



Characterization, biodegradation and cytotoxicity of thermoplastic starch and ethylene-vinyl alcohol copolymer blends

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ABSTRACT

Ethylene-vinyl alcohol samples containing 27 and 38 % ethylene were used to prepare blends containing 30 and 50 % thermoplastic starch (TPS) plasticized with glycerol. Their biodegradability and cytotoxicity were studied by different techniques (XRD, DSC, TGA, CA, ATR-FTIR, SEM). TPS presence significantly affected copolymer behavior, as confirmed by the appearance of O—H IR 1000–1170 cm⁻¹ bands and overall reduction of EVOH crystallinity, melting point, thermal stability and hydrophobicity. Biodegradation was more efficient in the presence of TPS and resulted in the formation of a robust biofilm by a consortium of three bacteria. A lower ethylene content facilitated biodegradation, making the material easier to metabolize. The mineralization percentages obtained after a 40-day bioassay at 45 °C were up to 66 % (EVOH-27/TPS 50:50). *In vitro* cytotoxicity assay demonstrated no cytotoxicity before and after biodegradation. EVOH/TPS blends are presented as a potential environmentally friendly alternative to pure synthetic polymers.

1. Introduction

Polymer blends are a growing field of research, since they allow different materials with special properties to be obtained, which represents an economic advantage over new materials (Basappa et al., 2022; Dong et al., 2021; Kopač, Ručigaj, & Krajnc, 2022). Polymer blends from renewable sources that are also biodegradable are increasingly in demand to mitigate the adverse environmental impact of petroleum-based polymers. Among different natural polymers, starch stands out for its properties, abundance, low cost, good melt-processing, and ease of extraction (Carvalho, Zambon, da Silva Curvelo, & Gandini, 2005; Sri-suwana & Baimark, 2022). Starch by itself is not a thermoplastic material, but the use of certain plasticizers allows its gelatinization making it a thermoplastic starch (TPS) (Lendvai, Apostolov, & Karger-Kocsis, 2017; Wang et al., 2021). This capacity gives it an interesting faculty to form blends with synthetic polymers such as poly(vinyl alcohol) (PVA) (Zhou et al., 2009), high-density (HPDE), low-density polyethylene (LPDE) (Kaseem, Hamad, & Deri, 2012), and ethylene vinyl acetate (EVA) (Bhattacharya, 1998; Diyana et al., 2021). These previously studied blends of TPS with synthetic polymers reduce the environmental impact of the synthetic ones. Within the synthetic polymers, a candidate to form

these blends would be ethylene vinyl alcohol (EVOH) (Jiang, Qiao, & Sun, 2006). This is a semi-crystalline copolymer commercially available with an ethylene content of 24–48 % mol (Gavara et al., 2016). The applications of EVOH copolymers vary from packaging, agriculture, construction or biomedical due to their low oxygen permeability coefficients (Maes et al., 2018; Marcano et al., 2022). It is known that a higher content of EVOH improves mechanical properties in mixtures where starch is involved (Jiang et al., 2006). However, there are no studies that indicate how the combination of these two polymers can influence the biodegradation of these mixtures and their cytotoxicity. The presence of a vinyl alcohol group improves its compatibility with TPS (Alves, Grande, & Carvalho, 2019) since both have reactive hydroxyl groups that improve the chemical reaction (Gulati, Lal, & Arora, 2019; Teodorescu, Bercea, & Morariu, 2018). Some studies address the ability of these polymers to form blends with other natural polymers (Jiang et al., 2006; Nogueira et al., 2012). However, the aforementioned studies do not cover whether the blends improve biodegradability or if they are cytotoxic. These factors are essential to indicate that the new polymers lower the environmental impact as this is one of the main drivers for their production. The importance of this is even more when these are mass-produced.

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The aim of this study was to prepare a series of novel EVOH/TPS blends with different ethylene content (27/38) and concentrations of TPS (30/50 and 70/30); and to investigate their biodegradability with specific microorganisms as well as the cytotoxicity of the blends. The differences in ethylene content between EVOH-27 and EVOH-38 were studied to determine if this influenced biodegradation. Blends with 70/30 concentrations of TPS have not been studied before; therefore the purpose was to do a comparative study with the 50/50 ratio, which is frequently studied in the literature. Biodegradable and non-cytotoxic blends would lower the environmental impact of such materials and especially for mass-production applications such as packaging, agriculture, and other industrial applications. The materials (EVOH and TPS blends, and PVOH) were biodegraded and compared with non-biodegraded samples. Samples were studied by means of XRD, DSC, TGA, CA, ATR-FTIR, SEM. Biodegradation of the materials was studied by determining the carbon dioxide during their mineralization by specific bacteria in a consortium (MIX) of *Bacillus subtilis*, *Bacillus licheniformis* and *Brevibacillus borstelensis* (Morro et al., 2016) using an indirect impedance technique. This technique has been previously applied and was proven useful in studying polymer biodegradation (Abrusci, Marquina, Del Amo, & Catalina, 2007; Abrusci, Palomar, Pablos, Rodríguez, & Catalina, 2011). The cytotoxicity study of the non-biodegraded and biodegraded samples was assessed by the reduction of the MTT reagent (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) for observing the cell viability of the polymer to evaluate the environmental impact of the samples (Morro et al., 2017).

2. Materials and methods

2.1. Materials

Ethylene-vinyl alcohol 27 % mol ethylene Aldrich 414077 and ethylene-vinyl alcohol 38 % mol ethylene Aldrich 414085, (CAS N° 25067-34-9) were used pure and prepared into blends as detailed in Section 2.2.

To produce TPS, potato starch 20 % amylase and 80 % amylopectin (Meritena® 400) from Syral Ibérica, S.A.U. (Zaragoza, Spain) was used. Glycerol Aldrich (CAS N° 56-81-5) was also used for obtaining gelatinized starch.

Poly(vinyl alcohol) (PVOH) powder Aldrich (CAS N° 9002-89-5) was used for comparison purposes in polymer characterization analysis.

