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Abstract: Biodegradable polymers are an active area of investigation, particularly ones that can be produced from sustainable, biobased monomers, such as copolymers of poly(butylene succinate) (PBS). In this study, we examine the enzymatic degradation of poly(butylene succinate-dilinoleic succinate) (PBS-DLS) copolymers obtained by “green” enzymatic synthesis using lipase B from Candida antarctica (CALB). The copolymers differed in their hard to soft segments ratio, from 70:30 to 50:50 wt.%. Enzymatic degradation was carried out on electrospun membranes (scaffolds) and compression-moulded films using lipase from Pseudomonas cepacia. Poly(e-caprolactone) (PCL) was used as a reference aliphatic polyester. The degradation process was monitored gravimetrically via water uptake and mass loss. After 24 days, approx. 40% mass loss was observed for fibrous materials prepared from PBS-DLS 70:30 copolymer, as compared to approx. 10% mass loss for PBS-DLS 50:50. Infrared spectroscopy (FTIR) and SEC analysis were used to examine changes in chemical structure. Differential scanning calorimetry (DSC) and scanning light microscopy (LSM) revealed changes in degree of crystallinity, and changes in surface morphology, consistent with a surface erosion mechanism. We conclude that the obtained copolymers are suitable for tissue engineering applications thanks to tuneable degradation and lack of acidification during breakdown.

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Enzymatic degradation of poly(butylene succinate) copolyesters synthesized with the use of Candida antarctica lipase B

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Supporting Information

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- SI.1 ¹H NMR analysis
- SI.2 ¹³C NMR analysis
SI.1 $^1$H NMR analysis

Table SI.1. Characteristic $^1$H NMR signals used for PBS-DLS 70:30 and 50:50 copolymers calculations

| Monomer   | Proton | ppm  | Number of H | Integral [I] |
|-----------|--------|------|-------------|--------------|
| PBS:DLS   | DS     | c    | 2.6         | 120          |
|           | BD     | b    | 1.7         | 110          |
| 70:30     | DLA-OH | f+h  | 1.25        | 148          |
| PBS:DLS   | DS     | c    | 2.6         | 269          |
|           | BD     | b    | 1.7         | 217          |
| 50:50     | DLA-OH | f+h  | 1.25        | 648          |

- From Table SI.1 experimental molar % of DS, BD and DLA-OH can be calculated from equations (SI 1-3):

  (SI 1) \[ [\text{Molar } \% \text{ DS}] = \frac{I_{2.6}}{I_{2.6} + I_{1.25} + I_{1.7}} ; \]

  (SI 2) \[ [\text{Molar } \% \text{ BD}] = \frac{I_{1.7}}{I_{1.7} + I_{1.25} + I_{1.7}} ; \]

  (SI 3) \[ [\text{Molar } \% \text{ DLA - OH}] = \frac{I_{1.25}}{I_{2.6} + I_{1.25} + I_{1.7}} \]

were Molar % DS, Molar% BD and Molar % DLA-OH are related to the experimental molar content of the succinate, butanediol and dilinoleic units on a PBS:DLS copolymer.

- In addition, $M_n$ can be approximately calculated with equation (SI 4):

  (SI 4) \[ \text{[DP}_{n+m} = \frac{I_{2.6}, I_{1.7}, I_{1.25}}{I_{3.68}, I_{1.30}, I_{1.28}} \]

were $I_{3.68}$ and $n_{3.68}$ are the integral and the number of protons of the CH$_2$ close to the OH end group, respectively.

- The DP of DS, BD and DLA-OH can be calculated taking into account the molar amount previously obtained with equation (SI 5):
\[(SI\ 5)\ [DP_n] = \frac{DP_{n+m}(\text{Molar}\ %\ \text{DS}+\text{Molar}\ %\ \text{BD})}{100};\ [DP_m] = DP_{n+m} - DP_n\]

*Mn* is obtained taking into account the DP, Molar % and the Molar mass of a hard segment composed by all the BD-DS units and a soft segment containing only DLA-OH as shown in equation (SI 6):

\[(SI\ 6)\ \left[ M_n \right] = (DP_n \cdot \text{Molar mass}_{BD-DS}) + (DP_{n+m} \cdot \text{Molar mass}_{DLA-OH})\]

were Molar mass\textsubscript{BD-DS} is 172 g/mol and Molar mass\textsubscript{DLA-OH} is 538 g/mol.

Finally, the experimental molar and weight % of hard and soft segments can be calculated as follows with equations (SI 7-10):

\[(SI\ 7)\ \left[ \text{Molar % HS} \right] = \frac{\frac{I_1}{I_{1.25}} \times 100}{\frac{I_{1.25}}{I_{1.7}}};\ (SI\ 8)\ \left[ \text{Molar % SS} \right] = 100 - \text{Molar % HS}\]

\[(SI\ 9)\ \left[ \text{Weight % HS} \right] = \frac{\text{Molar % HS} \cdot \text{Molar mass}_{HS}}{\text{Molar % HS} \cdot \text{Molar mass}_{HS} + (100 - \text{Molar % HS}) \cdot \text{Molar mass}_{SS}} \times 100\]

\[(SI\ 10)\ \left[ \text{Weight % SS} \right] = 100 - \text{Weight % HS}\]

**Table SI.2.** Theoretical Molar % and Weight % comparison with experimental values for PBS:DLS 70:30 and 50:50

|       | aMolar % BD:DS:DLA-OH | bMolar % BD:DS:DLA-OH | cMolar % HS:SS | dMolar % HS:SS | eWeight % HS:SS | fWeight % HS:SS | gMn (g/mol) |
|-------|----------------------|----------------------|--------------|--------------|----------------|----------------|--------------|
| PBS:D |                      |                      |              |              |                |                |              |
| LS 70:30 | 45:50:5 | 45:50:5 | 90:10 | 91:9 | 70:30 | 73:27 | 1159 |
|        |                      |                      |              |              |                |                |              |
| PBS:D |                      |                      |              |              |                |                |              |
| LS 50:50 | 50:39:11 | 50:41:9 | 79:21 | 82:18 | 50:50 | 55:45 | 2744 |

