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Biodegradation of Levan Polymer / Poly (Lactic Acid) (PLA) Blend

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Abstract. Poly (lactic acid) (PLA) is a biodegradable thermoplastic. With low elongation and poor degradation properties, many approaches have been developed to overcome these limitations, including copolymerization, blending and plasticizing to improve the mechanical properties. Levans are a group of fructan polymer with act as a good co-polymer, which can be produced by levansucrase using sucrose as substrate. In this study, co-solvent for levan/PLA blend film preparation, thermal properties and biodegradation of levan/PLA blend film were investigated. The result found that DMSO and CH₂Cl₂ were suitable for the co-solvent process. The Levan/PLA blend films were prepared with levan contents of 0, 0.5, 1, 2, 5, 10, 15 and 20 wt%. The biodegradation of levan/PLA blend film *in vitro* and in soil conditions showed that 15 wt% levan/PLA at 7 days and 10 wt% levan/PLA at 21 days were the highest weight loss, respectively. In addition, the morphological changes in the Levan/PLA blend film and thermal properties were analyzed by Scanning electron microscope (SEM) and Differential scanning calorimetry (DSC). The results from DSC present that the melting temperature of PLA was slightly decreased with increasing levan contents. Moreover, the holes from degradation were larger and the rougher surfaces were observed with levan contents.

1. Introduction

In recent years, there has been using biodegradable polymers from renewable resources in many fields such as medicine, agriculture, and packaging industry [1]. With environmentally friendly materials, they have been interesting to develop morphological, thermal and biodegradability properties. Poly (lactic acid) (PLA) is a biodegradable thermoplastic polymer, which can be obtained from 100% natural sources such as corn, tapioca, as well as from petrochemical resources [2]. PLA doesn't have good properties, especially low elongation, poor hydrophilicity, complicated processes for preparing high molecular-weight polymer and high price [3-6]. Copolymerization, blending and plasticizing methods can be helping to improve these poor properties of PLA [7-8]. Levans are composed of D-fructose units joined together by a β -(2, 6) glycosidic linkage in the main chain with β -(2, 1) linkage at branch points, which can be produced from plants and microorganisms. A superior thermal resistance with the high melting temperature (223°C) and non-toxicity, it has been using in many applications such as food, medicine and nanotechnology [9-12].



In this work presented herein focuses on a co-solvent for levan/PLA blend film preparation, thermal properties and biodegradation of levan/PLA blend film. Biodegradation was studied *in vitro* and in soil conditions. Morphology and thermal properties were determined by Scanning electron microscope (SEM) and Differential scanning calorimetry (DSC).

2. Experimental

2.1. Materials

Sucrose was used as a substrate for the levan production, purchased from Ajax Finechem Pty Ltd., Australia. *E. coli* BL-21 was used as an expression host and pETLSKK9 containing levansucrase gene (lsKK9) of *B. licheniformis* KK9 for levansucrase production. Glucose was used for levansucrase inducing, purchased from APS Finechem, Australia. IPTG was used as a promotor for levansucrase expression, purchased from Sigma-ALORICH, Switzerland. PLA was obtained from BC polymers marketing, Co., Ltd. Luria-bertani broth contain peptone, yeast extract and sodium chloride, purchased from Himedia Laboratories, BD, USA and Sigma-ALORICH, Switzerland, respectively. Other reagents were used to co-solvent for Lev/PLA blend production.

2.2. Levansucrase Activity Assay

The activity of levansucrase was measured by quantifying the reducing ability of free glucose from the reaction by the modified DNS method [13]. One unit was defined as the amount of levansucrase that produces 1 μ mole of reducing sugar (glucose equivalent) per min.

2.3. Levan production

The reaction mixture containing 8.75 % w/v sucrose, 3 U/mL levansucrase in 25 mM citrate buffer pH 6.0 and incubated at 37 °C, 150 rpm for 16-18 h. after that the levan was precipitated in acetone. The levan was filtered, dialyzed in water and lyophilized.

2.4. Levan/poly (lactic acid) blend preparation.

Solvents for levan and poly (lactic acid) dissolving were studied using DI water, acetone, dichloromethane and dimethyl sulfoxide. The optimum solvents for levan and PLA dissolving were used as co-solvent for Levan/poly (lactic acid) blend preparation. Polymer blend of levan and PLA were prepared. Levan/poly (lactic acid) blend were prepared at various levan/PLA mass ratios (0, 0.5, 1, 2, 5, 10, 15 and 20 wt%) using co-solvent for dissolving. Then place in the compression mold machine at 50 °C until the levan/poly (lactic acid) blend was molded. After that, cut the sample size 1x1 cm to study the biodegradation.

2.5. Biodegradation of Polymers via *in vitro* condition.

The polymer blend was degraded with *Penicillium sp.* and *Pseudomonas aeruginosa*. Preparation of *Penicillium sp.* and *Pseudomonas aeruginosa* for the biodegradation were cultured in medium containing 0.25 g yeast extract, 0.5 g (NH₄)₂SO₄, 0.5 g KH₂PO₄, 0.8 g K₂HPO₄, 0.1 g MgSO₄·7H₂O, 0.05 g NaCl, 0.01g CaCl₂·2H₂O, 0.005 g FeSO₄·7H₂O, 0.25 mg Na₂MoO₄·2H₂O and 0.25 mg MnSO₄ in 500 mL DI water at 37 °C, 250 rpm for 16-18 h. Dropping 5 μ L of 5 mL culture medium on polymer blend samples in tubes and incubated at 37 °C, 250 rpm for 3, 5, 7, 14 and 21 days. The polymer blend samples were measured weight loss and captured surface.

