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Biodegradation of poly(butylene succinate) film by compost microorganisms and water soluble product impact on mung beans germination

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ABSTRACT

The biodegradation of poly(butylene succinate) (PBS) films by compost microorganisms were studied for insight into the composition of water soluble products and their effect on the surroundings, including medium pH, microorganism viability and mung beans germination. After degradation by compost microorganisms adhering to the film surface, PBS films showed a lot of cracks and holes, accompanied by a continuous decrease of remaining mass and molecular weight. In the initial two weeks, the water soluble products acidified the medium from pH7.2 to pH5.2 and inhibited the microbe growth. With the assimilation of these products as carbon sources by microorganisms, the medium pH value gradually returned to neutral and the microbes fast proliferated as well. The ultra performance liquid chromatography-mass spectrum analysis confirmed that the water soluble products were composed of 1,4-butanediol (B), succinic acid (S), and their oligomers BS, BSB, SBS, BSBS, BSBSB and SBSBS, whose contents depended on their production, microbial assimilation and instability. The germination test revealed that the water soluble products themselves were little hazardous to mung beans, but the acidified medium significantly inhibited the mung beans germination. Fortunately, PBS-degrading microorganisms were able to recover the safe neutral environment by assimilating these acid products.

1. Introduction

The extensive use of non-degradable plastics such as polyolefins has produced lots of wastes and caused severe environmental pollutions [1–3]. The traditional solutions to these wastes, including landfill, incineration or recycling, show limits due to occupation of lands, release of poisonous gases, or high recycling cost [4]. For this reason, a lot of biodegradable polymers, especially synthetic aliphatic polyesters capable of degradation by environmental microorganisms, e.g. poly(lactic acid) (PLA), poly(3-hydroxybutyrate) (PHB), poly(3-hydroxylbutyrate-co-3-hydroxyvalerate) (PHBV), poly(ε-caprolactone) (PCL), poly(-butylene succinate) (PBS) and its copolymer poly(butylene succinate-co-butylene adipate) (PBSA), have been developed and widely studied [5–10]. The biodegradation of aliphatic polyesters is a complex biological process, involving three phases: biodeterioration phase where microorganisms adhere to the polymer surface, biofragmentation phase where polymers are degraded into small water soluble fragments by extracellular enzymes secreted by microorganisms, and assimilation phase where the water soluble fragments are assimilated by microorganisms producing carbon dioxide, water and biomass [11,12].

Essentially, the biodegradability of aliphatic polyesters is initiated by the hydrolysis of ester bonds, leading to the formation of small water soluble fragments (less than 500 in molecular weight) that may be assimilated by microorganisms and finally changed into eco-friendly products, i.e. carbon dioxide, water and biomass [13,14]. From final products of view, it is doubtless that these aliphatic polyesters are environmentally friendly, but it is worthwhile to note that those intermediate water soluble products, mainly composed of acids and alcohols, are in risk of acidifying the

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surroundings when diffusing into the environment. Thus, although the routine characterizations on surface morphology, molecular weight, weight loss, mechanical property et al., may reflect the existence of polymer degradation, the composition and microbial assimilation of water soluble products as well as their impact on the surroundings are necessary for in-depth understanding the real microbial degradation process and evaluating the eco-friendship of biopolymers.

Despite the microbial degradation of biopolymers in soil or compost may exhibit their real biodegradability in nature, the complex biological environment of soil or compost makes it impossible for the collection and analysis of water soluble products. Thereby, the biodegradation of biopolymers is often carried out by exposing biopolymers to an enzyme solution, followed by isolation and identification of the water soluble products using advanced analytic techniques, such as high efficiency liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) et al. [15,16]. However, the enzymatic degradation of biopolymers cannot reveal the real biodegradation process in the absence of microorganisms for lack of microbial assimilation, thus we have no ideas what will happen to the water soluble products and the surroundings during the degradation. This challenge moves us to provide an insight into the degradation of biopolymers by environmental microorganisms, the evolution of water soluble products and their impact on the surroundings, such as medium pH, microorganism viability and plant growth, in a microbial suspension for mimicking the naturally microbial degradation to the most degree.