2.2. Preparation of blends and films

Thermoplastic starch (TPS) and ethylene-vinyl alcohol (EVOH) blends were prepared according to the procedure described in the literature (Morro et al., 2016). TPS was first obtained by mixing starch powder, water, and glycerol as plasticizers in a 100:20:30 (w/v/v) ratio for 30 min to obtain a paste, which was converted to TPS by heating to 120 °C in a thermostatic bath with constant stirring for 30 min.

TPS and EVOH were mixed with different ethylene percentages; 27 % (EVOH-27) and 38 % (EVOH-38) and blended with starch as EVOH: TPS 70/30 and 50/50 for both ethylene concentrations. The blends were prepared by melt processing in a Thermo Scientific Haake MiniLab II extruder with two counter-rotating screws at a single temperature of 135 °C. The rotor speed and mixing time were 80 rpm and 10 min respectively. PVOH and 100 % EVOH-38 and EVOH-27 samples were not prepared using extrusion.

EVOH-38 and EVOH-27, PVOH and blend films (~110 µm) were prepared by compression molding (300 mg polymer) in an Atlas Auto T8 Specac press at 220 °C in one cycle: Melting (1.5 min at 0 t), compression (2 min at 2 t), and cooling (1 min between cooling plates at 20 °C).

2.3. Polymer characterization

Diffraction X-Ray patterns (XRD) were obtained using an X Bruker

D8 Advance diffractometer, with CuK radiation. The scanning speed and the step size were 0.5° min⁻¹ and 0.02° min⁻¹ respectively. All the experiments were conducted with 2θ varying from 10° to 40°.

Differential Scanning Calorimetry (DSC) was performed on a METTLER DSC-823e instrument (−65 to 200 °C) previously calibrated with an indium standard (T_m = 429 K, ΔH_m = 25.75 Jg⁻¹). Film samples (5 mg) were processed by heating or cooling at 10 °C/min rates under nitrogen (60 ml/min).

Thermogravimetric analysis (TGA) was conducted in a Perkin-Elmer TGA7 under dynamic conditions using nitrogen as inert gas at 10 °C/min heating rates to ramp the temperature from 25 °C to 700 °C. Data was processed using TA Universal Analysis software.

Contact Angle (CA) was measured with a CAM200 KSV instrument using ultrapure distilled water as a wetting fluid. Eight independent advanced contact angles were obtained, and the average values were taken. Surface tension (ST) was calculated as described in the literature (Morro et al., 2017).

Attenuated Total Reflectance/FT-Infrared Spectroscopy (ATR-FTIR). IR spectra were obtained using a Perkin Elmer BX-FTIR spectrometer coupled with an ATR accessory, MIRacle™-ATR from PIKE Technologies.

Scanning Electron Microscopy (SEM). Polymer surfaces were examined employing a SEM Philips XL30 model. The samples were coated with approx. 3 nm of gold/palladium using a Polaron SC 7640 sputter coater.

2.4. Bacterial strains

The assay was performed using a consortium MIX of *Bacillus subtilis* (AF318900), *Bacillus licheniformis* (GU137297), and *Brevibacillus borstelensis* (GU125631), capable of hydrolyzing starch and glycerol at 5 to 55 °C and isolated from a mixture of ethylene/butyl acrylate (EBA) and ethylene/acrylic acid (EAA) based polymers previously described in Morro et al. (2016). A mixture of the three identified bacterial strains, noted herein as MIX, was used for the biodegradation studies.

2.5. Biodegradation by indirect impedance technique

Aerobic biodegradation of film samples by the MIX consortium was performed at 45 °C, as it is an adequate temperature for polymer biodegradation by these microorganisms (Abrusci et al., 2011; Morro et al., 2016), in bioreactors with a capacity of 7 ml filled with 1 g of sterile silica as a sample support and 1.5 ml of a bacterial suspension in a minimal growth medium with a count of 2.5 × 10⁷ cells/ml.

Film samples (6 mg) were then added to the medium. These containers were placed in 20 ml disposable cylindrical cells filled with 1.5 ml of aqueous 2 g/l KOH solution and fitted with electrodes to measure impedance on a Bac-Trac 4300 (SY-LAB Geräte GmbH, Neupurkerdorf, Austria). The instrument monitors the relative change in the initial impedance value of the KOH solution, which is converted to carbon dioxide, by a calibration curve of impedance variation versus CO₂ concentration. Under our experimental conditions, the media impedance of the KOH solution remained constant in the absence of films, confirming the absence of organic contaminants. The experimental apparatus and procedure were described by Abrusci et al. (2007).

2.6. Cytotoxicity assay

Cytotoxicity of the polymers was assessed by the reduction of the MTT reagent (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma Aldrich) to formazan as described by Morro et al. (2017) using Jurkat cells (human T-cell lymphoblast-like cell line). Cells were seeded at a density of 1–10⁵ cells/ml on membrane disks previously placed in a 24-well culture plate. Samples were incubated for various times under the above conditions. Absorbance at 590 nm was measured using a LabTech LT-4000 spectrophotometer.

3. Results and discussion

3.1. Characterization of non-biodegraded EVOH and TPS-blends by XRD, DSC

EVOH, the TPS-blends and PVOH samples were prepared according to the procedure described in Section 2.2. Visual observation of EVOH and TPS-blends showed that all the compositions were quite homogeneous and translucent (Fig. 6.4).

The results of the TPS characterization and biodegradation study by the MIX consortium has been previously carried out by Morro et al. (2016). Fig. 1 shows the X-ray diffractograms of the non-biodegraded EVOH-38 and EVOH-27 copolymers and blends (EVOH-38/TPS 70:30 and 50:50), (EVOH-27/TPS 70:30 and 50:50) and PVOH. The polymers EVOH-38 (Fig. 1A) and EVOH-27 (Fig. 1B) both showed a peak at $2\theta = 20.4^\circ$, which is characteristic of the polyvinyl alcohol (PVOH) fraction (shown in Fig. 1) in the ethylene-vinyl alcohol (EVOH) copolymers (Assender & Windle, 1998). This peak decreased proportionally to TPS ratio, as TPS reduced the crystallization of the copolymer, which is consistent with other copolymers with natural fibers such as EVOH/piassava blends (Nogueira et al., 2012).