\(^a\)Initial and \(^b\)experimental (determined by \(^1\)H NMR) feed molar ratio of monomers. \(^c\)Initial and \(^d\)experimental (determined by \(^1\)H NMR) feed molar ratio between hard (HS) to soft segments (SS). \(^e\)Initial and \(^f\)experimental (determined by \(^1\)H NMR) feed weight ratio between hard (HS) to soft segments (SS). \(^g\)Determined by \(^1\)H NMR.
Si.2 $^{13}$C-NMR analysis

**Figure S1.** $^{13}$C NMR of poly(butylene succinate-co-dilinoleic succinate) 70:30 copolyester.

Enzyme-catalyzed poly(butylene succinate-co-dilinoleic succinate) (PBS:DLS) 70:30 and 50:50 copolymers ($^{13}$C-NMR, CDCl$_3$, $\delta$): 172.2 ppm (l; -CO-CH$_2$-CH$_2$-CO-, from DS), 64.3 ppm (a; -O-CH$_2$-CH$_2$-CH$_2$-CH$_2$O-, from BD), 29.2 ppm (c; -CO-CH$_2$-CH$_2$-CO-, from DS), 25.3 ppm (b; -O-CH$_2$-CH$_2$-CH$_2$-CH$_2$O-, from BD); 14.3 ppm (j; -CH$_2$-CH$_2$-CH$_3$ from DLA-OH), 22.7-31.9 ppm (d, e, f, g, h, i; -CH2- (aliphatic carbons), from DLA-OH).
Low intensity resonances ascribed to BD end-groups at 65 ppm (a₂; HO-CH₂-CH₂-CH₂O-) and 62.3 ppm (a₁; HO-CH₂-CH₂-CH₂-CH₂O-); low intensity resonances ascribed to ester end-groups from DS at 14.2 ppm (k; CH₃-CH₂-O-CO-), 29.16 (c₁; CH₃-CH₂-O-CO-CH₂-) and 172.3 (l₁; CH₃-CH₂-O-CO-). No end groups related to DLA-OH (CH₂-CH₂-OH) at 32.8 ppm and 63.0 ppm respectively were visible. In addition, a new resonance at 28.7 ppm (d; -O-CH₂-CH₂-) confirms the reaction of DLA-OH with DS corroborating the structure proposed on the ¹H NMR analysis. In addition, the split of the carbonyl carbon peak at 172.3 ppm is related to the different environments due to a DS unit linked either to BD or DLA-OH on both sides, or BD on one side and DLA-OH on the other side.
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Abstract: Biodegradable polymers are an active area of investigation, particularly ones that can be produced from sustainable, biobased monomers, such as copolymers of poly(butylene succinate) (PBS). In this study, we examine the enzymatic degradation of poly(butylene succinate-dilinoleic succinate) (PBS-DLS) copolymers obtained by “green” enzymatic synthesis using lipase B from Candida antarctica (CALB). The copolymers differed in their hard to soft segments ratio, from 70:30 to 50:50 wt.%. Enzymatic degradation was carried out on electrospun membranes (scaffolds) and compression-moulded films using lipase from Pseudomomas cepacia. Poly(e-caprolactone) (PCL) was used as a reference aliphatic polyester. The degradation process was monitored gravimetrically via water uptake and mass loss. After 24 days, approx. 40% mass loss was observed for fibrous materials prepared from PBS-DLS 70:30 copolymer, as compared to approx. 10% mass loss for PBS-DLS 50:50. Infrared spectroscopy (FTIR) and SEC analysis were used to examine changes in chemical structure. Differential scanning calorimetry (DSC) and scanning light microscopy (LSM) revealed changes in degree of crystallinity, and changes in surface morphology, consistent with a surface erosion mechanism. We conclude that the obtained copolymers are suitable for tissue engineering applications thanks to tuneable degradation and lack of acidification during breakdown.

Keywords: polyester copolymers; enzymatic degradation; nanofibers; electrospinning; fatty acid; poly(butylene succinate) (PBS)
1. Introduction

Biodegradable polymers are being constantly investigated for medical applications,[1] especially for tissue engineering (TE). Such polymers need to meet many important requirements to be successfully applied in TE. These include suitable chemical properties (non-toxicity, biodegradability, and ease of sterilization), physical properties (nanofibrous, elastic, and compliant), and biological properties (biocompatibility and biofunctionality).[2] Currently, the greatest attention is focused on a large group of aliphatic polyesters, including poly(lactic acid)(PLA), poly(glycolic acid)(PGA), poly (ε-caprolactone)(PCL)[3], and polyhydroxyalkanoates (PHA)[4], such as poly(hydroxybutyrate)(PHB). Overall, these biobased polymers see wide use in tissue engineering, due to their biodegradability, good biocompatibility, and suitable mechanical properties.

However, since the 1990’s, poly(butylene succinate) (PBS) and its copolymers have gained (renewed) prominence within this aliphatic polyester family, particularly for biomedical applications.[5–7] PBS offers good mechanical properties and biocompatibility, as well as better processability, easier obtainability, and higher elongation at break than other aliphatic polyesters. In particular, its high ductility suggests that PBS might be a good candidate for soft tissue repair. In terms of degradation, much like the other aliphatic polyesters, PBS is susceptible to hydrolysis and, in the environment, degradation of PBS by enzymes and microorganisms yields harmless products (carbon dioxide and water). However, (but again similarly to PLA, PGA, PCL and PHB) the crystallinity of PBS plays a major role in the degradation process[8] and may limit its suitability for soft-tissue repair and tissue engineering, due to its relatively slow degradation and resorption rate. In these applications, the polymer should combine appropriate mechanical performance and degradation profile: maintaining strength until the formation of new tissue can occur to replace the degraded material.