In this work, PBS film, from the condensation polymerization of 1,4-butanediol (B) and succinic acid (S), was chosen as the biodegradation sample because PBS has been recently regarded as the most competitive substituent of conventional plastics for its desirable biodegradability, low production cost, good mechanical properties similar to polypropylene, and easy processing like polyolefins [17–21]. The degrading microbes were selected from compost microorganisms, considering that compost, a mixture of various decaying organic substances with a high microbial diversity, is a good mimetic natural environment. The biodegradation of PBS film was performed in a microbial suspension. Besides the routine characterizations on surface morphology, mass loss, molecular weight and thermal property of PBS films during the microbial degradation, the composition of water soluble products and their effect on the medium pH, microorganism viability as well as the mung bean sprout growth were focused on.

2. Materials and methods

2.1. Materials

Dihydroxyl terminated PBS ($M_n = 6.0 \times 10^4$ Da) was provided by Shanghai Showa Highpolymer Co., LTD (China). The compost microorganism inocula (containing *Aspergillus*, *Bacillus*, *Penicillium* and *Thermopolyspora*) were provide by Nongguan Biology Co.Ltd (China). The basal medium (pH 7.2) containing 1.0 g $(NH_4)_2SO_4$, 0.2 g KH_2PO_4, 1.6 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.02 g $CaCl_2 \cdot 2H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.5 mg $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg $Na_2WO_4 \cdot 2H_2O$, and 0.5 mg $MnSO_4$ in one liter distilled water, was prepared following our previous work [10]. Mung beans were purchased from the market for the germination experiment. All other reagents and solvents used were of analytical grade.

2.2. Preparation of PBS films

PBS film was prepared by a casting method. Briefly, a 0.03 g ml^{-1} PBS solution in chloroform was casted in a Petri dish. The solvent was allowed to slowly evaporate at ambient temperature. After being dried at vacuum (0.5 mmHg), PBS film with a thickness of about 200 μm was obtained. Before use, the film was cut into 1 cm × 1 cm pieces.

2.3. Biodegradation of PBS film by compost microorganisms

The microorganisms were dispersed in 100 ml basal medium to get a suspension of 2.8×10^7 microorganisms ml^{-1}. About 300 mg PBS films sterilized by 75% (v/v) ethanol aqueous solution were added to this microbial suspension and incubated on a rotary shaker (130 rpm) at 30 °C for up to 10 weeks. The degradation of PBS film without microorganisms was performed as the control. The degradations of PBS film with and without microorganisms were defined as biotic and abiotic degradation respectively.

2.4. Scanning electron microscopy (SEM) observation

The surface morphologies of PBS film during the degradation were characterized by a field emission scanning electron microscope (Ultra™55 FESEM, Zeiss, Germany) using an accelerating voltage of 5 kV. At desired intervals, the films were ultrasonically washed at a low frequency of 50 Hz for 2 min to remove the possible residues of microorganism metabolites on the film surface, meanwhile the washout of adhered PBS-degrading microorganisms was avoided. Before observation, a gold layer was coated on the specimen surface by a sputtering device (BAL-TEC, SCD005, Finland).

2.5. Weight loss analysis

The weight loss percentage of PBS film during degradation was measured gravimetrically. At desired intervals, PBS film was taken out from the medium and thoroughly washed with distilled water, followed by drying to constant weight under vacuum (0.5 mmHg) at 50 °C in an oven. However, the weight of residue film may not be directly measured because the microorganisms tightly anchored to the film surface was unable to be completely removed by rinses. For this reason, the dry specimen were dissolved in chloroform and filtered by a 0.22 μm filter to remove the residue microorganisms. Then, the filtrate was dried to constant weight again, representing the weight of residue PBS film after degradation. The weight loss percentage was evaluated by the following equation:

$$\text{Weight loss}(\%) = \frac{(W_0 - W_t)}{W_0} \times 100\% \quad (1)$$

Where W_t was the residue weight of PBS film at t time, and W_0 was the original weight of PBS film before degradation.

2.6. Number-average molecular weight analysis

The number-average molecular weights of PBS film during the degradation were characterized by proton nuclear magnetic resonance (^1H NMR) in a 500 MHz Bruker AVANCE III (Bruker, Germany) spectrometer at room temperature using $CDCl_3$ as the solvent and tetramethylsilane as the internal standard.

2.7. Thermal property analysis

The thermal properties of PBS film during the degradation were studied by differential scanning calorimetry (DSC) (NETZSCH DSC 204F1 Phoenix, Germany) in a temperature range of 25 °C–150 °C using a heating or cooling rate of 10 °C min^{-1} and a nitrogen gas flow of 50 ml min^{-1}. The first run was used to erase any thermal