Furthermore, a shoulder ($2\theta = 21.4^\circ$) and other peaks ($2\theta = 23.9^\circ$) were observed in EVOH-27 and EVOH-38 copolymers and blends (EVOH-38/TPS 70:30 and 50:50 (Fig. 1A) and EVOH-27/TPS 70:30 and 50:50 (Fig. 1B)), both associated with polyethylene (PE) as described previously (Morro et al., 2016).

Gelatinization of TPS was confirmed by this technique, as the characteristic peaks of native crystalline starch ($2\theta = 15^\circ$, 18° and 23°) disappeared in the blend's samples EVOH-38/TPS 70:30 and 50:50 and EVOH-27/TPS 70:30 and 50:50 (Fig. 1A and B), respectively (Parada & Aguilar, 2012).

The DSC thermogram was used to determine the melting point (T_m) of the non-biodegraded EVOH-27 and EVOH-38 copolymers and its blends (EVOH-38/TPS 70:30 and 50:50, EVOH-27/TPS 70:30 and 50:50) and is shown in Fig. 2. For EVOH-38, the melting point was 172°C and for its blends EVOH-38/TPS 70:30 and 50:50 these were 164°C and 156°C respectively (Fig. 2A). EVOH-27 had a melting point of 180°C and for both its blends EVOH-27/TPS 70:30 and 50:50 this was $164 \pm 1^\circ\text{C}$ (Fig. 2B). This descent in T_m for the non-biodegraded blends was due to the presence of starch (TPS) that reduced the crystallinity of the blends Fig. 1, and prevented the EVOH from crystallizing, which caused the reduction of the melting temperature. As previously observed, the usage of other more crystalline polysaccharides such as microcrystalline cellulose and cellulose nanocrystals, in blends with EVOH did not modify the T_m with respect to EVOH without

polysaccharides (Graninger, Kumar, & Falzon, 2020).

3.2. Film characterization by TGA, CA, ATR-FTIR, SEM of the non-biodegraded and biodegraded of EVOH and TPS blends

The materials were characterized using different techniques before and after 7 days of biodegradation by the bacteria consortium MIX as this period is adequate for characterization comparison. Thermogravimetric analysis (TGA) was used to study the thermal stability of the non-biodegraded and biodegraded polymers EVOH-38 and EVOH-27 copolymers and blends (EVOH-38/TPS 70:30 and 50:50), (EVOH-27/TPS 70:30 and 50:50) and PVOH; data is shown in Fig. 3. The peaks of weight loss (T_{peak}) observed for all non-biodegraded and biodegraded samples below 200°C were related to the evaporation of water.

The non-biodegraded copolymers EVOH-38 and EVOH-27 (Fig. 3 A, D) showed a shoulder around 450°C , associated with PE (Martínez-Sanz, Lopez-Rubio, & Lagaron, 2013) which has tertiary structures that can interfere with thermal degradation. In addition, there is a lack of information on the kinetic parameters of thermal degradation of EVOH copolymers (Abbas-Abadi, 2021; Alvarez, Ruseckaite, & Vázquez, 2003). After biodegradation, the shoulder was more pronounced (459°C), which was due to a higher PE content and a slight overlap with the weight loss of the PVOH segments that had been partially biodegraded. This confirms that the polyethylene phase was not metabolized in the biodegradation time frame. The PVOH TGA showed a shoulder around $400\text{--}500^\circ\text{C}$ (Fig. 3G) which could be associated to the scission of other hydrocarbon related products (Sin, Rahman, Rahmat, & Mokhtar, 2011).

For the non-biodegraded blends (EVOH-38/TPS 70:30 and 50:50 in Fig. 3 B,C and EVOH-27/TPS 70:30 and 50:50 in Fig. 3 E,F), the T_{weak} of glycerol, which was used as a plasticizer for TPS, appears at 150°C (Zanela, Bilck, Casagrande, Grossmann, & Yamashita, 2018). However, after biodegradation of the blends, the peak associated with glycerol disappears, indicating complete biodegradation by the consortium and an efficient enzyme complex (Alvarez-Macarie, Augier-Magro, & Baratti, 1999; Meghji, Ward, & Araujo, 1990). Moreover, after biodegradation, the peak of starch, which occurs at about 300°C (Cyras, Manfredi, Ton-That, & Vázquez, 2008), appears earlier and with lower intensity, confirming the efficient biodegradation of TPS linked to the PVOH phase by hydrogen bonds.

The non-biodegraded PVOH (Fig. 3 G) had three maximum decomposition temperatures (266 , 354 , 455°C) which are characteristic of the polymer (Kim, Kwon, & Seo, 2014). Furthermore, biodegradation by the microorganisms in PVOH caused the disappearance of the first peak and displaced the other two due to the loss of molecular weight (334 and

