การย่อยสลายพอลิแลคติกแอซิดโดยกลุ่มจุลินทรีย์ในดินจากหลุมฝังกลบขยะ และตะกอนน้ำเสีย โรงงานปลาทูน่ากระป๋อง

Biodegradation of polylactic acid by indigenous microorganisms from landfill soil and wastewater sludge from Tuna processing and canning factory

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาความสามารถในการย่อยสลายพลาสติกชีวภาพพอลิแลคติกแอซิด (PLA) ด้วยกระบวนการทางชีวภาพโดยจุลินทรีย์ในดินจากหลุมฝังกลบขยะและตะกอนน้ำเสีย ซึ่งทำการทดสอบหาอัตราส่วนระหว่างดินจากหลุมฝังกลบขยะและตะกอนน้ำเสียที่เหมาะสมต่อการย่อยสลายPLAโดยตรวจวัดจากน้ำหนักของ PLA ที่หายไป เมื่อนำแผ่น PLA ฝังลงในดินจากหลุมฝังกลบขยะและตะกอนน้ำเสียที่อัตราส่วนต่างๆ ผลการศึกษาพบว่า ชุดทดลองที่มีอัตราส่วนของดินจากหลุมฝังกลบขยะและตะกอนน้ำเสียเท่ากับ 25:75 ค่าน้ำหนักที่หายไปของแผ่น PLA สูงที่สุดคือ 25.45% จากนั้นนำอัตราส่วนที่เหมาะสมนี้ไปศึกษาผลของการกระตุ้นการย่อยสลาย PLA ของจุลินทรีย์โดยการเติมสารอาหาร (คาร์บอน ไนโตรเจนและฟอสฟอรัส) ต่ออัตราการย่อยสลาย PLA พบว่า ชุดทดลองที่ไม่มีการเติมคาร์บอน ไนโตรเจนและฟอสฟอรัส (ชุดควบคุม) มีค่าน้ำหนักของแผ่น PLA ที่หายไปสูงที่สุดคือ 14.43% ผลการศึกษานี้แสดงให้เห็นว่าจุลินทรีย์ประจำถิ่นที่อยู่ในดินจากหลุมฝังกลบขยะและตะกอนน้ำเสียมีความสามารถในการย่อยสลาย PLA ได้ดีโดยไม่ต้องมีการเติมสารอาหารอื่นเพื่อเร่งการเจริญและการย่อยสลาย PLA

คำสำคัญ: พอลิแลคติกแอซิด / ดินจากหลุมฝังกลบขยะ / ตะกอนน้ำเสีย

Abstract

The aim of this research was to study the ability of indigenous microorganisms habited in sanitary landfill soil and wastewater sludge on the polylactic acid (PLA) biodegradation. The optimum ratio of sanitary landfill soil and wastewater sludge for PLA biodegradation was investigated by measuring the weight loss of PLA sheets which were buried in different ratios of sanitary landfill soil and wastewater sludge. The results found that the ratio of sanitary landfill soil and wastewater sludge at 25:75 had the highest percentage of PLA weight loss by 25.45%. Biostimulation of microbial acidity on PLA degradation with carbon, nitrogen and phosphorus addition was performed. The results found that the treatment without carbon, nitrogen and phosphorus addition (control treatment) had the highest percentage of PLA weight loss by 14.43%. It could be concluded that the indigenous microorganisms insanitary landfill soil and wastewater sludge had the good ability on PLA biodegradation without biostimulation with nutrients.

Keywords: Polylactic acid / Landfill soil / Wastewater sludge

INTRODUCTION

Nowadays, many plastic materials were produced from petroleum-based industry. The residues of the plastic wastes have caused many serious environmental pollution affecting human lives. Most of these wastes ended up in landfills because landfill disposal is the most common method used for the disposal of solid waste. The traditional polymers were recognized as the current environmental concerns in terms of environmental pollution, greenhouse gas emissions and the depletion of fossil resources (Jenck et al., 2004; Kummerer, 2007).

Polylactic acid (PLA) is abiodegradable aliphatic polyester synthesized from renewable resources. PLA becomes more attention as the green materials due to a process of the PLA production that was derived from polymerization process of lactic acid which obtained from several crop plants such as corn, cassava. However, the enormous consumption of bioplastics generates a huge quantity of bioplastic wastes and causes the emerging problems of solid waste management. The biodegradation of bioplastics in the environment involves several processes due to the complex structures of bioplastics. The natural microorganisms influence the abiotic and biotic factors, including physical, chemical and enzymatic reactions (Shah et al., 2008). Studies on the biodegradability and biodegradation mechanisms of polyesters play a significant role in the development of biodegradable polyesters. Furthermore, understanding the mechanisms will be very useful to minimize the harmful effects of polyesters upon disposal in the environment. Under appropriate conditions, PLA can be degraded by some microorganisms in the environment. Unfortunately, PLA-degrading microorganisms have rarely distribution in the natural environment (Tokiwa and Calabia, 2007).