It is well known that polyester properties are influenced by many variables, some of which are molar mass, chemical structure, stereochemistry, hydrophilic/hydrophobic balance or chain mobility.[9,10] Consequently, copolymerization[11–14], as well as nanoadditives,[15] are well-established strategies for tailoring the properties of PBS for various biomedical applications. Previously, we demonstrated that copolymerization of PBS with increasing amount of dlinoleic acid (a dimerized fatty acid derivative) yielded lower crystallinity, higher susceptibility to degradation, and increased elasticity.[16] These fatty acid derivatives are obtained from vegetable oils and, thus, this new family of PBS copolymers could be entirely biobased, because PBS monomers (diol, diacid/diester) are readily available from refined biomass and biobased PBS (as well as various copolymers) is already commercially accessible[17].

Sustainability and “green chemistry”[18] can be further embraced in polyester synthesis by employing enzymatic catalysts[19], which offer much greater selectivity. This approach offers with several additional advantages compared to traditional melt polycondensation[20], such as mild conditions reactions (low temperature and pressure), and no toxic organometallic initiators—particularly relevant to biomedical applications. Lipases, in particular, have been
demonstrated to be powerful biocatalysts for the synthesis of polyesters, when the proper conditions are used.[21–23] In previous work performed in our laboratory using immobilized lipase B from *Candida antarctica* (CALB) as a catalyst, we obtained PBS copolymers containing dimer linoleic diol (poly(butylene succinate-co-dilinoleic succinate), PBS-DLS) with well-defined structures and high reproducibility of the initial monomer feed ratio.[24]

Our primary goal has been to obtain unique PBS-DLS copolymers, able to form flexible and soft materials for application in scaffolds for soft tissue engineering. The introduction to fatty acid moieties (dilinoleic diol), as well as using enzymatic catalysis, can be expected to significantly influence the degradation profile of these copolymers. In this work, we examine the enzymatic degradation of PBS:DLS copolymers differing in the amount of dilinoleic diol building up the soft segments (30 and 50 wt%, respectively). In the body, a range of hydrolase enzymes, present in fluids and tissues or as part of the inflammatory response, can be involved in increasing the *in vivo* degradation rate, as compared to hydrolysis alone.[25] It is impossible to adequately mimic these complex (and dynamic) conditions *in vitro*, thus it is common and useful to use model enzyme systems to gain insight into the degradation behavior of new materials and plan future *in vivo* studies. Lipase, a hydrolase present in human serum and macrophages, is a common model enzyme system, as it is well-known to hydrolyze ester bonds of aliphatic polyesters in aqueous media[25] (in addition to the previously mentioned catalytic activity towards ester formation). In the present study, we utilized lipase from *Pseudomonas cepacia*, one of the most commonly used lipases for *in vitro* enzymatic degradation, thanks to sufficient stability and activity to yield reproducible studies.[11] The degradation process was monitored by weight loss, water uptake, and changes in medium pH as a function of degradation time, as well as Fourier Transform Infrared Spectroscopy (FTIR) and Laser Scanning Microscopy. Additionally, Differential Scanning Calorimetry (DSC) and Size Exclusion Chromatography analysis were performed to compare samples before and after degradation.

2. Materials and Methods

Two aliphatic PBS copolyesters containing dimer linoleic diol moieties (PBS-DLS), differing in hard to soft segments ratios (70:30 and 50:50 wt.%), were synthesized using immobilized CALB (Fermase CALB™10000, kindly provided by Enzyme Catalyzed Polymers LLC, Akron, OH) as a catalyst. The synthesis was carried out using a two stage method and worked-up accordingly to the procedure described previously[24] to obtain samples with white powder appearance after 96 h. Briefly, 1,4-butanediol (BD, ≥99%, Sigma-Aldrich), dimer linoleic diol (DLA-OH, dimer alcohol ≥96.5%, Pripol 2033, Croda), and diethyl succinate (DS, ≥99%, Sigma-Aldrich) were dissolved in diphenyl ether (in appropriate ratios to obtain desired hard to soft segment ratio of the copolymer) with addition of CALB as catalyst. The mixture was reacted at atmospheric pressure at 80 °C under N₂. The temperature was slowly increased to 95 °C and after 2 hours (half of the theoretical amount of ethanol was collected) the pressure was reduced to 600 Torr. After 18 hours the pressure was gradually reduced to 2 Torr and maintained for 72
hours (96 h of total reaction time). After this time, the cooled products were dissolved in chloroform and filtered to remove the CALB. Products were concentrated using a rotary evaporator and precipitated by drop-wise addition into cold methanol, under continuous stirring. Finally, the polymers were filtered, washed well with cold methanol, and dried in a vacuum oven at 40 °C. The mass feed of all monomers was varied in order to obtain two materials with butylene-succinate (BS) units as hard segments and succinate-dimer linoleic diol units (DLS) as soft segments in approximately 50:50 and 70:30 wt% ratios. The obtained copolymers with hard to soft segments ratios of 70:30 and 50:50 wt.% will be abbreviated in this work as PBS-DLS 70:30 and PBS-DLS 50:50, respectively. The structural formula of the copolymers comprising hard and soft segments respectively is illustrated in Fig. 1.