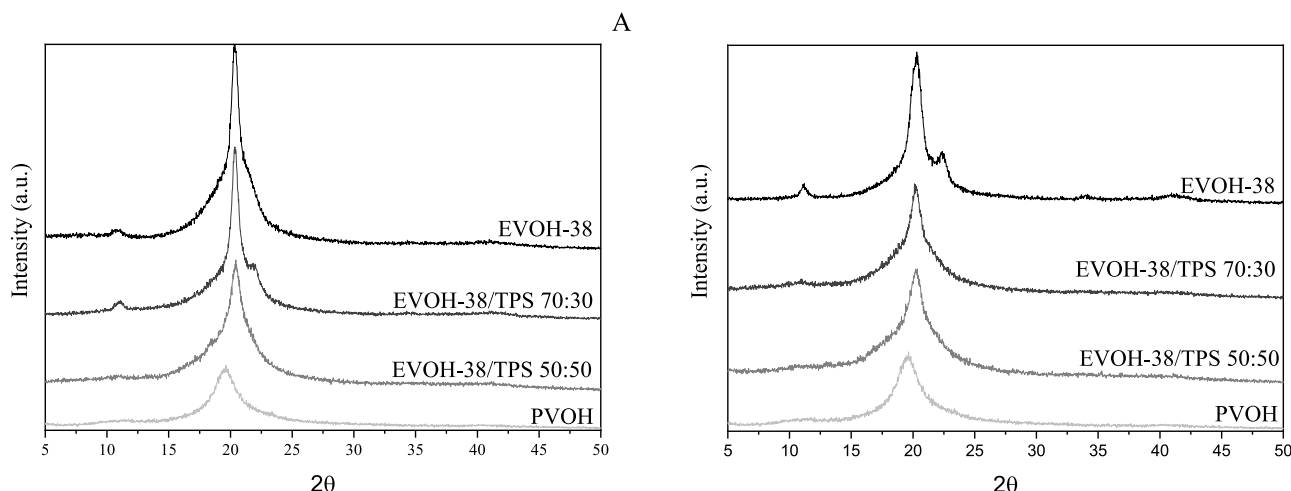


Fig. 1. X-ray diffraction patterns of EVOH-38 (A), EVOH-27 (B) and their corresponding blends with TPS, and PVOH.

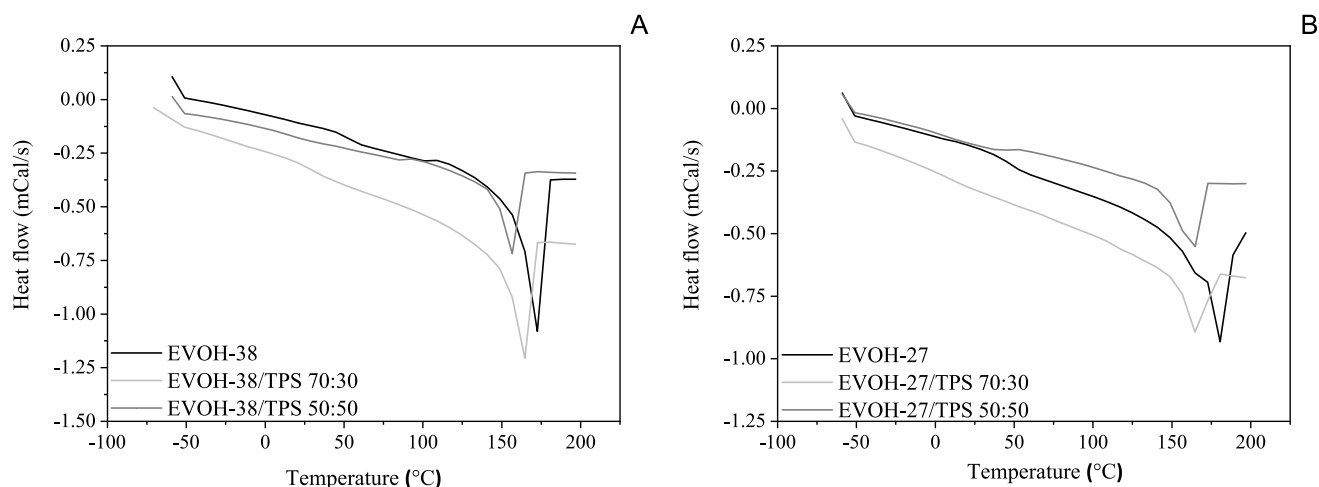


Fig. 2. DSC data of the non-biodegradable copolymers and their TPS-blends, EVOH-38 (A) and EVOH-27 (B).

