Understand Science, Apply Technology,
Create Innovation for Sustainable Society

17-19 OCTOBER 2017
STT43 PROCEEDINGS BOOK

การประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 43 (วทท 43)
เข้าใจวิทยาศาสตร์ เข้าถึงเทคโนโลยี สร้างนวัตกรรม นำสังคมยั่งยืน

PROCEEDINGS BOOK

The 43rd Congress on Science and Technology of Thailand (STT43)

"Understand Science, Apply Technology, Create Innovation for Sustainable Society"

October 17-19, 2017
Venue: Chaloem Rajakumari 60 Building
Chulalongkorn University, Thailand

Organized by:
The Science Society of Thailand under the Patronage of His Majesty the King
In Association with
Chulalongkorn University

E_003_PF: ENHANCED BIODEGRADATION OF POLYLACTIC ACID SHEETS BY ADDITION OF MICROBIAL CONSORTIA

Wisuda Pattanasuttichonlakul[1*], Benjaphorn Prapagdee[1], Narongrit Sombatsompop[2], Achara Ussawarujikulchai[1]
[1] Faculty of Environment and Resource Studies, Mahidol University, Salaya, Nakhonpathom 73170, Thailand
[2] Polymer Processing and Flow (P-PROF) Research Group, Division of Materials Technology, School of Energy, Environment and Materials, King Mongkut's University of Technology Thonburi, Thungkru, Bangkok 10140, Thailand
*e-mail: cobo_new@hotmail.com

Abstract: The purposes of this research study were to study the effect of ultraviolet (UV) treatment on PLA degradation and to evaluate the potential of microbial consortia from organic fertilizers and wastewater treatment sludges on accelerating PLA degradation under soil burial exposure. PLA sheets from commercial PLA cool beverage cups were exposed to UV for 30, 60, 90, 120, and 150 min, respectively and then buried in agricultural soil. The results found that the increasing UV exposure time increased the percentages of PLA weight loss. Moreover, the addition of microbial consortia from organic fertilizers and wastewater treatment sludges for accelerating PLA degradation in soil were studied. The results found that PLA sheets were highly degraded in the soil added with wastewater treatment sludge from dairy factory. The percentage of weight loss reached 100% at 15 days after soil burial. Therefore, wastewater treatment sludge from dairy factory was a good source of microbial consortia for acceleration PLA degradation.

Introduction: Polylactic acid (PLA) is a one type of the bioplastics, most beneficial for the production, eco-friendly for food packaging and can be decomposed into carbon dioxide and water. The lactic acid is derived from renewable resources such as fermented starches and polysaccharides (corn and sugar beets)[1]. PLA packaging has been used widely as a foil wrap, bottles, containers, and PLA films can be produced a shopping bags and food containers[2]. In Thailand, PLA has been started to use in terms of packaging to replace petroleum plastics such as yogurt cup, cold beverage cup which are claimed that it is made from 100% plant. Although PLA is a biodegradable polymer, the complete disappearance of PLA in nature environmental may take several year[3]. Therefore, the treatment and disposal of used PLA packagings have been concerned.

Several methods have been developed to evaluate and quantify biodegradability under different disposal conditions, such as in compost, soil, marine water, wastewater treatment plant and anaerobic digestor[4]. However, Tokiwa and Jarerat (2004)[5] reported that the rate of bioplastics degradation in the landfill for 20 months in descending order as follows: Polyhydroxybutyrate(PHB) = Polycaprolactone (PCL)> Polybutylene succinate(PBS)>PLA. Therefore, this research focused on the study of effect of UV exposure for pretreatment before soil burial on the degradation of commercial PLA cool beverage cups. The performance of microbial consortia from organic fertilizer (cow manure and compost) and wastewater sludges (from diary factory, rice vermicelli factory and coconut factory) on the accelerating PLA degradation under soil burial exposure. These findings should be further applied for treatment of commercial PLA plastic waste.

