



Characterization of polyhydroxyalkanoates synthesized from microbial mixed cultures and of their nanobiocomposites with bacterial

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The present work reports on the production and characterization of polyhydroxyalkanoates (PHAs) with different valerate contents, which were synthesized from microbial mixed cultures, and the subsequent development of nanocomposites incorporating bacterial cellulose nanowhiskers (BCNW) via solution casting processing. The characterization of the pure biopolyesters showed that the properties of PHAs may be strongly modified by varying the valerate ratio in the poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) copolymer, as expected. Increasing the valerate content was seen to greatly decrease the melting temperature and enthalpy of the material, as well as its rigidity and stiffness, resulting in a more ductile behaviour. Additionally, the higher valerate PHA displayed higher permeability to water and oxygen and higher moisture sensitivity. Subsequently, BCNW were incorporated into both PHA grades, achieving a high level of dispersion for a 1 wt.-% loading, whereas some agglomeration took place for 3 wt.-% BCNW. As evidenced by DSC analyses, BCNW presented a nucleating effect on the PHA matrices. BCNW also increased the thermal stability of the polymeric matrices when properly dispersed due to strong matrix–filler interactions. Barrier properties were seen to depend on relative humidity and improved at low nanofiller loadings and low relative humidity.

Introduction

Over the past decades, as a result of the growing environmental awareness, a great interest has been focused on the development and optimization of sustainable biodegradable polymeric materials produced from renewable resources, especially within the packaging applications field. Among the wide variety of biopolymers which have been developed and studied during the past years, biopolyesters such as polylactic acid (PLA) and polyhydroxyalkanoates (PHAs) have attracted special interest since they are synthesized from renewable resources and present promising properties.

PHAs are a family of biopolyesters produced by a wide variety of bacteria as carbon and energy storage materials [1]. Within this range of materials, the homopolymer poly(3-hydroxybutyrate) (P3HB) has been more extensively studied since it presents mechanical properties similar to those of conventional petroleum-based polymers. In addition, this material possesses a relatively high melting and glass transition temperature [2,3], as well as great stiffness since it possesses a relatively high crystallinity [4]. Nevertheless, it presents some drawbacks, such as excessive brittleness and low thermal stability, making it unstable during melt processing and limiting its applicability. To overcome these issues, two main approaches have been typically developed. The first one consists in blending PHB with other polymers such as poly(vinyl

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alcohol) (PVA) [5], poly(methyl acrylate) (PMA) [6] or poly(ethylene oxide) (PEO) [7]. In that case, the second polymer must be miscible with PHB and it should be preferably biodegradable. As an alternative, the properties of the homopolymer can be modified by incorporating different monomer types during bacterial fermentation. Copolymers of hydroxybutyrate (HB) with hydroxyvalerate (HV), that is poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), present lower crystallinity for HV contents up to 40–50 mol% [8–10] and therefore, decreased stiffness and brittleness. Additionally, the incorporation of HV units results in reduced melting temperature without reducing the thermal stability of the material [11,12], hence widening the processing window of the material. By contrast, a decrease on the crystallinity is known to impair the barrier properties of polymeric materials [13], which might be a disadvantage for the use of PHBV in packaging applications.

The industrial production of PHAs has been typically done by using pure microbial cultures. Nevertheless, this process involves high operational costs which account for nearly 11% of total production costs [14]. The use of expensive pure substrates and the requirement of sterile conditions and extensive reactor maintenance are the main causes for these high operational costs. During the last years, extensive research has been carried out to decrease costs by increasing the production volumes and using cheaper substrates. In this context, the use of microbial mixed cultures is an interesting alternative. The selection of microorganisms for microbial mixed cultures is done on the basis of their high capacity for PHA storage. This is done by imposing alternate carbon substrate availability conditions (also known as feast and famine conditions). Since several PHA producing microorganisms which can adapt to changes in the substrate are selected, it is possible to use cheap mixed substrates or even wastewaters. In addition, large scale fermentations may be produced without the need for sterile conditions.

