallinity and strain at failure were observed. Significant residual

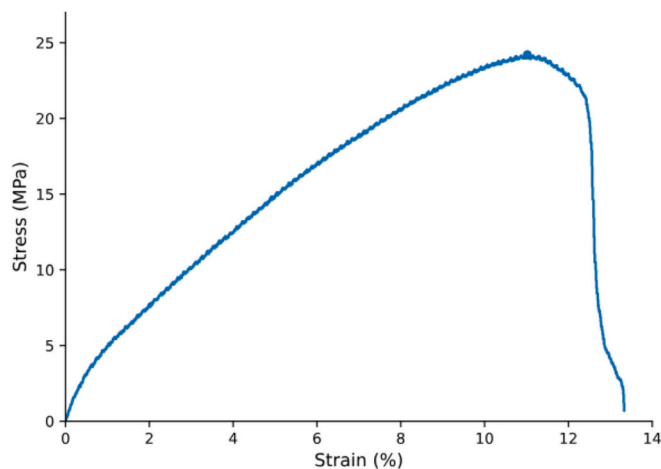


Fig. 8. Stress-strain curve of the hot-pressed P(3HB) film obtained from uni-axial tensile testing.

chloroform is considered unlikely, as TGA (Fig. S3), showed no additional low-temperature mass loss, and the film was subsequently processed at 170 °C.

Although the elongation at break is relatively high for neat P(3HB), values of a similar order have been reported for P(3HB) subjected to other processing routes. Longé et al. [43] reported an elongation at break of $11 \pm 1.6\%$ for neat processed P(3HB), while Lee et al. [44] reported $12.41 \pm 1.00\%$ for neat P(3HB) after melt processing. Although differences in polymer grade, processing history and testing conditions preclude a direct comparison, these results indicate that elongation values in this range are not unprecedented for processed P(3HB).

P(3HB) films can exhibit tensile strengths in the approximate range of 20–40 MPa and relatively high Young's moduli up to several GPa, depending on processing history and crystalline state [35]. Kurusu et al. [45] reported 600 ± 40 MPa (modulus), 24 ± 0.3 MPa (tensile strength), and $6.8 \pm 0.1\%$ (elongation at break) for compression-molded films of sugarcane-based bacterial P(3HB) (Biocycle® B100, PHB Industrial, Brazil) closely aligning with the mechanical range obtained in this study. Das et al. [46] observed higher stiffness (1860 MPa) but lower elongation (2.1%), while Boyandin et al. [47] reported even higher moduli (3147 MPa) with limited strain-at-break ($\sim 1.5\%$), reflecting the intrinsic brittleness often associated with highly crystalline PHB films. Comparing with films of P(3HB) produced by *B. thailandensis* E264, Hernández-Alonso et al. [48] reported 1194 ± 42 MPa (modulus), 10 ± 3.1 MPa (tensile strength), and $0.9 \pm 0.1\%$ (elongation). Despite the lower Young's modulus, the film produced from the cotton-based towels' hydrolysate exhibited markedly higher tensile strength and substantially improved elongation at break. Relative to neat P(3HB) films reported by Laoutid et al. [39], which showed 1840 ± 115 MPa, 42 ± 2.4 MPa, and $4.7 \pm 0.3\%$, the film produced in this study displayed lower stiffness but a higher deformation capacity, suggesting reduced brittleness under the applied testing conditions.

From a broader materials perspective, the mechanical performance of the present P(3HB) film can be benchmarked against polymer films commonly used in packaging and technical applications. For instance, Żotek-Tryznowska et al. [49] reported that polyethylene (PE) films exhibited a Young's modulus of 122 ± 17 MPa, a tensile strength of 19 ± 1 MPa, and an elongation at break of $407 \pm 49\%$. In contrast, biaxially oriented polypropylene (BOPP) films showed significantly higher stiffness (1686 ± 129 MPa), tensile strength (90 ± 9 MPa), and elongation ($141 \pm 20\%$) [49]. Compared with these materials, the P(3HB) film produced in this work exhibits substantially higher stiffness and strength than PE films, while remaining considerably less stiff and less ductile than BOPP films. Overall, *B. thailandensis* P(3HB) film bridges the mechanical gap between flexible polyolefin films (e.g., PE) and rigid engineering thermoplastics (e.g., BOPP). Its combination of intermediate stiffness (~ 0.56 GPa), competitive tensile strength (~ 27 MPa), and higher deformation capacity relative to many reported P(3HB) homopolymers suggests that it may serve as a bio-based alternative for applications requiring moderate mechanical resistance without extreme rigidity.

4. Conclusions

This study explores a complete valorization route for cotton-rich post-consumer textiles, integrating mechanical defibrillation, alkaline pretreatment, enzymatic hydrolysis and microbial biopolymer synthesis. By upcycling cotton-rich waste textiles into P(3HB), this approach envisages both environmental and economic benefits. Based on the overall process performance, the proposed technological route yielded approximately 0.146 g of recovered P(3HB) per g of cotton towel initially processed. In addition, the produced P(3HB) exhibited molecular, thermal and structural properties comparable to those reported for other bacterial and commercial P(3HB) grades, while the hot-pressed films showed mechanical performance within the range described for P(3HB) materials, with notably improved ductility compared with many

neat P(3HB) films. Overall, these findings demonstrate the feasibility of upcycling household cotton-based textiles into a biodegradable thermoplastic with properties comparable to those of established P(3HB) materials, reinforcing the potential of textile waste as a sustainable feedstock for the production of high-value biobased biodegradable polymers.

CRedit authorship contribution statement

Thomas Rodrigues: Writing – original draft, Methodology, Investigation, Formal analysis. **João Matias:** Writing – original draft, Methodology, Investigation, Formal analysis. **Patrícia Moura:** Writing – review & editing, Conceptualization. **Mariana Abreu:** Methodology. **Francisco Gírio:** Resources, Funding acquisition. **Adelaide Braga:** Writing – review & editing, Writing – original draft. **Juliana C. Dias:** Writing – review & editing, Writing – original draft, Conceptualization. **Ana Rovisco:** Writing – review & editing, Methodology, Investigation. **Elvira Fortunato:** Writing – review & editing, Resources, Funding acquisition. **Cristiana A.V. Torres:** Writing – review & editing, Resources, Funding acquisition. **Maria A.M. Reis:** Writing – review & editing, Resources, Funding acquisition. **Susana Marques:** Writing – review & editing, Writing – original draft, Resources, Funding acquisition, Conceptualization. **Filomena Freitas:** Writing – review & editing, Writing – original draft, Resources, Project administration, Funding acquisition, Conceptualization. **Carla J. Silva:** Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material includes Fig. S1 and S2, which provide additional information for the Materials and Methods section, and Fig. S3 that provides additional data. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.susmat.2026.e02262>.

Data availability

Data will be made available on request.

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