Methodology

Study of the effect of UV treatment on the degradation of the degradation of PLA sheets: PLA sheets were prepared by cutting from commercial PLA cool beverage cups at the size of (2×3 cm). PLA sheets were exposed to UV-C (wavelength 253.7 nm) lamp for 0, 30, 60, 90, 120, 150 min, respectively. The molecular weights of PLA sheet before and after UV treatment for 120 and 150 min were analysed using Gel Permeation Chromatography (GPC). After that, the UV-treated PLA sheets were buried in the 300 g of agricultural soil in 500 mL-Duran bottle at 58 ± 2°C, 40% of moisture content and air flow rate of 25 mL/min [6] (Figure 1). Non-UV-treated PLA sheets were used as the control treatment. Then, PLA sheets were collected after soil burial for 5, 10 and 15 days, respectively and gently washed with distilled water. After air drying, the appearance of the PLA sheet was observed. In addition, PLA sheets were kept in desiccator for 24 h before weighing. The percentage of PLA weight loss was calculated by following equation (Eq.1)[7]. The optimum time of UV exposure which provided the highest the percentage of PLA weight loss PLA will be selected for test in the further experiments.

$$\%\ \text{PLA weight loss} = \frac{W\ (before) - W\ (after)}{W\ (before)} \times 100 \qquad \text{(Eq.1)}$$

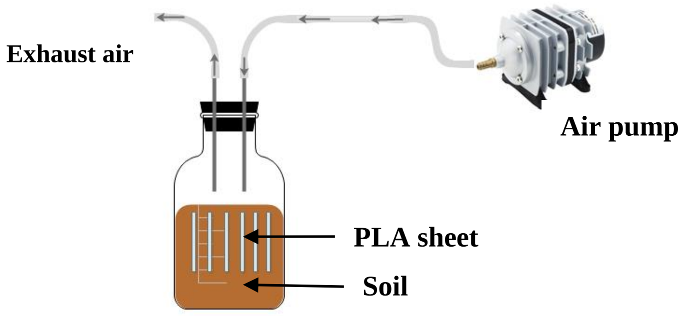


Figure 1 PLA biodegradation in glass vessel containing the soil mixture

Study of the efficiency of microbial consortia from different sources on PLA degradation under soil burial: To prepare the soil mixture for soil burial experiment, agricultural soil and each source of microbial consortia, including cow manure, compost, wastewater treatment sludge (2 organic fertilizers and 3 wastewater sludges) were mixed at the ratio of 80:20. The moisture content in the soil mixture was adjusted to 40% by adding sterile distilled water. This experiment was divided into 6 treatments as shown in Table 1. UV-treated PLA sheets (at the optimum time of UV exposure) were buried in each composition of the soil mixtures at $58 \pm 2°C$ under aeration condition. PLA sheets and the soil mixture were randomly collected at 15, 30 and 45 days, respectively. The weights of PLA sheets were measured and calculated the percentage of PLA weight loss using Eq.1. The appearance of PLA sheets was observed. Soil pH was analysed using pH meter. The number of bacteria and fungi in the soil mixture were enumerated by plating on nutrient agar (NA) and potato dextrose agar (PDA).

Table 1 Experimental treatments for the study of the effect of source of microbial consortia on PLA biodegradation

Treatment	Detail
T1	PLA sheet + soil (control)
T2	PLA sheet + cow manure
T3	PLA sheet + compost
T4	PLA sheet + wastewater sludge (dairy factory) or WWD
T5	PLA sheet + wastewater sludge (rice vermicelli factory) or WWR
T6	PLA sheet + wastewater sludge (coconut factory) or WWC

Results and Discussion

Effects of UV exposure on PLA degradation : The molecular weight of PLA sheet without UV treatment was 161436 daltons. After UV treatment for 120 and 150 min, the molecular weights of PLA sheets reduced by 96499 and 81046 daltons, respectively. Figure 2 shows the percentages of weight loss of PLA sheets with UV treatment at exposure times for 0, 30, 60, 90, 120 and 150 min after soil burial for 15 days. The percentages of weight loss of PLA with UV treatment for 120 and 150 min and soil buried for 15 day were significantly higher than of without UV treatment and other UV exposure times. However, the percentages of weight loss of PLA sheets with UV treatment for 120 and 150 min were not significantly different at $p < 0.05$.