This research investigated the ability of indigenous microorganisms in sanitary landfill soil and sludge from wastewater treatment plant on the PLA biodegradation. Biostimulation of microbial growth and PLA degradation activity with nutrients was investigated. These findings could be applied for further PLA waste treatment and disposal. However, to test the PLA biodegradation can change the source of microorganisms. It could be from fertilizer or other.

OBJECTIVES OF THIS STUDY

1. To study the ability of microbial consortia from sanitary landfill soil and wastewater sludge on PLA biodegradation.
2. To investigate the optimum ratio of sanitary landfill soil and wastewater sludge on PLA biodegradation.
3. To study the effect of biostimulation with nutrients on PLA biodegradation.

SCOPES OF THE STUDY

1. This experimental study was carries out in Laboratory of Environmental Biotechnology, Faculty of Environment and Resource Studies, Mahidol University.
2. Landfill soil was collected from sanitary landfill site at Nakhonpathom province.
3. Wastewater sludge from activated sludge treatment was collected from Tuna processing and canning factory at Samutsakhon province.
4. PLA was bought from Natureworks, USA and the PLA biodegradation process was performed at ambient condition during May – October 2014.

LITERATURE REVIEW

Polymer degradation occurs mainly through scission of the main chains or side chains of macromolecules. In nature, polymer degradation is induced by thermal activation, hydrolysis, biological activity (i.e., enzymes), oxidation, photolysis, or radiolysis. Because of the coexistence of biotic and nonbiotic processes, the entire mechanism of polymer degradation could, in many cases, also be referred to as environmental degradation. A variety of chemical, physical and biological processes and thus different degradation mechanisms can be involved with the degradation of a polymer. Environmental factors not only influence the polymer to be degraded, they also have a crucial influence on the microbial population and on the activity of the different microorganisms themselves. Parameters such as humidity, temperature, pH, salinity, the presence or absence of oxygen and the supply of different nutrients have important effects on the microbial degradation of polymers, and so these conditions must be considered when the biodegradability of plastics is tested. The process is also dependent upon the chemical and physical characteristics of the polymer. These include diffusivity, porosity, morphology, cross linking, purity, chemical reactivity, mechanical strength, thermal tolerance, and resistance to electromagnetic radiation (Muller, 2008).

Rudeekit et al. (2008) conducted a biodegradation test of PLA under wastewater treatment, landfill, composting plant and controlled composting conditions. They results found that the PLA sheets had noticeable white spots on the surface after a 1-month exposure to wastewater treatment conditions. However, the biodegradation of PLA was more rapid under composting plant conditions at high temperature (50-60 ºC) and relative humidity (> 60%). The PLA sample in sheet form became brittle and started to break into small pieces after testing for 8 days. This is because the degradation temperature at a land composting plant is higher than the glass transition temperature of PLA. The large volume of water in contact with PLA in the wastewater treatment conditions, because the degradation temperature is lower than the glass transition temperature the degradation rate is lower than that under composting plant conditions. When the PLA sheets were buried in the landfill conditions, they degraded more slowly than those in the composting condition. This is because of the higher temperature and humidity in the composting plant conditions, which help the PLA to degrade rapidly. In the landfill conditions, it required 6 months for major fragmentation to occur and 15 months for there to be some disappearance. In contrast, PLA under composting plant conditions showed disappearance in merely 30 days. In conclusion, the degradability of PLA is dependent on the hydrolysis and cleavage of ester linkages in the polymer backbone to form oligomers.

MATERIALS AND METHODS

1. Sample collection and preparationof the landfill soil and wastewater sludge

Landfill soil was collected from landfill site at Nakhonpathom province. Wastewater sludge was collected from Tuna processing and canning factory at Samutsakhon province. All samples were kept in plastic bag at -20 ºC for further use. Landfill soil and wastewater sludge were air-dried, crushed, sieved through a 2-mm screen, and then pulverized to pass a 0.2-mm mesh sieve. The soil properties, including pH, organic matter, nitrogen, phosphorus, potassium, texture, moisture content and number of viable bacterial, fungal cells were analysed.