![Poly(butylene succinate-co-dilinoleic succinate)](image)

**Figure 1.** Chemical structure of synthesized PBS-DLS copolymers showing the composition of hard segments (red) and soft segments (blue)

### 2.1 Characterization of PBS:DLS enzymatically obtained copolymers

The chemical structures of purified PBS-DLS 70:30 and 50:50 copolymers were verified by nuclear magnetic resonance (\(^1\)H NMR, \(^{13}\)C NMR) and Fourier transform infrared spectroscopy (FTIR). The \(^1\)H NMR (128 scans, 1 second relaxation delay) and \(^{13}\)C NMR (5120 scans, 1 second relaxation delay) spectra were recorded in CDCl\(_3\) on a TM Bruker DPX 400 (400 MHz) spectrometer with tetramethylsilane (TMS) as internal reference. FTIR transmission spectra (4000 to 400 cm\(^{-1}\), 2 cm\(^{-1}\) resolution, 32 scans), were obtained on a Bruker ALPHA spectrometer with an Attenuated Total Reflectance (ATR) cell. Intrinsic viscosity ([\(\eta\)]) measurements were performed using an Ubbelohde capillary viscometer (\(K = 0.00342\)) immersed in a water bath at 25 °C. Samples were dissolved in chloroform (0.125 g/25 cm\(^3\)) and results were calculated using the Solomon–Ciuta equations (1-3) [26].

\[
\eta = \frac{\sqrt[2]{2(\eta_w - \ln \eta_r)}}{C} \quad (1)
\]

\[
\eta_w = \eta_r - 1 \quad (2)
\]

\[
\eta_r = \frac{t_1}{t_o} \quad (3)
\]

\(t_o\) – average flow time for solvent [s]; \(t_1\) – average flow time for polymer solution [s]; 
\(c\) – concentration of polymer solution [g/100 ml]
Qualitative size exclusion chromatography (SEC) measurements in order to better understand and confirm the degradation results, were performed in a set-up consisting on an Agilent 1260 Infinity Isocratic Pump, a Wyatt OPTILAB T-rEX interferometric refractometer, a Wyatt DAWN HELOS-II multi-angle static light scattering detector (MALS) with a built-in dynamic light scattering (DLS) module, an Agilent 1260 Infinity Standard Autosampler and 6 StyragelVR columns (HR6, HR5, HR4, HR3, HR1, and H0.5). The columns were thermostatted at 35 °C and tetrahydrofuran (THF), continuously distilled from CaH₂, was used as the mobile phase at a flow rate of 1 mLmin⁻¹. Previous to the analysis, samples were dissolved in THF (the same employed as mobile phase in the instrument) at 2-3 mgmL⁻¹ and filtered. dRI signal was analyzed qualitatively.

2.2 Sample preparation for degradation studies
Commercial PCL was as a reference and positive control (CAPA 6430, Perstorp, Mn = 43,000 g/mol), as it has well-established biomedical applications[3], while PBS homopolymer is very resistant to enzymatic degradation,[10,27] due to combination of high crystallinity and high melting temperature. The PCL and enzymatically synthesized PBS-DLS 70:30 and 50:50 were processed both by hot-press melting and electrospinning, in order to assess the degradation profiles of the materials in the shape of films and fibrous membranes (scaffolds). Thin polymer films (100 µm) were prepared in a Remi-Plast hot-pressing machine (Poland) at temperature of 120 °C. Electrospun mats of similar thickness (~100-150 µm) were prepared by means of a standard horizontal electrospinning setup equipped with an ASCOR AP14 (Poland) pump, a Gamma High Voltage Research (USA) voltage source connected to a 21 gauge needle, and a grounded flat collector covered with aluminum foil. PCL polymeric solution was prepared by dissolving 20 wt% of the material in a DMF:DCM 5:5 v/v solvent mixture. PBS-DLS 70:30 and PBS-DLS 50:50 solutions were prepared by dissolving 25 and 35 wt% respectively in a CHCl₃:MeOH 7:3 v/v solvent mixture. All solutions were stirred at room temperature overnight and polymers were pre-dried in a vacuum oven before their preparation. The electrospinning process parameters are summarized in Table 1. The concentrations and parameters used are optimal for obtaining fibers free of defects or drops. The obtained mats were dried under vacuum for 48 h in order to eliminate any residual solvents. Samples in the shape of discs (6 mm diameter) were punched-out from the polymeric film and mats to perform the degradation studies.
Table 1. Electrospinning conditions for PCL, PBS:DLS 70:30 and PBS:DLS 50:50 copolymers.

| Material         | Rate [ml/h] | Distance [cm] | Voltage [kV] | Solvents/Concentration (v/v, wt%) | Temp [°C] | Humidity [%] |
|------------------|-------------|----------------|--------------|-----------------------------------|-----------|--------------|
| PBS:DLS 70:30    | 1           | 15             | 15           | CHCl₃:DCM(7:3)/25                | 23        | 34           |
| PBS:DLS 50:50    | 1           | 15             | 15           | CHCl₃:DCM(7:3)/35                | 23        | 28           |
| PCL              | 3           | 18             | 18           | DMF:DCM(1:1)/20                  | 25        | 30           |

2.3 Enzymatic degradation

Enzymatic degradation was carried out inside a laminar flow cabinet.[28] The degradation media consisted of a solution of lipase enzyme (EC3.1.1.3, from *Pseudomonas cepacia* (Sigma Aldrich, Poznań, Poland)) (25 units/mL) prepared in Dulbecco’s PBS buffer (Sigma Aldrich, Poznań, Poland) (pH range 7.1-7.2), with sodium azide added to avoid bacterial contamination. Each sample, in the form of a 6 mm diameter disc, was placed in the well of a 48-well plate and covered with 1.5 mL of degradation media. Plates were then incubated at 37 °C on a rocker (60 rpm) for 24 days and samples were collected for analysis at 24 h, 48 h and every 96 h thereafter. In order to maintain enzymatic activity, the degradation medium was changed every 48 h. At the specific time points, samples were removed, washed with distilled water, and weighed (n=3).

2.4 Characterization of processed materials before and after degradation

Water uptake and weight loss were determined gravimetrically using an analytical balance with an accuracy of 0.01 mg. At the specific time points, samples were removed from the media, washed thoroughly with distilled water, and weighed wet. Then discs dried at 40 °C under a pressure of ~30 mmHg for 10 days and weighed dry. The initial mass, before degradation, was indicated as \(m_0\), while the wet mass, immediately after degradation was indicated as \(m_w\) (wet), and the mass after drying as \(m_d\).