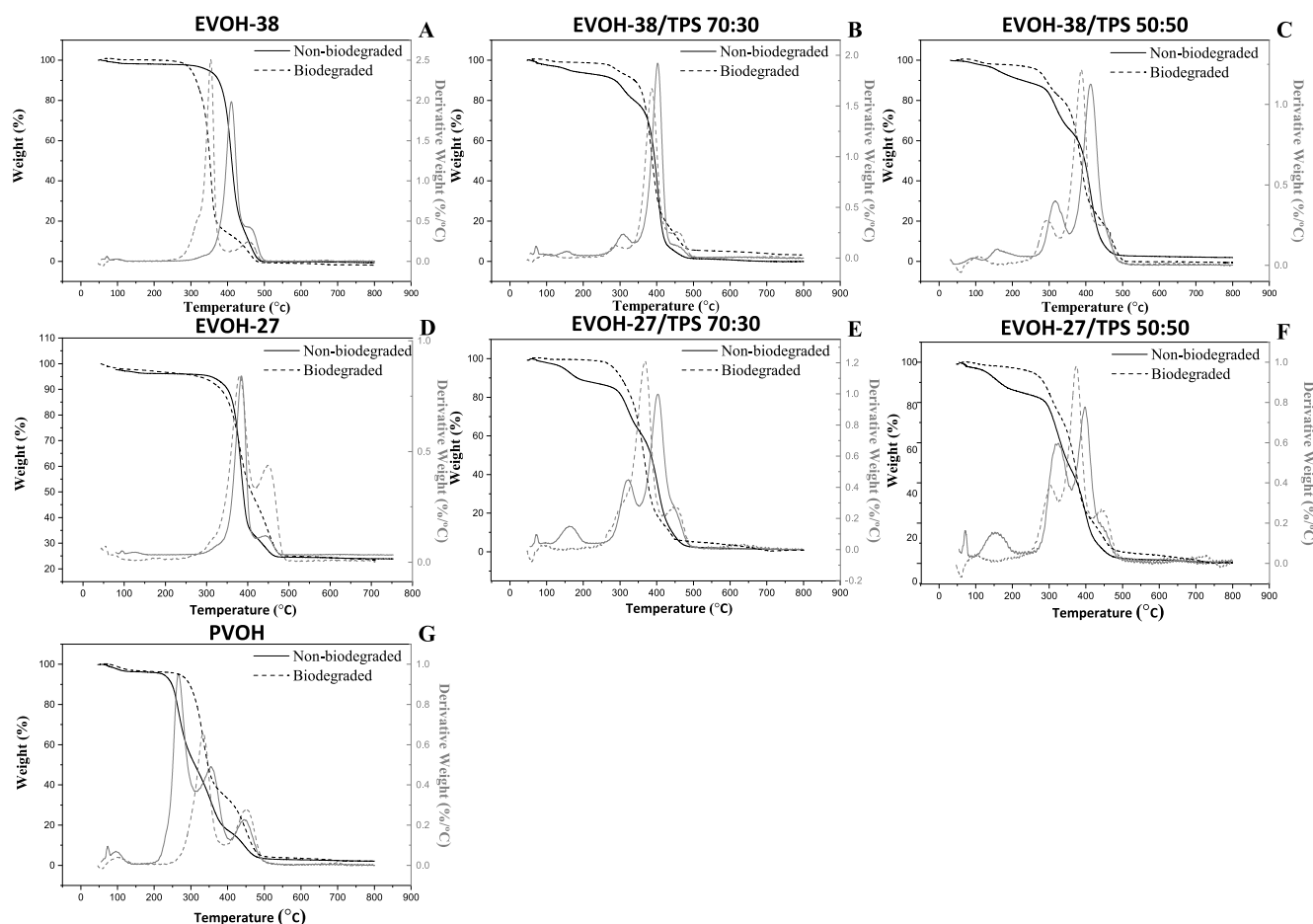


Fig. 3. Thermogravimetric analysis of the polymers EVOH-38 (A), EVOH-38/TPS 70:30 (B), EVOH-38/TPS 50:50 (C), EVOH-27 (D), EVOH-27/TPS 70:30 (E), EVOH-27/TPS 50:50 (F), PVOH (G) for non-biodegraded and biodegraded films.

452 °C).

Contact angles (CA) were measured on non-biodegradable polymers EVOH-38 and EVOH-27 copolymers and blends (EVOH-38/TPS 70:30 and 50:50), (EVOH-27/TPS 70:30 and 50:50) and biodegraded (Fig. 4). The non-biodegradable EVOH-38 and EVOH-27 exhibited contact angles greater than 100°, which identified them as highly hydrophobic polymers (Karthick & Maheshwari, 2008). However, for the non-

biodegradable blends (EVOH-38/TPS 70:30 and 50:50) and (EVOH-27/TPS 70:30 and 50:50), the incorporation of the TPS led to a lower contact angle, under 95°, resulting in more hydrophilic polymers (Morro, Catalina, Sanchez-León, & Abruscí, 2019). In the case of the biodegradable polymers EVOH-38 and EVOH-27, the contact angle decreased to 67° and 61°, respectively. For the EVOH-38/TPS 70:30 and EVOH-27/TPS 70:30 blends, this tendency was maintained with a

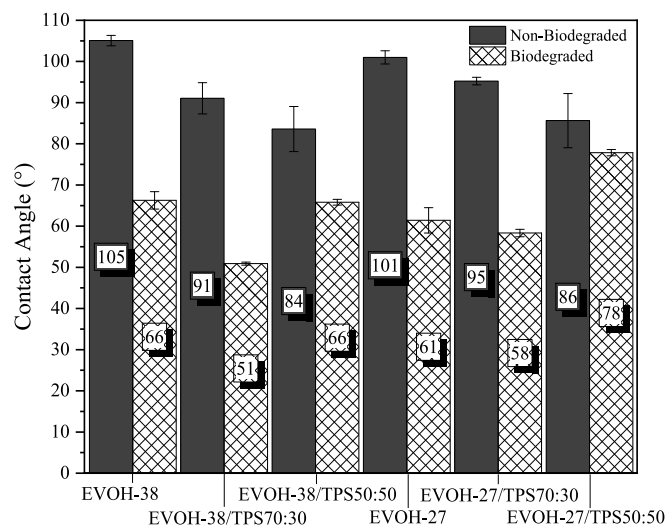


Fig. 4. Average contact angles of EVOH-38, EVOH-27, and their TPS blends films.

pronounced decrease of the contact angle to 50° and 58°, respectively. This decrease of the contact angle is due to oxidation on the polymer surface as a result of the biodegradation, which increases the hydrophilic character of the blends. In the case of the biodegraded EVOH-38/TPS 50:50 and EVOH-27/TPS 50:50 blends, the decrease in contact

angle was 65° and 77°, respectively, and therefore less than the TPS 70:30 blends. This could be due to a larger TPS content in the TPS 50:50 blends. The biodegradation of TPS and PVOH resulted in the exposure of the PE and increased the hydrophobicity of the blend (Silvia, Hasanah, & Zainuri, 2019). The contact angle of PVOH could not be measured due to the high hydrophilia of the non-biodegraded and biodegraded polymer.

The non-biodegraded and biodegraded EVOH-38, EVOH-27 films, and its blends EVOH-38/TPS 70:30 and 50:50; and EVOH-27/TPS 70:30 and 50:50 were analyzed using ATR-FTIR spectroscopy. The spectra obtained are shown in Fig. 5 A,B. In the non-biodegraded copolymers, several characteristic IR bands were found (Fig. 5 A,B). At 3300 cm^{-1} there was a band associated with the alcohol bond, at 1090 cm^{-1} the band was associated with C—C, C—O and C—O—C bonds and at 2900 cm^{-1} the band was associated with methylene, all of these are in turn associated with the PVOH group (Morro et al., 2016; Zanela et al., 2018). There was also a band around 1000–1170 cm^{-1} that was very pronounced in the TPS blends (EVOH-38/TPS 70:30 and 50:50 and EVOH-27/TPS 70:30 and 50:50), confirming the presence of starch (Capron, Robert, Colonna, Brogly, & Planchot, 2007).

In biodegraded materials (Fig. 5 A,B), the intensity of all bands decreased indicating an efficient biodegradation by the bacterial consortium MIX. In the case of the band situated at 2900 cm^{-1} , this was reduced drastically in the case of the TPS blends. This indicates that the interaction of TPS with PVOH could have improved the biodegradation of the latter by MIX consortium.