2. Study of the optimum ratio of landfill soil and wastewater sludge on PLA biodegradation using soil burial test

The soil mixtures of various ratio of landfill soil and wastewater sludge, including 100:0, 75:25, 50:50, 25:75 and 0:100 were prepared and placed in the glass vessel. PLA sheets were buried in the soil mixture. All glass vessels were placed under ambient conditions with aeration and control the moisture from the air-flow with the column of silica gel. This experiment was conducted for 45 days. Each piece of PLA sheet and the soil mixture in the glass vessel were randomly collected every 15 days and measured as follows:

- The percentage of PLA weight loss

PLA sheet which was taken out of the glass vessel was washed with distilled water several times to remove attached soil and then kept in desiccator before weighting. The percentage of weight loss of each PLA sheet was calculated as showed in following equation.

$$\%\ \text{Weight loss} = \frac{\text{W (before) - W (After)}}{\text{W (before)}}\ 100$$

- Physical surface appearance of degraded PLA sheet

Surface appearance of the degraded PLA sheet after biodegradation under soil burial condition was observed using a light microscope.

- Viable bacterial and fungal cells in the soil mixture

A 1 g of the soil mixture in glass vessel was randomly collected every 15 days for 45 days and suspended in 0.85% sterile normal saline. Soil suspension was thoroughly mixed using vortex mixer. A 100 µL of soil suspension was aseptically transferred and spreaded on Plate Count Agar (PCA) and Potato Dextrose Agar (PDA). Appeared bacterial colonies on PCA and PDA plate were counted and calculated the number of viable bacterial cells in the soil mixture.

3. Study of the effect of biostimulation with nutrients on PLA biodegradation

This experiment used glucose (C6H12O6) as carbon source, ammonium sulfate ((NH4) 2SO4) as nitrogen source and sodium dihydrogen phosphate (NaH2PO4) as phosphorus source. Organic carbon, nitrogen and phosphorous in the optimum ratio of the soil mixture selected from the method No.2 were analyzed. The different amounts of C6H12O6, NaH2PO4 and (NH4)2SO4 were added tothe soil mixture to give 4 ratios of carbon, nitrogen and phosphorous as follows: T1) without addition (control treatment), T2) 100:20:2, T3) 100:10:2.5, T4) 100:15:5 and T5) 100:10:2.PLA sheets were buried in the soil mixture at different ratio of C:N:P. Glass vessel was placed in the ambient condition with aeration. PLA sample was collected and measured similar to the methods previously described in the method No.2.

RESULTS

1. The physical, chemical and biological characteristics of landfill soil and wastewater sludge

The experimental results of physical, chemical and biological properties of landfill soil and wastewater sludge are presented in Table 1.

Table 1 The physical, chemical and biological properties of landfill soil and wastewater sludge

Sample	Texture	Organic carbon (%)	Total nutrient (%)			pH	Moisture	Viable cells	
			N	P	K	(1:5 w/v H_2O)	(%)	Bacteria (log CFU/g soil)	Fungi (log CFU/g soil)
Landfill soil	Loam Sand (%) 38.42, Silt (%) 38.92, Clay (%) 22.66	4.44	0.32	0.21	0.83	7.59	2.82	6.17	<0.1
Wastewater sludge	-	26.30	4.56	1.10	0.34	6.73	11.25	9.38	6.59
Ratio 25:75	-	30.85	5.00	1.25	-	6.90	9.64	-	-

2. The optimum ratio of the soil mixture on PLA biodegradation

The degree of biodegradation of all ratios of the soil mixture increased with time, except ratio at 100:0 and 75:25. After 45 days of soil burial exposure, the highest percentage of PLA weight loss was found in the soil mixture at the ratio of 25:75 by 23.45% and the lowest percentage of PLA weight loss was found at the ratio of 100:0 by 0.17% (Figure 1).