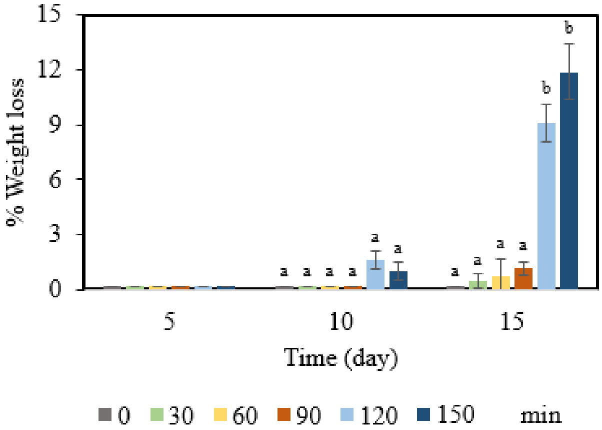
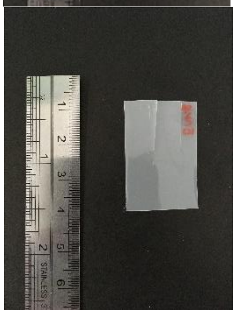
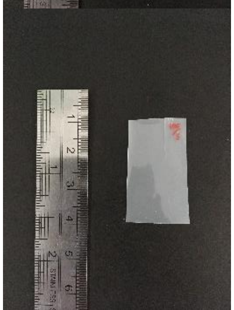
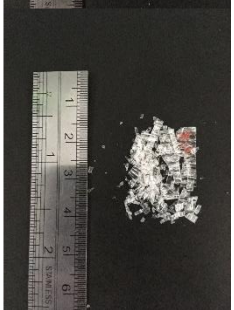
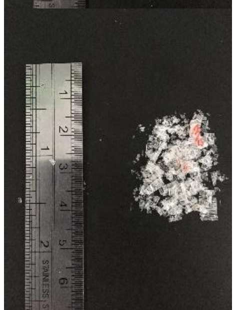
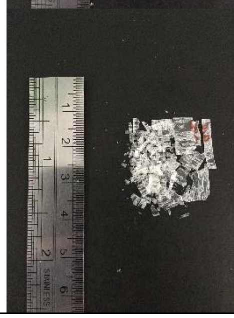
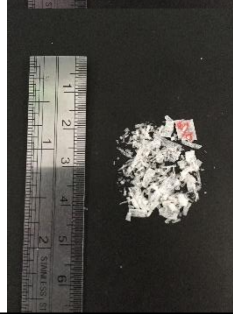


Figure 2 Percentages of weight loss of PLA sheets at various times of UV treatment after soil burial in agriculture soil (A different lowercase letter above a bar in the graph denotes a significant difference at $p < 0.05$ among different treatments.)

Table 2 shows the appearance of PLA sheets with and without UV treatment after soil burial for 15 days. The results found that the color of PLA sheets in all treatments changed from a clear color to white opaque. PLA sheets with UV treatment for 120 and 150 min became more brittle at 10 days. A significant increase in fragmentation of PLA sheets was observed in PLA sheets with UV treatment for 120 and 150 min compared to non-UV-treated PLA. Our findings indicated that the percentages of weight loss increased with increasing UV exposure time and the molecular weight of PLA decreased with increasing UV exposure time. Our results corresponded with the study of Zhang et al. (2013)[8], who reported that UV treatment of compostable PLA decreased the molecular weight and the mass decreased linearly when UV exposure time was increased.

Table 2 The digital photographs of the PLA sheets at various exposure time of UV treatment after soil burial for 15 days

UV exposure time (min)	Time of soil burial (day)		
	5	10	15
0			
30			
90			
120			
150			

Efficiency of microbial consortia on PLA degradation under soil burial condition: Figure 3 shows the percentages of weight loss of PLA sheets with UV treatment for 120 min after soil burial in the mixtures of agricultural soil and each source of microbial consortia, including cow manure, compost, WWD, WWR and WWC at 58 ± 2°C. The results found that the soil added with WWD had the highest percentage of PLA weight loss by 100% of PLA weight loss at the first of 15 days of soil burial. There was no remained PLA fragment in the soil mixture of WWD at 15 days. The pH of the soil mixture of WWD was presented in Table 3. The pH in the soil and the soil mixture were changed from acidic or neutral to alkaline, except the soil mixture of WWR. Interestingly, the pH of the soil mixture of WWD had more alkaline (up to pH 9.0) at 30 days of incubation. Our findings indicated that the alkaline pH should accelerate PLA degradation. Liang et al. (2016)[9] reported the optimum pH of PLA depolymerase enzyme produced from TKU015 was 10. Nakamura et al. (2001)[10] reported that the optimum pH of PLA depolymerase enzyme produced from *Amycolatopsis* sp. ranged from 9.5-10.5 in PLA-emulsified agar plates.