Scanning electron microscopy (SEM) micrographs of non-biodegraded, biofilm formation and biodegraded EVOH-27, EVOH-38,

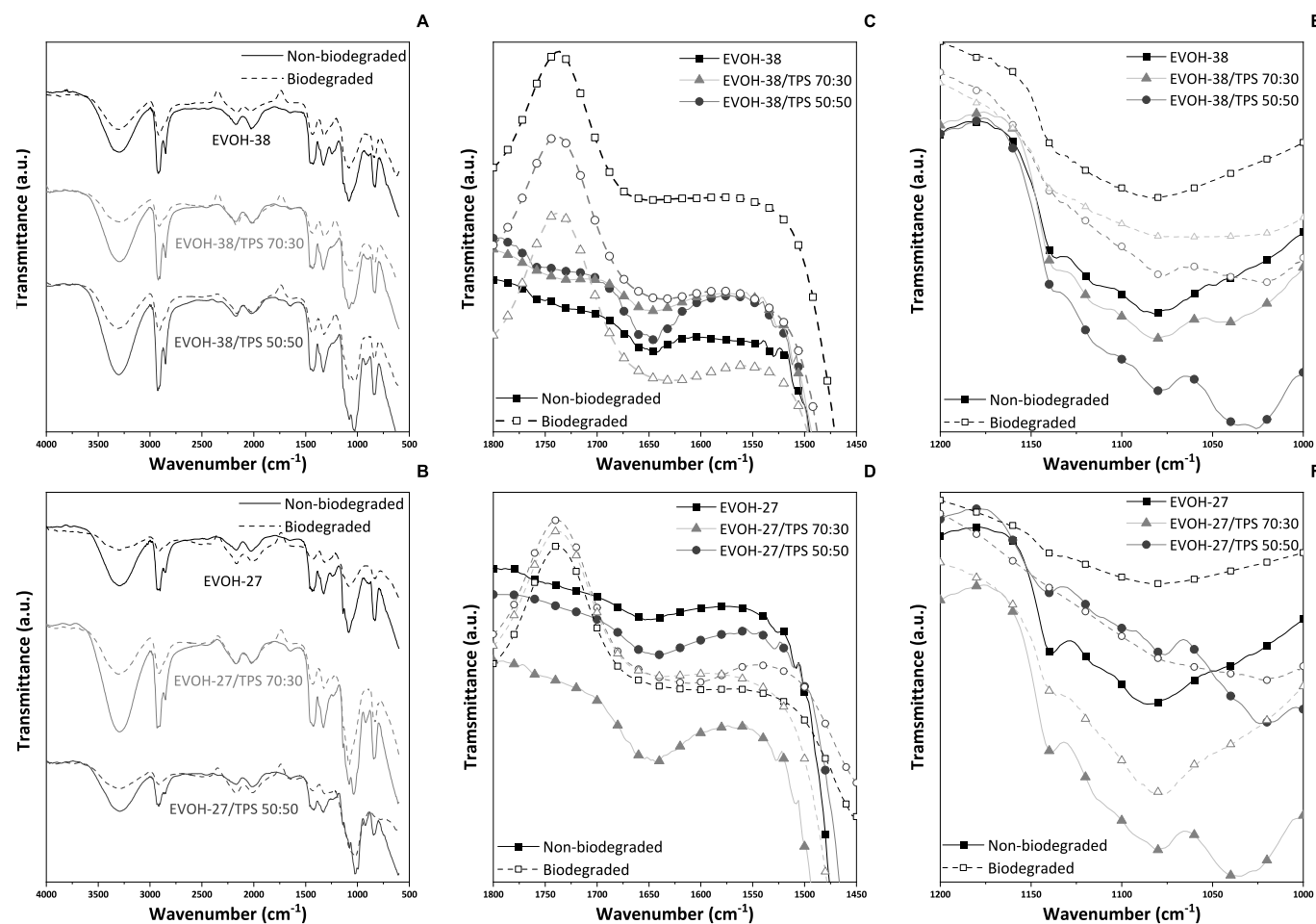


Fig. 5. FTIR-ATR spectra of EVOH-27, EVOH-38 and blends for non-biodegraded and biodegraded (after 7 days biodegradation by MIX consortium) samples. (A, B). Detailed EPS-associated bands of EVOH-27, EVOH-38, and blends for 1800 cm^{-1} to 1450 cm^{-1} (C, D) and 1200 cm^{-1} to 1000 cm^{-1} (E, F).

TPS-blends and PVOH are shown in Fig. 6. In the microphotographs of the non-degraded copolymers (Fig. 6.1), EVOH-38 (Fig. 6.1A) and EVOH-27 (Fig. 6.1D) presented smooth surfaces. However, in its blends EVOH-38/TPS 70:30 (Fig. 6.1B), EVOH-38/TPS 50:50 (Fig. 6.1C), EVOH-27/TPS 70:30 (Fig. 6.1E) and EVOH-27/TPS 50:50 (Fig. 6.1F) small bulges were observed as the starch content increased. These in turn corresponded with small TPS particles on the surface as the starch increased the domain size which allowed its availability to the microbial attack (Bootklad & Kaewtatip, 2013; Imam et al., 1992).

Microphotographs of the biofilm formation were taken for all materials (Fig. 6.2). The MIX consortium colonized all films, but the biofilm formation was larger in hydrophilic copolymers (Fig. 4) that contained TPS (Fig. 6 B–F). These results were confirmed through the ATR-FTIR spectroscopy analysis (Abrusci et al., 2013) (Fig. 5 C,D,E,F). Characteristic bands for the exopolymers (EPS) were found that had been produced by the microorganisms. The band 1037 cm^{-1} (Fig. 5 E,F) corresponding to C-O-C and C-O-P was associated with the presence of polysaccharides (Vijayabaskar, Babinastarlin, Shankar, Sivakumar, & Anandapandian, 2011), and the bands 1600 to 1500 cm^{-1} (Fig. 5 C,D) was associated to the presence of amides I and II (Verhoef et al., 2005).

This confirmed that the microorganisms easily colonized the materials.