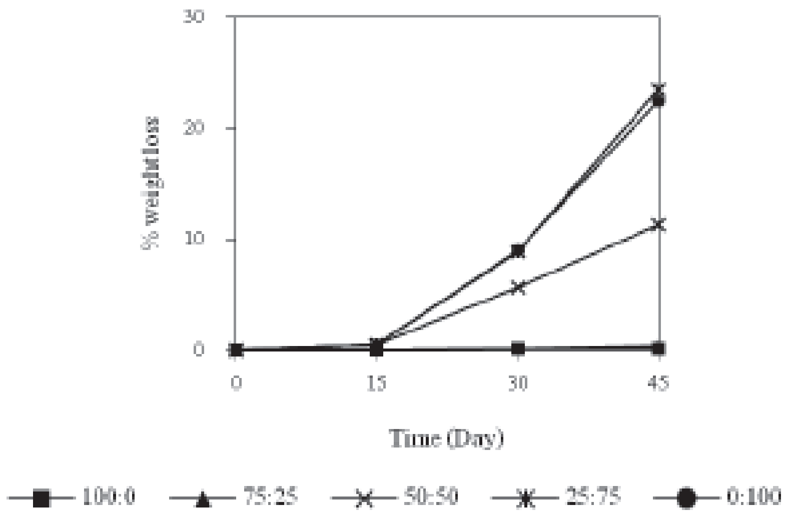
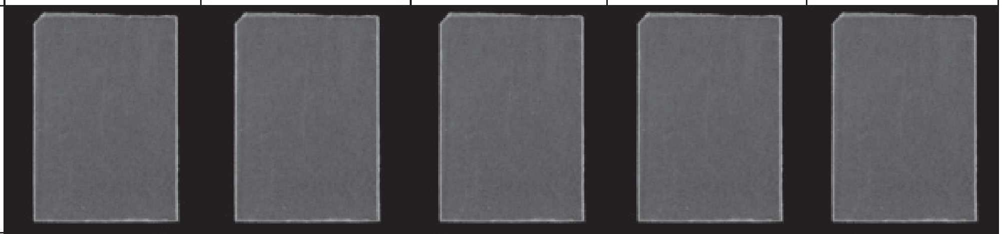
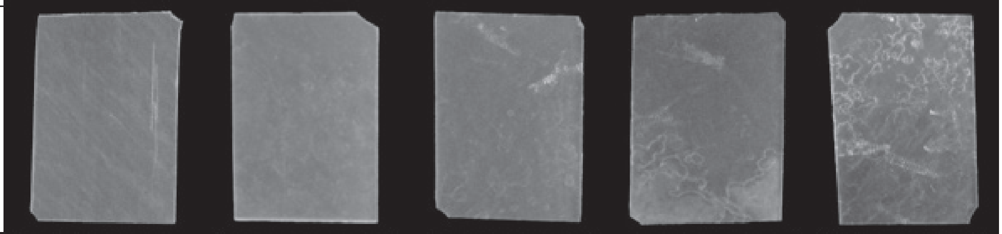
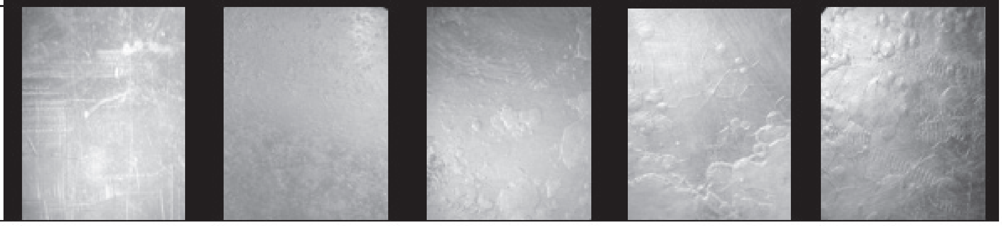


Figure 1 Percentages of PLA weight loss in different ratios of the soil mixtures

Table 2 shows the digital photographs of PLA sheets before and after the soil burial exposure at the ambient condition. The original shape of PLA sheet was visually inspected and clear seen as a transparent sheet at the beginning of soil burial test. After soil burial exposure, the PLA sheets were visually inspected. After 15 days, the PLA sheet changed from transparent to white opaque. The PLA sheet subsequently became brittle and started cracked after 45 days. At 10x magnification photograph of the degraded PLA sheet showed that all ratios began to become opaque.

Table 2 The digital photographs of the PLA sheets before and after soil burial exposure in various ratios of soil mixture

Time	Ratio of landfill soil and wastewater sludge				
	100:0	**75:25**	**50:50**	**25:75**	**0:100**
Day 0					
Day 45					
Light microscope 10X					

At the beginning period of the soil burial test, the amount of fungal cells in soil mixture in the ratio of 100:0, 75:25, 50:50, 25:75 and 0:100 were 2.70, 3.50, 5.73, 5.84 and 6.35 log CFU/g, respectively. After 45 days, the results showed that amounts of fungal cells in the ratio of 50:50, 25:75 and 0:100 were significantly decreased (Figure 2A). The ratio of 100:0 and 75:25 were not different in term of the amount of bacteria in all ratios (Figure 2B). But, the amount of bacteria in soil mixture ratio of 25:75 has more microbial population than those of other ratios.