The water uptake (P) was then calculated from equation:

\[ P = \frac{m_w - m_d}{m_d} \cdot 100\% \quad (4) \]

The weight loss (D) was calculated from equation:

\[ D = \frac{m_0 - m_d}{m_0} \cdot 100\% \quad (5) \]

Changes in the chemical structure of films and fiber membranes (scaffolds) before and after degradation were evaluated by ATR-FTIR spectroscopy (measured under previously mentioned conditions).
TA Instruments Q1000). Approximately 3 mg of sample (sealed in aluminum pans), were subjected to a triple heating-cooling-heating cycle -90°C, to 190°C at a rate of 10°C/min under N₂ atmosphere. Glass transition temperature (T_g), melting temperature (T_m) and crystallinity were evaluated from the heating run and before/after degradation. In all the cases, glass transition temperature (T_g) was calculated as the midpoint of the transition. The crystalline phase content in the hard segments (X_ch %) for PBS:DLS 70:30 and 50:50 was calculated following the equations 6 and 7, while the total content of crystalline phase in PCL (X_c,tot %), was calculated from equation 8:

\[
X_{ch\%} = (0.7 \times \Delta H_m / \Delta H^0_m) \times 100 \tag{6}
\]

\[
X_{ch\%} = (0.5 \times \Delta H_m / \Delta H^0_m) \times 100 \tag{7}
\]

\[
X_{c,tot\%} = \Delta H_m / \Delta H^0_m \times 100 \tag{8}
\]

where \(\Delta H^0_m\) is \(\Delta H_m = 110.3\) J/g-1 for 100% crystalline PBS[29] and \(\Delta H_m = 135.3\) J/g-1 for 100% crystalline PCL [30]. Changes in the surface morphology (roughness, cracks, pores) were examined by Laser Scanning Microscopy (LSM) using a Keyence VK9700 LSM equipped with a 408 nm violet laser. Images were collected at maximum resolution (2048 X 1536) with a 10% and 30% intensity setting of the laser for the 20x (0.3 NA) and 50x (0.95 NA) objectives respectively.

3. Results

3.1 Chemical structure analysis

\(^1\)H NMR and \(^{13}\)C NMR results confirmed the successful synthesis of the CALB catalyzed PBS:DLS copolymers. Fig. 2 presents the \(^1\)H NMR spectrum of PBS-DLS 70:30 copolymer.

![Figure 2](image_url)

**Figure 2.** \(^1\)H NMR of poly(butylene succinate-co-dilinoleic succinate) 70:30 copolyester.
In $^1$H NMR, signals appearing at: $\delta ^1$H=4.12 ppm are due to four protons from BD adjacent to the oxygen (a), $\delta ^1$H=2.63 ppm are signals from four protons from DS (c), $\delta ^1$H=1.72 ppm are from BD (b). Following signal are characteristic for soft segments DLS units: $\delta ^1$H=1.62 ppm (20H, from DLA-OH, g+h), $\delta ^1$H=1.25 (52H, from DLA-OH, f+h), 0.85 (10H, from DLA-OH, i+j). Low intensity resonance ascribed to the BD end-groups appears at 3.68 ppm (2H, I, normalized to value 2.00) ($^{13}$C NMR confirmed the absence of DLA-OH end groups. Figs. S2-S3). Diethyl succinate end-groups at 4.16 ppm and at 1.25 are partially/total overlapped with BD and DLA-OH signals respectively.

As detailed in the Supporting Information, analysis of the $^1$H NMR spectra showed that the final achieved copolymer compositions were in a good agreement with the initial monomer feed (BD:DS:DLA-OH molar feed/compositions; PBS-DLS 70:30 initial 45:50:5, final 45:50:5; PBS-DLS 50:50 initial 50:39:11, final 50:41:9) (SI Fig. S1-2; SI Table S1, S2).[24]. In addition, the number-average molecular weight ($M_n$) calculated from $^1$H NMR (Supporting Information) resulted in an $M_n = 11590 \text{ g/mol}$ for PBS-DLS 70:30 and $M_n = 27440 \text{ g/mol}$ for PBS-DLS 50:50. The higher value obtained for PBS-DLS 50:50 is on account of the higher amount of DLA-OH present in the polymer backbone. An analogous relationship was also observed for the intrinsic viscosity measurements, where for PBS-DLS 70:30 $h=0.40 \text{ dl/g}$ and for 50:50 copolymer $h=0.56 \text{ dl/g}$.

Taking into account the related literature and our previous experience with enzymatically synthesized PBS and PBS copolymers, lowering of the vacuum in a stepwise manner and the usage of high boiling point and high log P (octanol/water partition coefficient of diphenyl ether 4.21) solvent, helps the formation of a high molecular weight materials avoiding premature deactivation of the catalyst and precipitation of the system for medium conversions.[24,31-33]

Thus, the synthesis conditions chosen helped to retain the initial desired ratio of hard to soft segments and favour the formation of high molecular weight PBS:DLS materials that ensured their further processing into fibers and films.