The incorporation of nanoparticles, such as nanoclays and cellulose nanocrystals, is an efficient strategy to tailor the properties of polymeric materials and, consequently, many works have already reported on the production of PHBV nanocomposites [15–19]. The reinforcement of PHBV with cellulose nanowhiskers (CNW) may present particular interest since it would allow the development of fully biodegradable nanocomposites produced from renewable resources. CNW are typically produced by applying an acid hydrolysis process which leads to preferential digestion of cellulose amorphous domains. The native cellulose is, in most cases, extracted from vegetal resources, such as cotton, flax or hemp, since it is the major cell wall component. However, it can also be extracted from algae and marine animals, such as tunicin, as well as synthesized by some bacterial species, such as *Gluconacetobacter xylinus*. These bacteria are able to produce a layer of nearly pure bacterial cellulose (BC) in the liquid/air interface in a culture medium rich in polysaccharides. Bacterial cellulose presents several advantages over other cellulosic resources, such as its high purity and crystallinity, resulting in bacterial cellulose nanowhiskers (BCNW) with a highly crystalline structure [20] and high aspect ratio [20–22].

To the best of our knowledge, there are no previous works which report on the use of BCNW as nanofiller in PHBV nanocomposites. In addition, there is very scarce literature on the characterization of PHBV grades produced by microbial mixed cultures. The mechanical properties of several PHBV grades with HV contents

ranging from 12 mol% to 72 mol% have been very recently evaluated [23], however the barrier properties of these materials have not been reported previously. However, the reinforcement of commercial PHBV grades with plant-derived CNW has been already reported in several studies [15,17–19]. Due to the highly hydrophilic character of cellulose nanocrystals, they present low compatibility with PHAs and, therefore, attempts to prepare nanocomposites by melt compounding resulted in high levels of nanofiller agglomeration [15]. In contrast, it was possible to attain a good level of dispersion for nanocomposites prepared by solution casting with loadings of up to 2.3 wt.-% CNW [17] and up to 5 wt.-% CNW containing ca. 30 wt.-% PEG as compatibilizer [15] and the incorporation of CNW gave rise to improved mechanical properties. Nonetheless, these previous works focused on the mechanical performance of nanocomposites and did not investigate the effect on barrier properties.

The present work reports on the production and characterization of PHA nanocomposites incorporating BCNW. PHAs with two different valerate contents were produced by microbial mixed cultures and nanocomposites were generated by solution casting. The effects of both valerate content and BCNW addition on the morphology, thermal, mechanical and barrier properties were investigated throughout this work. These materials could have potential interest in fully biobased higher barrier PHA-based food packaging applications.

Materials and methods

Materials

Two different PHA grades, with different valerate (HV) contents, were used throughout this study. PHBV7 (7.3 mol% valerate content) and PHBV40 (40.3 mol% valerate content) were synthesized by using mixed microbial cultures as specified below.

Sulphuric acid 96%, acetone 99.5% and chloroform 99% were purchased from Panreac (Barcelona, Spain).

Synthesis of PHAs

PHAs were produced by using a lab-scale multi-stage process, consisting of a sequencing batch reactor (SBR), a PHA-accumulating reactor, and a final polymer extraction stage. The SBR operated under “feast and famine” conditions to select mixed microbial cultures in microorganisms with high storage ability. This enriched microbial consortium was fed with synthetic volatile fatty acid (VFA) mixtures in a fed batch reactor to produce PHA.

For the production of low valerate PHA, the SBR was fed with a mixture of acetic (0.87 C mol/C mol VFA) and propionic (0.13 C mol/C mol VFA) acid at an organic load rate (OLR) of 260 C mmol/L d. The length of the SBR cycle was six hours, each cycle consisting of an initial feed phase (10 min), a first reaction phase (70 min), a withdrawal phase of the mixed liquor (1 min), and a second reaction phase (279 min). All the other SBR operating conditions were as described elsewhere [24]. The biomass discharged from the SBR was sent to the PHA-accumulating reactor (0.3 L working volume), operated in batch mode with each batch lasting six hours. Simultaneously with the discharge of the biomass from the SBR, the batch reactor was fed for 30 min with acetic and propionic acid (with same composition as above) at an overall OLR of 890 C mmol/L d. This very high OLR, along with the lack of nitrogen, were used as a tool to enhance PHA accumulation.

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