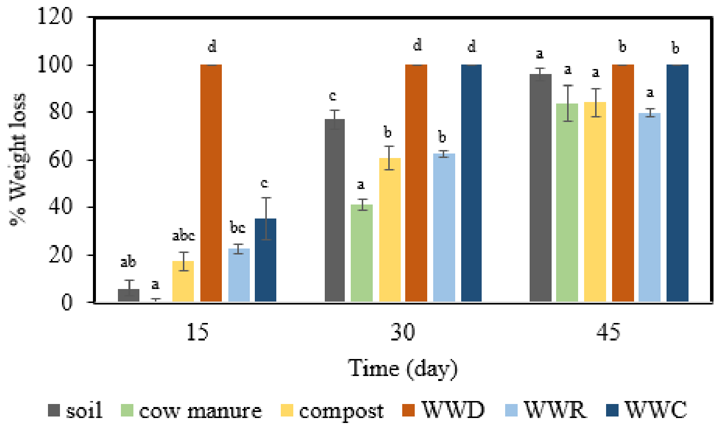


Figure 3 Percentages of weight loss of PLA sheets after UV treatment and soil burial in the soil mixtures with different sources of microbial consortia (A different lowercase letter above a bar in the graph denotes a significant difference at $p < 0.05$ among different treatments.)

Table 3 pH of the soil and soil mixed with each microbial consortium

Treatment	pH at different incubation periods (day)			
	1	15	30	45
Soil (Control)	7.3	7.6	8.1	7.8
Cow manure	7.4	7.3	7.6	7.2
Compost	7.5	7.5	8.1	7.7
WWD	6.6	8.5	9.0	8.8
WWR	6.1	5.6	6.2	5.2
WWC	6.7	8.3	8.7	8.4

Table 4 The digital photographs of the PLA sheets after UV treatment and burial in the soil mixtures with different sources of microbial consortia

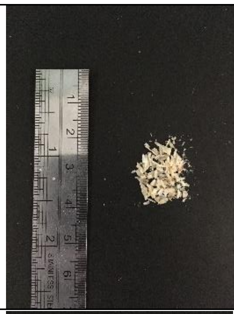
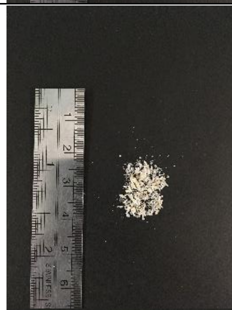
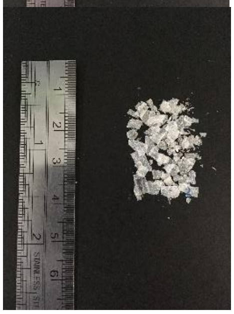
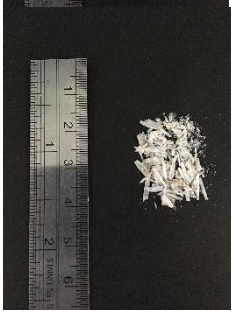
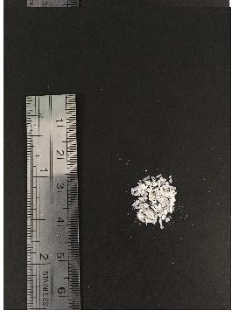
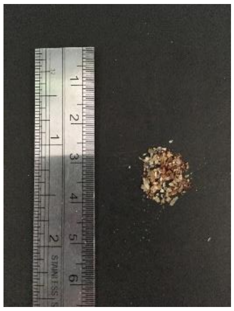
Treatment	Time of soil burial (day)		
	15	30	45
Soil			
Soil with Cow manure			
Soil with compost			
Soil with WWD		Totally degraded	
Soil with WWR			
Soil with WWC		Totally degraded	