### 3.2 Enzymatic degradation of film and fibrous materials

The progress of enzymatic degradation was monitored for film and fibrous materials differing in segmental composition. The surface area available for degradation plays an important role, as in contrast to hydrolytic degradation, which for PBS copolymers is typically a bulk process, enzymatic degradation can be expected to occur via surface erosion.[5] Here, the mass loss and water uptake values for bulk materials (films, ~100 µm) were low and almost constant over the observation period of 24 days: mass loss was at the same level of 3.2% for both PBS-DLS 70:30 and PBS-DLS 50:50 copolymers, while water uptake was higher for PBS-DLS 70:30 (8.7%) than for PBS-DLS 50:50 (5.4%). The difference in water uptake, while small, can be explained by the higher content of hydrophobic fatty acid sequences in PBS-DLS 50:50 and higher $M_n$ as compared to PBS-DLS 70:30. In the case of electrospun mats, the fibrous morphology had a very pronounced effect on water uptake and mass loss—dramatically increasing both, as compared to bulk materials—as can be seen from Fig. 3a. For the PBS-DLS 70:30 copolymer, containing
higher amount of short butylene succinate hard segments, it can be clearly seen, that the much greater surface area of fibrous materials along with the segmental composition has resulted in much higher water uptake, ranging from 400% to 600%, as compared to approx. 100% for PBS-DLS 50:50, which contains more long chain hydrophobic fatty acid sequences. As can be seen from Fig. 3b, fibrous mats show increasing mass loss over time: after 24 days, mass loss exceeds 40% for PBS-DLS 70:30 and is approx. 10% for PBS-DLS 50:50. The much more rapid degradation of fibrous mats, as compared to films, is likely due to the much greater effective surface area, in agreement with a surface erosion mechanism.

Figure 3. Water uptake (a) and mass loss (b) for electrospun fiber mats of PBS-DLS 70:30 and PBS-DLS 50:50 (mean±SD, n=3).

Importantly, no acidification of the degradation media was observed during the degradation process of films (Fig. 4) and electrospun mats (Fig. 5), for either copolymer composition. The reference material and positive control PCL exhibits very fast degradation, along with a sharp
drop in pH (as low as pH = 6). Again, the degradation proceeds much faster for electrospun PCL mats: these materials completely disintegrate after 24 hours.

![Figure 4](image_url)

**Figure 4.** Changes in the pH of degradation media during degradation for PBS DLS 70:30, PBS DLS 50:50, and PCL films.

![Figure 5](image_url)

**Figure 5.** The changes in the pH of degradation media during degradation for PBS DLS 70:30, PBS DLS 50:50, and PCL electrospun fiber mats.

In order to reveal changes in Mₙ of degraded samples, SEC was carried out and dRI signal was analyzed qualitatively. Fig. 6 shows the traces before and after degradation for films materials. The trace of PBS-DLS 70:30 before degradation is multimodal, clearly showing the populations with various molecular weights, typical of polycondensation reactions. After degradation (24 days), no differences are observed for PBS-DLS 50:50 (Fig. 6b), while PBS-DLS 70:30 (Fig. 6a) showed some molecular weight breakdown, especially from the higher molecular weight
populations. These results are consistent with a surface erosion mechanism, as one would expect small molecular weight breakdown products to be dissolved in the media.

Figure 6. High resolution SEC chromatograms of films a) PBS DLS 70:30 and b) PBS DLS 50:50, before (solid line, red) and after (dashed line, blue) degradation (24 days)

Chemical structure of materials was monitored with infrared spectroscopy (ATR-FTIR) (here we show only PBS-DLS 70:30, Fig. 7). Three characteristic regions of both PBS-DLS 70:30 and PBS-DLS 50:50 copolyesters are highlighted: 1) the two peaks at 2920 cm$^{-1}$ and 2853 cm$^{-1}$ corresponding to absorption by methylene -CH$_2$- stretching groups of the aliphatic soft segments, 2) the peak at 1718 cm$^{-1}$ corresponding to absorption by carbonyl C=O bond stretching, and 3) the peak at 1153 cm$^{-1}$ corresponding to the ester C-O-C bond stretching. As can be seen from Fig. 7 (PBS-DLS 70:30, bulk material (films) in region 1, at 2920 cm$^{-1}$ and 2853 cm$^{-1}$, there is only a slight increase in absorbance after 24 days of degradation, which is consistent with SEC observation where we do not see changes in $M_n$ and confirms that the polymer is degrading via surface erosion.

Figure 7. FTIR-ATR spectra of PBS DLS 70:30 films, before and after the enzymatic degradation. Inset (1): 2920 cm$^{-1}$, 2853 cm$^{-1}$ correspond to aliphatic soft segments backbone. Inset (2): peaks corresponding to ester carbonyl backbone: 1718 and 1731 cm$^{-1}$
Fig. 8 shows changes in IR spectra for films prepared from PCL homopolymer. As can be seen from this figure in region 1, two peaks, 2942 cm\(^{-1}\), 2862 cm\(^{-1}\), indicate the absorbance by stretching of methylene -CH\(_2\)- groups of aliphatic soft segments. The peak in region 2, at 1731 cm\(^{-1}\), and the peak at 1159 cm\(^{-1}\), corresponds to C=O and C-O-C bonds, respectively, confirming the presence of carbonyl bonds of ester groups. Due to rapid degradation of the material, it was only possible to obtain spectra after 24 h and 48 h. For PCL films, the absorbance in region 1, at 2942 cm\(^{-1}\) and 2862 cm\(^{-1}\), increases following degradation. In region 2, the peak at 1731 cm\(^{-1}\) there is a shift of the spectrum to the lower values and band at 1159 cm\(^{-1}\), shows an increase in absorbance as observed after 24 hours of degradation.

**Figure 8.** FTIR-ATR spectra of PCL films, before and after the enzymatic degradation, Inset (1) show changes in peaks at 2942 and 2862 cm\(^{-1}\) ascribed to aliphatic chains. Insets 2: peak corresponding to C=O bonds of ester groups.

