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New blends of ethylene-butyl acrylate copolymers with thermoplastic starch. Characterization and bacterial biodegradation

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ABSTRACT

Ethylene-butyl acrylate copolymer (EBA) with 13% of butyl acrylate content was used to produce blends with 10, 30 and 60% of thermoplastic starch (TPS) plasticized with glycerol. Ethylene-acrylic acid copolymer (EAA) was used as compatibilizer at 20% content with respect to EBA. The blends were characterized by X-ray diffraction, ATR-Fourier Transform Infrared Spectroscopy (ATR-FTIR), Scanning Electron Microscopy (SEM), water-Contact Angle measurements (CA), Differential Scanning Calorimetry (DSC) and Stress–strain mechanical tests. Initiated autoxidation of the polymer blends was studied by chemiluminescence (CL) confirming that the presence of the polyolefin-TPS interphase did not substantially affect the oxidative thermostability of the materials. Three bacterial species have been isolated from the blend films buried in soil and identified as *Bacillus subtilis*, *Bacillus borstelensis* and *Bacillus licheniformis*. Biodegradation of the blends (28 days at 45 °C) was evaluated by carbon dioxide measurement using the indirect impedance technique.

1. Introduction

In recent times, biodegradable materials have gained academic and industrial importance particularly for the protection of the environment from ever-increasing plastics waste. Partially biodegradable materials prepared by blending natural biodegradable and non-biodegradable synthetic polymers can reduce the volume of plastics waste by their non-complete environmental biodegradation. These blends exhibit economic advantages and superior properties than the completely biodegradable ones due to the overall properties imparted by the commercial polymer used as a blending component. Thermoplastic starch (TPS) is a promising biodegradable natural polymer of low cost with traditional polymers and particularly in polyolefins (Da Róz, Ferrera, Yamajai, & Carvalho, 2012; Rodriguez-Gonzalez, Ramsay, & Favis, 2003; Taguet, Huneault, & Favis, 2009; Cerclé, Sarazin, & Favis, 2013; Taguet, Bureau, Huneault, & Favis, 2014) such as polyethylene (LDPE), ethylene-vinyl acetate (EVA) or ethylene-vinyl alcohol (EVOH) copolymers. The main drawback of these materials is the poor compatibility between the hydrophilic starch and hydrophobic polymers. One way to increase compatibility in starch blends is to use a compatibilizer containing groups capable of hydrogen bonding with the starch hydroxyl groups. Poly (ethylene-co-acrylic acid) (EAA) is such an example and compatibilization with starch is due to a helical V-type complex formation (Shogren, Thompson, Green, Gordon, & Cote, 1991) and this copolymer has been used as a compatibilizer, typically of 20% of acrylic acid content.

Recently, ethylene-butyl acrylate copolymers (EBA) have been increasingly used in outdoor applications as a base polymer film due to some advantages compared to polyethylene. Owing to the presence of butyl ester polar groups in their structure, EBA performance improves significantly in terms of cohesion and adhesion to different substrates, resulting from its greater polarity and lower crystallinity. These properties can provide higher compatibility with the majority of organic additives and polymers. This fact also increases the dispersibility and the maximum concentration of additives in the polymeric matrix, improving film quality and enhancing service life.

Numerous studies have been done to investigate the microbial biodegradation of starch- plastic materials (Swanson, Shogren, Fanta, & Imam, 1993). These studies involve blends with polyolefins like LDPE (Danjaji, Nawang, Ishiaku, & Mohd, 2002; Tena-Salcido, Rodríguez-González, Méndez-Hernández, & Contreras-Esquivel, 2008), EVOH (Simmons & Thomas, 1995) and EVA (Da Róz et al., 2012), but there is no related work made with EBA-starch blends.

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The aim of this study was to prepare a series of EBA/TPS blends using the copolymer EAA as compatibilizer and varying the starch content in order to investigate their behaviour in microbial biodegradation using previously isolated bacterial strains from biofilm formation under exposure to soils. The materials were studied by means of X-ray diffraction, DSC, FTIR, DMTA and Stress–strain mechanical tests. Also, the blends were studied by the chemiluminescence technique and results pertaining to the thermal oxidative stability of EBA/TPS blends are presented.

Three bacterial species have been isolated from films buried in soil located in Murcia, Spain. These were found attached to the polymer and identified as *Bacillus subtilis*, *Bacillus borstelensis* and *Bacillus licheniformis*. Biodegradation of the materials was studied by determining the carbon dioxide during their mineralization by the bacteria using an indirect impedance technique. This technique has been previously employed and was proven useful in studying polymer biodegradation (Abrusci, Marquina, Del Amo, & Catalina, 2007; Abrusci et al., 2011).

