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Accelerating biodegradation of PLA using microbial consortium from dairy wastewater sludge combined with PLA-degrading bacterium

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ABSTRACT

This work mainly aims to develop an efficient method for accelerating degradation of commercial polylactic acid beverage cups (cPLA). Ultraviolet (UV) irradiation was used to reduce the molecular weight of cPLA. The cPLA sheets were buried in soil amended with various sources of microbial consortium, including cow-manure compost, green yard-waste compost, and wastewater sludges from dairy, rice vermicelli and coconut milk factories. Interestingly, a complete degradation of cPLA sheets was found after 15 days of burial in the soil with dairy wastewater sludge, having high total nitrogen content. The dominant bacterial genus in the soil mixture of agricultural soil and dairy wastewater sludge was *Actinomadura*. In addition, there were the significant increases in the percentages of biodegradation and weight loss of cPLA and neat PLA (nPLA) in the soil with dairy wastewater sludge and *Pseudomonas geniculata* WS3, compared to the uninoculated control, indicating a synergistic action of the microbial consortium in sludge and *P. geniculata* WS3 on PLA biodegradability. The cPLA was more difficult to degrade than nPLA. In conclusion, UV-C irradiation of PLA followed by burying in a soil mixture of dairy wastewater sludge with *P. geniculata* WS3 inoculation is an efficient method for accelerating degradation of PLA waste.

1. Introduction

Petroleum-based plastics are commonly used worldwide for packaging, e.g., food, beverage, cosmetics, pharmaceutical products, accounting for approximately 30–40% of all petroleum-based plastics (Ghosh et al., 2013). The extensive production and use of petroleum-based plastics poses a significant threat to the environment because they degrade slowly and accumulate in the environment, posing an increasing ecological threat to terrestrial and marine wild life (Siven, 2011; Emadian et al., 2017). Ideally, packaging must be resistant during use and have biodegradable properties at the end of their useful life. Among several types of biodegradable plastics, polylactic acid (PLA) has received much attention as a green material because it derives from renewable resources, e.g., corn and cassava, and it has excellent properties, e.g., high mechanical strength, high modulus, and transparency (Qi et al., 2017). Recently, PLA production has increased worldwide, in particular for use in food packaging containers and films, resulting in greater PLA waste contamination in the environment (Karamanlioglu et al., 2017).

However, complete degradation of PLA in natural environments may take up to several years (Qi et al., 2017). Tokiwa and Jarerat (2004) reported the rate of bioplastic degradation in a landfill, in descending order, as follows: Polyhydroxybutyrate (PHB) = Polycaprolactone (PCL) > Polybutylene succinate (PBS) > PLA. Therefore, it is desirable to develop better treatment methods for degrading used PLA packaging. The degradation rate of polymers depends on their molecular weight. Polymers with high molecular weights had slower degradation rate than those with low molecular weights (Tokiwa and Calabia, 2006). The biodegradability of polymers can be changed by exposure to abiotic factors (e.g., mechanical, light, thermal, and chemicals) and these factors are useful for initiating the biodegradation process (Lucas et al., 2008). Ultraviolet (UV) irradiation at a wavelength of 254 nm (UV-C) can break the long PLA chains and reduce the average molecular weight of PLA (Jeon and Kim, 2013). The exposure of PLA waste to UV-C radiation prior to burial under soil should increase the rate of PLA degradation.

The biodegradability of PLA in the soil takes a long time compared to other biodegradable polyesters because PLA is resistant to microbial attack and the limited distribution of PLA-degrading microorganisms in the soil (Pranamuda et al., 1997; Ohkita and Lee, 2006; Tokiwa and Calabia, 2006). Therefore, the addition of a microbial consortium in the soil might help to speed up PLA biodegradation. When composts from

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organic waste and dairy manure were used as a microbial inoculum for testing of PLA degradation, it was found that PLA can be degraded through composting under aerobic conditions (Itävaara et al., 2002; Ahn et al., 2011). Yagi et al. (2014) reported that PLA biodegradation rate in anaerobic sludge at 37 °C was very slow because only smaller molecular weight PLA, caused by hydrolysis, was degraded by the microorganisms in the sludge. Isolation and screening of PLA-degrading microorganisms, including actinomycetes, bacteria and fungi have been performed by several investigators (Karamanlioglu et al., 2014; Penkhrue et al., 2015; Satti et al., 2017). However, studies of PLA-degrading microorganisms have been mainly conducted in controlled laboratory conditions (Qi et al., 2017). A few researchers have studied the acceleration of PLA biodegradation by inoculating a pure culture of aerobic degrading microorganisms, e.g., bacterium (Jeon and Kim, 2013), actinomycetes (Apinya et al., 2015), in the compost or the soil. Qi et al. (2017) stated that future research of PLA biodegradation should change from the use of a single culture system to a co-culture system under aerobic condition because most PLA-degrading microorganisms are aerobic microorganisms.

Our study aims to establish an efficient simulated method of PLA degradation based on UV radiation and a combined use of a specific microbial consortium and a PLA-degrading bacterium under aerobic condition. We hypotised that UV irradiation combining to the inoculation of PLA-degrading bacteria and consortia from composts or wastewater sludges might significantly enhance the PLA biodegradation process in soil. Therefore, the effect of UV irradiation on the molecular weight of PLA before soil burial was studied. The addition of a microbial consortium from composts and wastewater sludges in soil to accelerate PLA biodegradation was carried out. In addition, soil inoculation with a specific microbial consortium and a PLA-degrading bacterium, *Pseudomonas geniculata* WS3 to accelerate PLA biodegradation under both mesophilic and thermophilic conditions was investigated. Our findings can be applied for accelerating the elimination of PLA waste in the environment.