2.6. Biodegradation of Polymers: *in soil* condition.

The polymer blend was placed and filled soil in a porous plastic box. Embedding and watering a porous plastic box 1 time per day for 21 days. The polymer blend samples were measured weight loss and captured surface.

3. Results and discussion

3.1. Optimum solvents for the levan / poly (lactic acid) blend preparation

Co-solvent for the levan / poly (lactic acid) blend was investigated. Dimethyl sulfoxide (DMSO) and dichloromethane (CH_2Cl_2) showed a great levan/poly (lactic acid) dissolving property. These results indicate that optimum dissolution of levan in DMSO was 11.4 % and PLA in CH_2Cl_2 was 20% w/v. Further, both types of solvent mix very well and homogeneously.

3.2. Levan /poly (lactic acid) blend preparation

The mass ratios of levan/PLA (0, 0.5, 1, 2, 5, 10, 15 and 20 wt%) were investigated. At 0 wt% levan, the PLA polymer was transparent but other mass ratios (0.5, 1, 2, 5, 10, 15 and 20 wt%) showed that the levan/PLA blends were not transparent.

3.3. Biodegradation via in vitro condition

The results from the biodegradation revealed that the holes from biodegradation were observed after 3 days on 15 and 20 wt% levan in PLA. While the other sample ratios were observed the holes in several days. Except the pure PLA sample, the surface did not biodegrade in 21 days. %Weight loss showed that at 0, 0.5, 1 and 2 wt% levan in PLA had the weight loss in range 0 – 15 wt% in 21 days, which compared with 10, 15 and 20 wt% levan in PLA had more increasing trend in range 25 – 60 wt% in 7 days and decreasing trend in range 20 – 50 wt% after 7 days (Figure 1). The highest weight loss of levan/PLA was 56.55 % at 15 wt% levan in 7 days biodegradation test. However, the weight loss of levan/PLA blend via *in vitro* condition may cause be swelling and hydrolysis from water in solution.

3.4. Biodegradation in soil condition

The results from the biodegradation showed that the holes from biodegradation were clearly observed on 10, 15 and 20 wt% levan in PLA. While the other sample ratios were observed the slightly holes. %Weight loss indicated that at 0, 0.5, 1 and 2 wt% levan in PLA had the weight loss in range 0 – 8 wt% in 21 days, which compared with 10, 15 and 20 wt% levan in PLA had more increasing trend in range 10 – 45 wt% in 21 days (Figure 2). The highest weight loss of levan/PLA was 41.86 % at 10 wt% levan in 21 days of biodegradation test. Previously report, the biodegradation rate of P(3HB, 4HB)/PLA have highest increasing in 5 months [14]. These results indicated that the levan contents act as a key to biodegradability of PLA via *in vitro* and soil conditions.

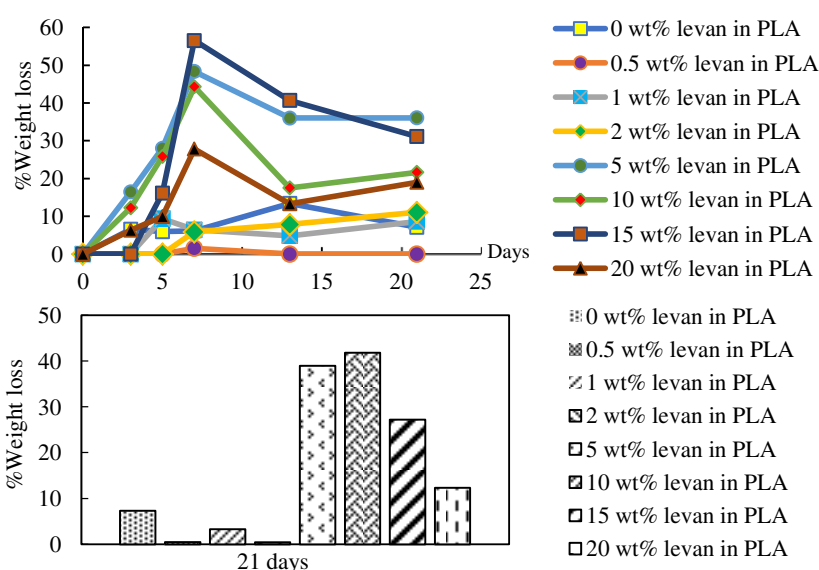


Figure 1. %Weight loss of levan/PLA blend from biodegradation via in vitro condition

Figure 2. %Weight loss of levan/PLA blend from biodegradation in soil condition for 21 days

3.5. Effects of levan on thermal properties of levan/PLA blend

The results from Differential Scanning Calorimetry (DSC) presented that the melting temperature of levan/PLA blend was slightly decreased from 154.8 to 154.5 °C. This mean there was no significant effect of levan on melting behavior. But the levan content showed the area under a graph of melting point (T_m) less than the PLA without levan. This result indicated that the levan/PLA blend had less crystalline and more amorphous than PLA without levan. According to crystallization temperature (T_c), which showed that the levan/PLA blend had slightly decreased crystalline when prepared with the PLA without levan, resulting in an easy biodegradation.

4. Conclusion

The biodegradation of levan/PLA blend was investigated. It was found that newly prepared polymer blend for the fast biodegradation in both *in vitro* and soil conditions. The biodegradation showed that the holes from biodegradation were clearly observed. At 15 wt% levan in PLA had highest degradation was 56.55 % via *in vitro* condition in 7 days. In part of soil condition, at 10 wt% levan in PLA had highest degradation was 41.86 % in 21 days. DSC measurements revealed the melting temperature of levan/PLA blend was slightly decreased from 154.8 to 154.5 °C. However, the levan/PLA blend still need to be improved to obtain efficiently in application.

5. References

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