2. Experimental

2.1. Materials and polymer characterization

The Ethylene/butyl acrylate (EBA) with a content of 13% (w/w) in butyl acrylate (BA) was supplied by Repsol (ALCUDIA® EBA PA-1303, density 0.925 g ml^{-1}, Melt Flow Index MFI = 0.3 g/10 min). The Ethylene/acrylic acid (EAA) of 20% of acrylic acid content (PRIMACOR® 5980I, density 0.958 g ml-1, Melt Flow Index MFI = 14 g/10 min) was supplied by Dow Ibérica (Tarragona, Spain). Native potato starch, 20% of amylose and 80% amylopectin (Meritena®400), was supplied by Syral Ibérica, S.A.U. (Zaragoza, Spain) and Glycerol (CAS N° 56815) and Magnesium nitrate hexahydrate (CAS N° 13446-18-9) were purchased from Aldrich.

Diffraction X-Ray patterns (XRD) were obtained using an X Bruker D8 Advance diffractometer, with CuKα radiation. The scanning speed and the step size were 0.5° min^{-1} and 0.02° min^{-1}, respectively. All the experiments were carried out with 2θ varying from 10° to 40°.

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.

Contact Angle (CA) was measured using a CAM200 KSV equipment using Millipore grade distilled water as wetting liquid. Five independent advanced contact angles were measured and the average values were taken. The surface tension (ST) was calculated as described in the literature (Fowkes, 1964).

Dynamic Mechanical Thermal Analysis (DMTA) was performed on a METTLER-TOLEDO DMA/SDTA861^e instrument (tensile mode, frequency of 1 Hz) in the temperature range from −130 °C to 65 °C and heating rate of 2 °C min^{-1}.

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

Tensile properties. Tensile strength (σ) and retention of elongation at break (ε) were determined using a dynamo-metric MTS Q-Elite apparatus (room temperature, stretching speed of 50 mm/min). Before testing, all compression moulding films (200 μm thickness) were stored under controlled atmosphere by a saturated $Mg(NO_3)_2.6H_2O$ solution (R.H. 53% at 25 °C). Standard dumbbell probes (5 specimens of 35 mm) were tested and the average values were taken.

Differential Scanning Calorimetry (DSC) was performed on a METTLER DSC-823e instrument (30–180°C) previously calibrated with an indium standard (T_m = 429 K, ΔH_m = 25.75 Jg^{-1}). Film samples (10 mg) were heating or cooling at 5 °C/min rates under nitrogen. Polyethylene crystallinity (χ_c %) were determined using the area of the melting endothermic peak and related to a reference of 293 Jg^{-1} for crystalline polyethylene (Flory & Vrij, 1963).

2.2. Preparation of blends

TPS was obtained by mixing starch powder, water and glycerol in the ratio of 100:20:30 (w/v/v), respectively, for 30 min to obtain a paste which was transformed to TPS by heating at 120 °C in thermostatic bath with continuous stirring for 30 min.

EBA and TPS were mixed in different ratios maintaining a constant EAA at 20% (see Table 1 for compositions). The blends were prepared by melt processing in a Haake MiniLab mixer extruder with two counter-rotating screws at 135 °C of temperature. The rotating speed of the rotor and the mixing time were respectively 80 rpm and 10 min.

Polymer films (200 μm) were made by compression moulding (1 g of blended powder) in a Collin-200 press 170 °C and pressure cycle: 2 min at 0 bar and 1 min at 200 bar. All blends contained a 20% of EAA respect to EBA as compatibilizer and samples will be referred as EBA% TPS, being% the percentage of TPS content (w/w) in the blend.

2.3. Isolation of bacterial strains

Film samples were scattered in November in an agricultural soil (Torre Pacheco, Murcia, Spain). Films collected after 7 days for microbial identification were cultured in trypticase soya agar (TSA) and grown at different ranges of temperature and their ability to hydrolyze starch and glycerol was tested as described in Abrusci et al. (2004).

2.4. PCR amplification of 16S rRNA

DNA from the enrichment culture was extracted using an Ultra Clean microbial DNA isolation kit (MO BIO Labs., Inc., Solana Beach, CA), following the manufacturer's directions, and purified using a DNA purification JetQuick kit (Genomed). The sequences obtained (about 1453 nt) were compared to those available in the GenBank database (National Center for Biotechnology Information) using the Basic Local Aligment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast/) algorithm to identify known sequences with high similarity.

The selected sequences were aligned with CLUSTAL X (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997). The bacteria isolated from the EBA60 samples and identified were *B. subtilis*, *B. borstelensis*, *B. licheniformis*. A mixture of the three identified bacterial strains, noted herein as MIX, was used for the biodegradation studies.

2.5. Bioassay procedure and indirect impedance technique

Aerobic biodegradation of film samples by bacteria were conducted at 45 °C in bioreactors of 7-ml of capacity filled with 1 g of sterile silica and 1.5 ml of bacterial suspension in minimal growth medium of 2.5×10^7 cells/ml concentration, prepared as described in Abrusci et al. (2011). After that, discs of film samples (4 mg) were added to the medium. These containers were introduced in 20-ml disposable cylindrical cells charged with 1.5 ml of 2 g/l KOH aqueous solution and provided with electrodes to measure impedance on a Bac-Trac 4300 (SY-LAB Geräte GmbH, Neupurkerdorf, Austria). The device monitors the relative change in the initial impedance value of KOH solution which is converted to concentration of carbon dioxide by a calibration curve of impedance variation versus