In Fig. 6.3, the microphotographs are shown after 7 days of biodegradation by the bacteria consortium MIX. In all films, (Fig. 6.3 (A–G)) there was a general deterioration. In the mixes with TPS, EVOH-38/TPS 70:30 (Fig. 6.3B), EVOH-38/TPS 50:50 (Fig. 6.3C), EVOH-27/TPS 70:30 (Fig. 6.3E) and EVOH-27/TPS 50:50 (Fig. 6.3F), holes appear due to colonization and the gradual biodegradation of TPS by the microorganism. This was confirmed by the results described in the following section.

3.3. Biodegradation by indirect impedance technique

The biodegradation study was undertaken using indirect impedance measurements (Abrusci et al., 2007; Corrales et al., 2012). Biodegradation of the EVOH-38, EVOH-27 polymers, and their blends EVOH-38/TPS 70:30 and 50:50, EVOH-27/TPS 70:30 and 50:50, and PVOH by the bacterial consortium MIX was conducted at 45°C (Abrusci et al., 2012). Data is shown in Fig. 7.

In the case of the EVOH-38, EVOH-27 and PVOH samples, the biodegradation percentages at 7 days were 3.8, 5.2 and 8.0 %, respectively.

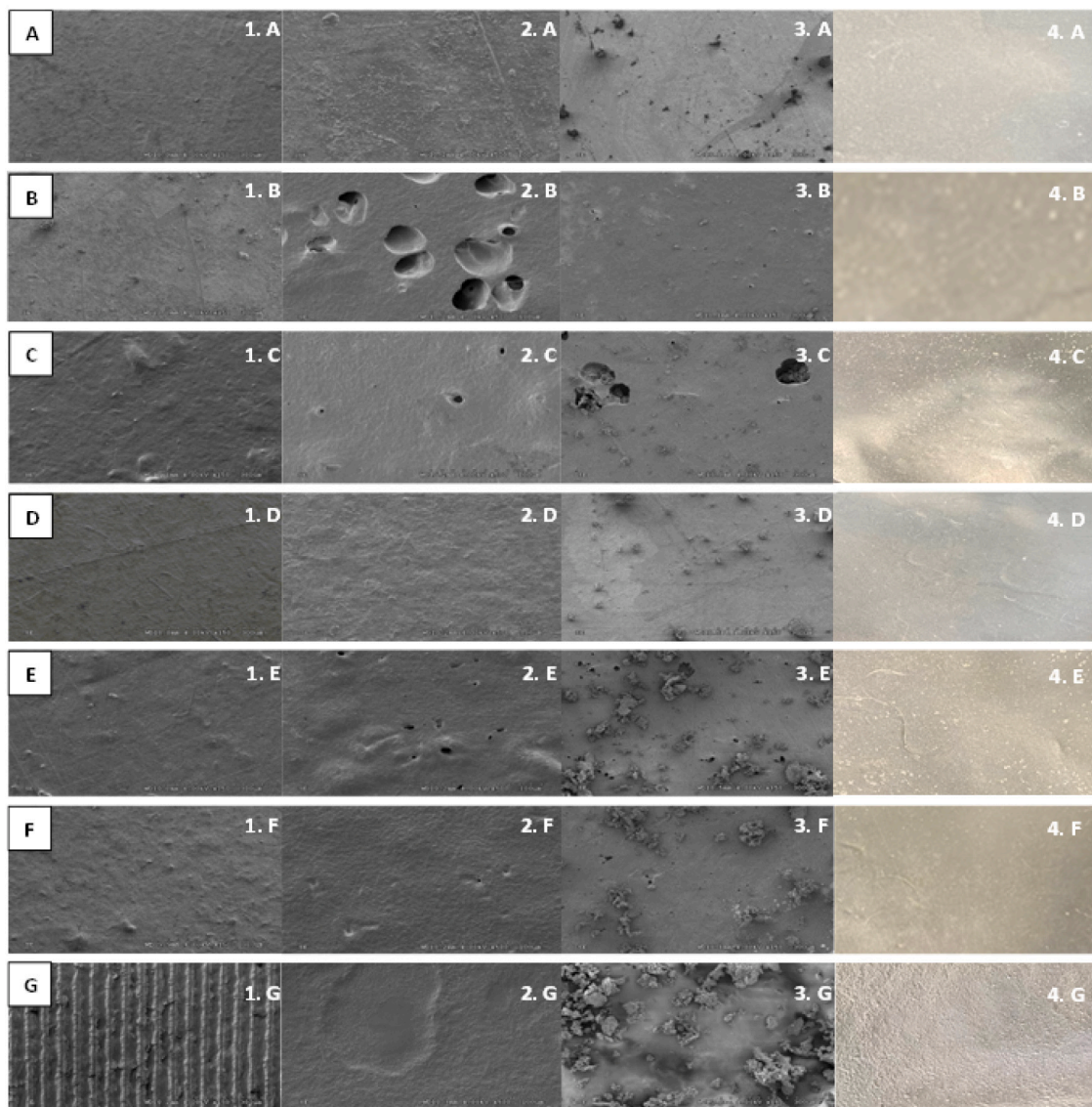


Fig. 6. SEM micrographs of EVOH-38 (A), EVOH-38/TPS 70:30 (B), EVOH-38/TPS 50:50 (C), EVOH-27 (D), EVOH-27/TPS 70:30 (E), EVOH-27/TPS 50:50 (F), PVOH (G) for non-degraded (1), biofilm formation (2), biodegraded (3) by the consortium MIX of *B. subtilis*, *B. licheniformis* and *B. borstelensis* and (4) film photographs.