### 3.3 Thermal properties and surface morphology of films and fiber materials

Table 2 summarizes the thermal properties of the synthesized copolymers and Figure 9 shows their DSC traces. As can be seen, both copolymers are semicrystalline as the thermal behavior is characterized by the presence of a glass transition at around -40 °C and an endothermic peak at higher temperatures related with the melting of crystalline part. However, compared to the values of PBS homopolymer (Tm=113 °C)[34], the increase in DLA-OH content leads to a melting point decreasing in temperature value and lower in enthalpy (\(\Delta H_m\)). Further, PBS-DLS 50:50 has a broad melting peak and reaches the lowest value of melting enthalpy, probably due to a decrease in the perfection of the crystalline phase. In addition, the glass transition step height is more evident for this system due to the higher amount of amorphous part (soft segments). Although PBS homopolymer, as well as both copolymers, have the capability of crystallize before melting (cold-crystallization), the copolymerization with DLA-OH affects the area under the exothermic peak, (\(\Delta H_{cc}\)) lowering the ability of the butylene succinate sequences.
to crystallize. In addition, the DSC curve for PBS-DLS 50:50 shows a reduction in the cold crystallization temperature at 3 °C, suggesting that the highest amount of DLA-OH increased the chain mobility of the system.[35] Finally, from the cooling run, can be seen that the T<sub>c</sub> behaves similarly to the T<sub>m</sub>, decreasing as a function of the DLA-OH content. Regarding the T<sub>s</sub> values, the DSC results showed just one T<sub>s</sub> value for the PBS-DLS 70:30 and PBS-DLS 50:50 copolymers (Table 2), with both having an intermediate value between T<sub>s</sub> of PBS homopolymer (~43 °C) [27] and neat DLA (~55 °C) [36]. Due to this, and taking into account the synthesis procedure, the most likely segmental distribution for the obtained copolymers is a random distribution.

**Table 2.** Thermal behaviour of PBS-DLS 70:30 and 50:50 copolymers as synthesized (powder)

| Sample            | T<sub>c</sub> (°C) | ΔH<sub>c</sub> (J/g) | T<sub>g</sub> (°C) | ΔH<sub>g</sub> (J/g) | T<sub>cc</sub> (°C) | ΔH<sub>cc</sub> (J/g) | T<sub>m</sub> (°C) | ΔH<sub>m</sub> (J/g) | X<sub>c,h</sub> (%) |
|-------------------|---------------------|----------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|------------------|
| PBS-DLS 70:30     | 51.8                | 62.6                 | -43.3              | 4.4                 | 78.8                | 4.4                 | 101.3               | 57.3                | 33.6             |
| PBS-DLS 50:50     | 13.9                | 43.5                 | -46.4              | 2.9                 | 5.8                 | 84.2               | 45.1                | 17.8                |

Crystallization temperature (T<sub>c</sub>); glass transition temperature (T<sub>g</sub>); cold-crystallization temperature (T<sub>cc</sub>); melting temperature (T<sub>m</sub>); crystallization, cold-crystallization and melting enthalpies (ΔH<sub>c</sub>, ΔH<sub>cc</sub> and ΔH<sub>m</sub>); crystallinity from hard segments (X<sub>c,h</sub>); crystallinity total content (X<sub>c,tot</sub>); calculated from the DSC cooling run; calculated from the second DSC heating run using equations 6-7;

**Figure 9.** DSC thermograms. Left: first (solid line) and second heating (dashed line) scans of polymer powders (after synthesis) of PBS-DLS 50:50 (blue) and PBS-DLS 70:30 (red) copolymers. Right: cooling scan of polymer powders (after synthesis) PBS-DLS 50:50 (blue) and PBS-DLS 70:30 (red) copolymers.

With respect to the processed materials, films and fibers, before and after degradation, the thermal behavior was analyzed from the first heating. The characteristic transition temperatures and DSC traces are presented in Table 2 and Figs. 10 (films) and 11 (fibers). The processing parameters (hot melt pressing versus electrospinning from solution) did not strongly
affect the melting temperature, $T_m$. However, the processing of materials did have an effect on the crystallization temperature $T_c$, which was found to be higher for PBS-DLS 70:30 film, as compared to the fibrous mat fabricated from this copolymer. On the other hand, for PBS-DLS 50:50, with a lower content of crystallizable hard segments, higher $T_c$ was observed for fibrous and film materials, as compared to the as-synthesized copolymer (powder). Regarding the crystallinity, it appears that during the electrospinning process the molecules of PBS-DLS had less time to crystallize, compared to the film forming process, which allows for the crystallization of the PBS, as can be seen from the calculated $X_{c,h}$ values and the DSC cooling process of the powder materials (Fig. 9) that can simulate the cooling during the hot-press film forming.[35]

Table 3. Thermal behaviour of PBS-DLS 70:30 and 50:50 copolymers before and after degradation.

| Sample       | Before degradation | After degradation |
|--------------|--------------------|-------------------|
|              | $T_g$ ($^\circ$C) | $T_m$ ($^\circ$C) | $\Delta H_m$ (J/g) | $X_{c,h}$ (%) | $X_{c,tot}$ (%) | $T_c$ ($^\circ$C) | $T_g$ ($^\circ$C) | $T_m$ ($^\circ$C) | $\Delta H_m$ (J/g) | $T_c$ ($^\circ$C) | $T_g$ ($^\circ$C) |
| PCL film     | -61.2              | 65.0              | 77.8              | -            | 57.5            | 24.9            | -71.2             | n.o.              | 61.4            | 58.7            | 30.1            | n.o.              |
| PBS-DLS 70:30| -42.8              | 97.0              | 83.3              | 53.0         | -              | 51.7            | -43.3             | -                 | -               | -               | -               | -                 |
| Powder       |                    |                   |                   |              |                |                 |                   |                   |                 |                 |                 |                   |
| PBS-DLS 70:30| -43.0              | 99.0              | 50.1              | 33.0         | -              | 44.9            | -43.8             | -35.1             | 100.8          | 56.8            | 50.2            | --               |
| Fiber        |                    |                   |                   |              |                |                 |                   |                   |                 |                 |                 |                   |
| PBS-DLS 70:30| -40.1              | 102.0             | 52.6              | 33.4         | -              | 51.0            | -42.4             | -43.7             | 100.5          | 60.8            | 49.5            | -43.5             |
| Film         |                    |                   |                   |              |                |                 |                   |                   |                 |                 |                 |                   |
| PBS-DLS 50:50| -47.0              | 84.2              | 61.1              | 27.7         | -              | 14.0            | -46.4             | -                 | -               | -               | -               | -                 |
| Powder       |                    |                   |                   |              |                |                 |                   |                   |                 |                 |                 |                   |
| PBS-DLS 50:50| -44.1              | 89.3              | 41.6              | 18.8         | -              | 21.0            | -44.6             | -41.6             | 84.7            | 47.7            | 36.0            | -45.7             |
| Fiber        |                    |                   |                   |              |                |                 |                   |                   |                 |                 |                 |                   |
| PBS-DLS 50:50| -40.6              | 84.8              | 59.6              | 27.0         | -              | 24.6            | -44.7             | -42.2             | 84.0            | 60.4            | 29.2            | -44.1             |