2. Materials and methods

2.1. PLA materials, sources of microbial consortium and PLA-degrading bacterium

Two types of PLA materials were used: PLA cool beverage cups (cPLA) and PLA (nPLA) prepared from PLA granules 2003D (Ingeo™, Natureworks LLC, USA). The weight-average molecular weights (M_w) of cPLA and nPLA were 179954 and 168749 Da, respectively. Five sources of microbial consortia were obtained from cow manure compost (CMC), green yard-waste compost (GYC), wastewater sludges from dairy factory (DWS), coconut milk factory (CWS) and rice vermicelli factory (RWS) and were obtained from a local dairy farm, composting facility plant at Mahidol University (Nakhon Pathom, Thailand) and the aerobic wastewater treatment plants from factories located at Nakhon Pathom province, Thailand. *P. geniculata* WS3, a mesophilic PLA-degrading bacterium, was obtained from the Laboratory of Environmental Biotechnology, Faculty of Environment and Resource Studies, Mahidol University (Nakhon Pathom, Thailand). *P. geniculata* WS3 produces protease and PLA-degrading enzyme (Bubpachat et al., 2018).

2.2. UV irradiation of cPLA

Sheets of cPLA were prepared by cutting PLA cool beverage cups into pieces having dimensions of approximately $20 \times 30 \times 1\ mm^3$ (W × L × H). The cPLA sheets were exposed to UV-A (at a wavelength of 340 nm) and UV-B (at a wavelength of 310 nm) for 120 min and exposed to UV-C (at the wavelength of 254 nm) for 0, 90, 120 and 150 min, respectively at room temperature. The number-average molecular weights (M_n) and a weight-average molecular weight (M_w) of cPLA before and after UV-C exposure were determined by gel permeation chromatography (GPC) with a refractive index detector (Waters 2414, USA) (Lucas et al., 2008). GPC column was eluted with tetrahydrofuran at a flow rate of 1.0 mL min^{-1} at 40 °C and polystyrene standards were used for column calibration.

2.3. Effects of microbial consortium on cPLA biodegradation

2.3.1. Preparation and characterization of the soil, composts and wastewater sludges

The agricultural soil, composts (CMC and GYC) and three wastewater sludges (DWS, RWS and CWS) were air-dried at room temperature for 3 weeks. To determine viable bacterial population, the samples were suspended in normal saline and spread on nutrient agar (NA) (Difco, USA). The samples were ground and sieved through a 2-mm sieve before analyses of physical and chemical properties. The soil pH was measured at a ratio of 1:1 (w/v) of soil and deionized water using a pH meter (F2 FiveGO™, Mettler Toledo, Switzerland). Organic matter and total nitrogen contents were analysed using the Walkley-Black titration method (Walkley and Black, 1934) and the Kjeldahl method (Black, 1965), respectively.

2.3.2. Preparation of the soil mixture

The agricultural soil and each source of microbial consortium were mixed at the ratio of 80:20 by weight. The soil mixtures with different microbial consortia were filled in a 500 mL-Duran bottle. The water holding capacity in the soil mixture was adjusted to 40% by adding sterile distilled water.

2.3.3. The experimental set-up of PLA degradation

The UV-treated cPLA sheets were vertically buried in 300 g of the soil mixture of each microbial consortium in a 500-mL Duran glass vessel. Air was supplied to the glass vessel via stainless pipes at a flow rate of 25 mL min^{-1}. The cPLA sheet was placed into a small net to facilitate sample collection due to its excessive disintegration (Shah et al., 2008). Non-UV-treated PLA sheets were used as the control treatment. All glass vessels were placed in a water bath at a temperature of 58 ± 2 °C according to the standard test methods of ASTM D5338-11 (2003). The soil moisture content was maintained at approximately 40% by adding sterile distilled water. Then, cPLA sheets were collected after soil burial for 15, 30 and 45 days, respectively and gently washed with distilled water. After air drying, the appearance of the cPLA sheet was observed. In addition, the cPLA sheets were kept in desiccator for 24 h before weighing. The percentage of cPLA weight loss was calculated. The pH and viable bacterial cells in the soil mixture were measured using pH meter and standard plate count technique, respectively. The percentages of cPLA weight loss, pH and viable bacterial cells were expressed as mean values and standard deviations ($n = 3$). One source of microbial consortium which provided the highest percentage of cPLA weight loss was selected for further experiments.

2.4. Analysis of bacterial community in the soil mixture

The bacterial community in the soil mixture was analysed using 16S amplicon sequencing (Kozich et al., 2013; Walters et al., 2015). Briefly, genomic DNA in the soil mixture was extracted using PowerSoil® DNA Isolation kit (MO BIO Laboratories, USA). PCR amplification was carried out for the 16S rRNA gene with the forward F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′) and reverse R (5′-TCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGG GTATCTAATCC-3′) primers (Walters et al., 2015). The PCR amplification conditions were the initial denaturation at 95 °C for 3 min, followed by 25 cycles of the denaturation step at 95 °C for 30 s, the annealing step at 55 °C for 30 s and the extension step at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The two-side ends of sequencing primers and Illumina sequencing adapters were tagged using a Nextera XT Index Kit (Illumina, USA) before PCR amplification.