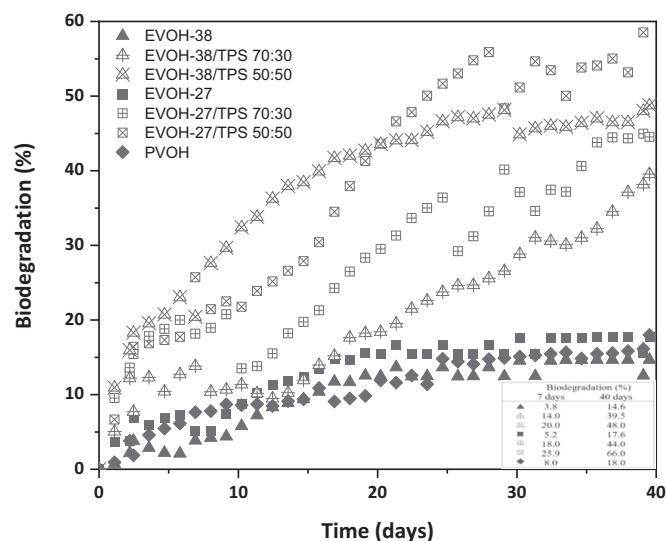


Fig. 7. Biodegradation percentage of EVOH-38, EVOH-27 and their TPS-blends and PVOH after 40 days at 45 °C by the consortium MIX of *B. subtilis*, *B. licheniformis* and *B. borstelensis*.

respectively. After 40 days, the biodegradation was 14.6, 17.6, and 18 %, respectively. These results suggested that the PVOH present in the EVOH-38 and EVOH-27 copolymer was the susceptible component to biodegradation by MIX. This is due to the fact that the *Bacillus* genre has an extracellular enzymatic system that is remarkably effective in biodegrading PVOH (Kawai & Hu, 2009). In the case of the EVOH-38/TPS 70:30 and EVOH-27/TPS 70:30 blends, the biodegradation in 7 days was 14 and 18 %, respectively. After 40 days, the biodegradation of these blends was of 39.5 and 44 %, respectively. For the EVOH-38/TPS 50:50 and EVOH-27/TPS 50:50 blends, the biodegradation in 7 days was 20 and 25.9 %, respectively, and after 40 days it was of 48 and 66 %, respectively. For all studied EVOH/TPS blends, an exponential biodegradation dependent on TPS concentration was observed as these blends were more hydrophilic (Fig. 4). It was also found that a lower EVOH content resulted in higher biodegradation activity as observed for the EVOH-38 and EVOH-27 copolymers as well as for the blends. The lower ethylene content makes the material easier to metabolize. The efficient biodegradation of these polymers was incremented by the capacity of the bacterial consortium MIX and in the formation of a robust biofilm (Abrusci et al., 2013). These results indicated that MIX contains highly effective enzymes such as α -amylases, which are required for TPS biodegradation (Konsula & Liakopoulou-Kyriakides, 2004; Morro et al., 2016). This efficacy was confirmed in the biodegradation process (Fig. 7) since the metabolic activity of the consortium remains constant throughout the study period.

3.4. Cytotoxicity of EVOH and TPS-blends films

EVOH-38 and EVOH-27 copolymers and blends (EVOH-38/TPS 70:30 and 50:50), (EVOH-27/TPS 70:30 and 50:50), and PVOH were evaluated for cytotoxicity with Jurkat cells after 24 h for non-biodegraded samples and after 40 days of biodegradation. The results are shown in Fig. 8. Most polymers, both biodegraded and non-biodegraded, presented cell viability over 90 % except non-biodegraded PVOH that presented 69 % cell viability after 24 h. After 40 days biodegradation, PVOH exhibited a cell viability of 95 %. This could be due to the fact that the high molecular weight of the polymer affects the proliferation of the Jurkat cells in suspension. The molecular weight of the polymer decreased after biodegradation, and therefore the cytotoxic effect on the cells was also lessened (Morro et al., 2017). These results indicated that the EVOH blends do not exhibit cytotoxicity and

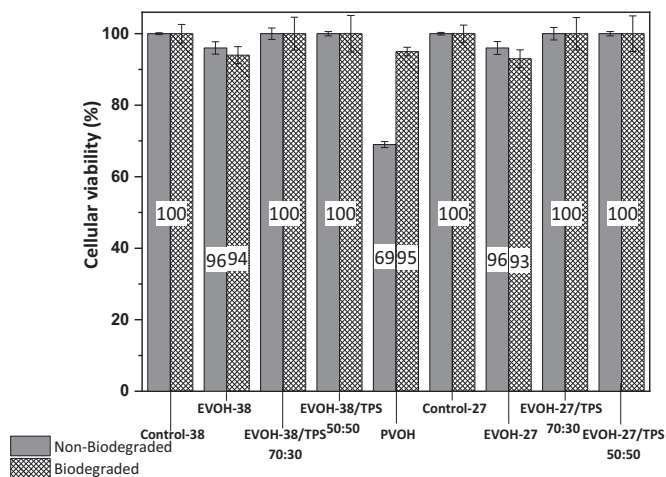


Fig. 8. Cellular viability in percentage for the cytotoxic assay post 24 h and 40 days inoculation of Jurkat cells with EVOH copolymers and blends.

are suitable for food and beverage packaging (Graninger et al., 2020).

4. Conclusion

The characterization study of the ethylene-vinyl alcohol (EVOH) and thermoplastic starch (TPS) blends showed that all the compositions were quite homogeneous, confirming overall gelatinization of TPS. The presence of TPS in the blends resulted in a lower fusion temperature and an increase in hydrophilicity with respect to the copolymers without TPS. The biodegradation of these polymers was more efficient as the blend composition enabled the formation of a robust biofilm by bacterial consortium MIX. The mineralization percentages obtained after a 40-day bioassay confirmed complete biodegradation of the TPS phase and partial biodegradation of the PVOH phase. PVOH was the most susceptible component to biodegradation from all the non TPS-blended copolymers. *In vitro* cytotoxicity assay demonstrated the non-cytotoxicity of the samples before and after biodegradation. These EVOH/TPS combinations improved the biodegradability of the polymers and are biologically safe.

CRediT authorship contribution statement

C. Pérez-Blanco: Formal analysis, Investigation, Resources, Writing – original draft. **E. Huang-Lin:** Formal analysis, Resources. **C. Abrusci:** Conceptualization, Formal analysis, Investigation, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

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.

Data availability

The data that has been used is confidential.

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