Glass transition temperature ($T_g$); crystallization temperature ($T_c$); melting temperature ($T_m$); melting enthalpy ($\Delta H_m$); crystallinity from hard segments ($X_{c,h}$); crystallinity total content ($X_{c,tot}$); calculated from the first DSC heating run; calculated from the DSC cooling run; calculated from the second DSC heating run; calculated from the first DSC heating run using equations 6-7; calculated from the first DSC heating run using equation 8; n.o.-transition not observed on the thermograms.
Meanwhile, as can also be seen from DSC thermograms (Fig. 10 and 11), the degradation process has an effect on melting enthalpy, $\Delta H_m$. In general, for processed PBS-DLS 70:30 fibers and films after degradation, there is an increase in the melting enthalpy, as a direct consequence of the degradation affecting mainly the amorphous part. The same trend occurs for the PBS-DLS 50:50 fibers; however, after degradation the films retain the same $\Delta H_m$ as before, which is in agreement with the overall results (mass loss, LSM and SEC) indicating slower degradation for this material. Interestingly, as can be seen from the inset in Figure 11, the degradation process has a greater effect on the $T_g$ of the fibers, compared to the as-synthesized powder. In particular, for the PBS-DLS 70:30 fibers, there is a clear split into 2 different $T_g$, one at -35 °C, closer to that of PBS homopolymer, and the other at -58 °C, closer to that of neat DLA. This is direct evidence of the hydrolysis of the linkage between copolymer hard and soft segments contributing to the highest degradation rate observed for this material. This phenomenon appears to start to occur in the PBS-DLS 50:50 fibers after degradation, but is not as evident as in the PBS-DLS 70:30.

**Figure 10.** Left: First heating scan of films before (solid line) and after (dashed line) degradation with a $T_g$ region detail of degraded samples. Right: Cooling scan of films before (solid line) and after (dashed line) degradation. PBS-DLS 50:50 (blue) and PBS-DLS 70:30 (red) copolymers.
Figure 11. Left: First heating scan of fibers before (solid line) and after (dashed line) degradation with a $T_g$ region detail of degraded samples. Right: Cooling scan of fibers before (solid line) and after (dashed line) degradation. PBS-DLS 50:50 (blue) and PBS-DLS 70:30 (red) copolymers.

Changes in the surface morphology of samples before and after degradation are shown in Figs. 12-15. Polymeric films were prepared by melting in hot press, followed by cooling. The LSM micrographs of both bulk materials (Figs. 12a and 13a) before degradation show surfaces with cracks, reflecting the Teflon foil surface used during hot pressing. After degradation (Fig. 12b and Fig. 13b), the PBS-DLS 70:30 copolyester exhibits banded morphology, attributed to the spherulites formed during the crystallization. However, the typical periodic distance along the radial direction is not observed. It is possible that the amorphous region of the spherulites degraded, while the crystalline component still remains after the degradation. The surface morphology of the PBS-DLS 50:50 copolyester does not show as pronounced spherulitic forms (Fig. 13b). Further, this copolyester shows holes due to the degradation and many un-banded structures. This surface morphology can be related to higher content of hydrophobic fatty acid soft segments, which may be resistant to enzymatic lysis. The LSM micrographs are consistent with the DSC analysis, which indicated lower crystallinity of PBS-DLS 50:50 copolyester.
Figure 12. LSM micrographs of PBS DLS 70:30 films a) before and b) after 24 days of degradation.

Figure 13. LSM micrographs of PBS DLS 50:50 films a) before and b) after 24 days of degradation.

The morphology of electrospun mats is presented in Figs. 14 and 14. Following degradation, micrographs of PBS-DLS 70:30 copolyester (Fig. 14) reveal many regions with disrupted fibres, thus reflecting the observations from mass loss and water uptake, which indicated more pronounced degradation of these samples after 24 days. In the case of PBS-DLS 50:50 (Fig. 15), more fibres are visible and fewer differences from the pre-degradation micrograph can be observed. This is consistent with the previous results and explained by the greater proportion of hydrophobic fatty acid soft segments that may segregate to the surface.
4. Conclusions

Our work describes the enzymatic degradation of poly(butylene succinate-dilinoleic succinate) (PBS-DLS) copolymers, which can be synthesized from sustainable, biobased monomers using enzymatic catalysis. The incorporation of dimer fatty acid soft segments yields thermoplastic elastomers with good processability, well-suited to biomedical applications. The enzymatic degradation studies presented here indicate that an increase in fatty acid soft segments is associated with lower water uptake and mass loss, indicating slower degradation. Additionally, preparing samples in the form of electrospun fiber mats had a marked influence on degradation, indicating that the effective surface area plays a pronounced role, consistent with an erosive degradation mechanism. Importantly, we do not observe any trend towards acidification of the media during the degradation process. Overall, we conclude that these copolymers are well suited to applications in biomedicine, such as tissue engineering, thanks to their attractive processability (melt compression moulding and electrospinning) and tuneable degradation behaviour, which does not generate acidic breakdown products.
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