Table 4 illustrates the appearance of PLA after soil burial for 45 days. The results found that the change of PLA color from a clear color to white opaque was observed in all treatment at 15 days. More cracks and fragmentations of PLA sheets were also observed since 15 days of soil burial. In particular, PLA sheets in the soil mixture of WWD and in the soil mixture of WWC were totally degraded and no remained PLA sample in the soil mixture at 15 and 30 days, respectively. These findings indicated that WWD and WWC were the good sources of microbial consortia for acceleration PLA degradation. WWD and WWC highly contained protein and lipid, respectively that might be enriched for protease- and lipase-producing bacteria. Protease and lipase enzymes have been reported to be able to degrade PLA[5,11]. However, the number of viable bacterial cells (cultured in NA at pH 7.0) in the soil mixtures during soil burial reduced with times as a result of alkaline (in the soil mixture of WWD and WWC) and acidic (in the soil mixture of WWR) conditions in the soil mixture. These conditions were not suitable for general bacterial growth and survival[12]. In addition, the amounts of fungi in the soil mixture of all wastewater sludges were lower than 100 CFU/g of soil due to alkaline condition and acidic condition. The amounts of fungi in the soil (control), the soil mixture of cow manure and the soil mixture of compost increased with time. Our results suggest that the highly degradation of PLA sheets in the soil mixture of WWD and WWC might involve the group of alkalophilic or alkalotolerant microorganisms that should be further investigated.

Conclusion: The UV treatment on PLA sheets decreased PLA molecular weight and promoted PLA degradation under soil burial exposure at 58 ± 2°C. The optimum time of UV exposure was 120 min. The percentages of PLA weight loss of PLA sheets buried in the soil mixture of WWD and the soil mixture of WWC were 100% at 15 and 30 days of soil burial, respectively. The pH in the soil mixtures of WWD and WWC increased to be alkaline soil. The good source of microbial consortia for accelerating PLA degradation was wastewater treatment sludge from dairy factory, followed by wastewater treatment sludge from coconut factory. It could be concluded that UV treatment and the addition of wastewater treatment sludge from dairy factory is an appropriate method for accelerating PLA degradation under soil burial condition.

References:

1. Luzi F, Fortunati E, Puglia D, Petrucci R, Kenny J, Torre L. Polym. Degrad. Stab. 2015;121: 105-15.
2. Ingrao C, Tricase C, Cholewa-Wójcik A, Kawecka A, Rana R, Siracusa V. Sci. Total Environ. 2015;537:385-98.
3. Jeon HJ, Kim MN. Int. Biodeterior. Biodegrad. 2013;85:289-93.
4. Mohee R, Unmar G, Mudhoo A, Khadoo P. Waste Manage. (Oxford). 2008;28(9):1624-29.
5. Tokiwa Y, Jarerat A. Biotechnol. Lett. 2004;26(10):771-7.
6. Ghorpade VM, Gennadios A, Hanna MA. Bioresour. Technol. 2001;76(1):57-61.
7. American Society for Testing and Materials (ASTM). Standard Test Method for Wood Preservatives by Laboratory Soil-block Cultures. Pennsylvania: ASTM International; 1999.
8. Zhang C, Rathi S, Goddard J, Constantine K, Collins P. J. Emerg. Invest. 2013.1-6.
9. Tzu-Wen Liang, Shan-Ni Jen, Anh Dzung Nguyen and San-Lang Wang. MDPI. 2016:8:98
10. Nakamura K, Tomita T, Abe N, KamioY. Appl. Environ. Microbiol. 2001.67:345-53.
11. Williams D. Enzymic hydrolysis of polylactic acid. Engineer. Med. 1981;10(1):5-7.
12. Kale G, Kijchavengkul T, Auras R, Rubino M, Selke S, Singh S. Macromol. Biosci. 1998;135(1):193-204.

Acknowledgements: This research is supported by Thailand Research Fund (TRF) under the Directed Basic Research Grant and Faculty of Environment and Resource Studies of Mahidol University Alumni Association.