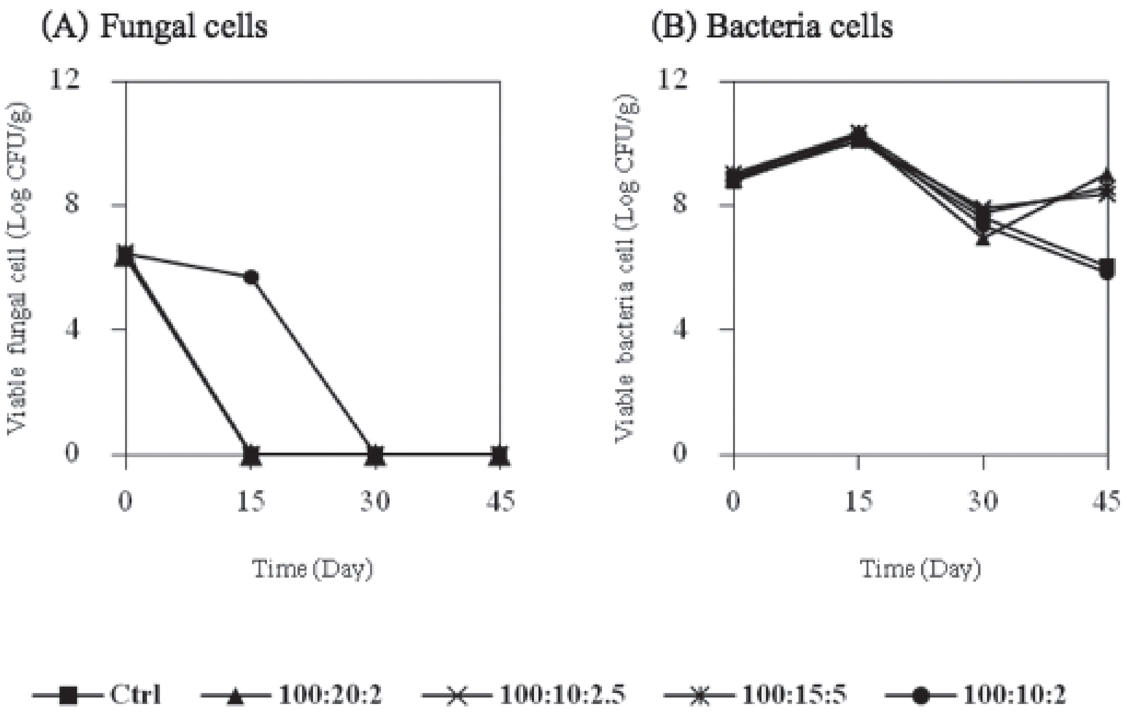


Figure 2 The number of viable cell in soil mixture (A) Fungal cells (B) Bacterial cells

3. The effect of biostimulation with carbon, nitrogen and phosphorus on PLA biodegradability by indigenous microorganisms

Figure3 shows the percentage of PLA weight loss in the soil mixture with different C:N:P ratios for 45 days. After 45 days, the highest percentage of PLA weight loss was found in the soil mixture without biostimulation with nutrients (control treatment) by 14.43%.

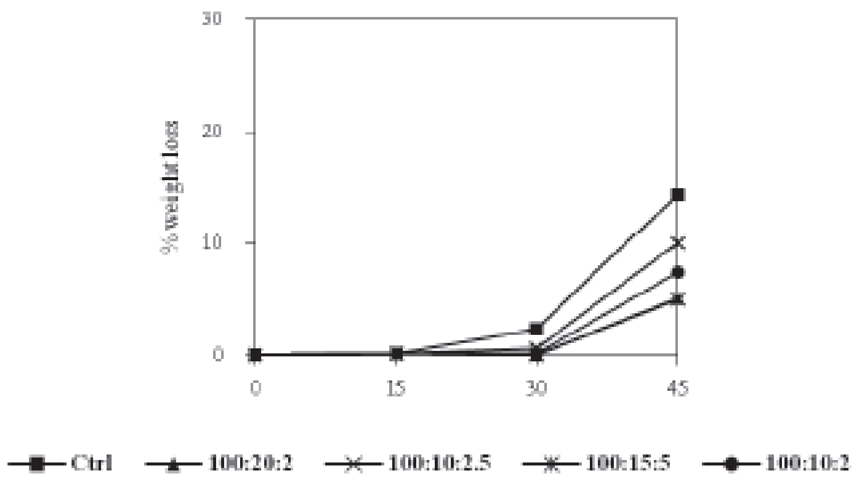
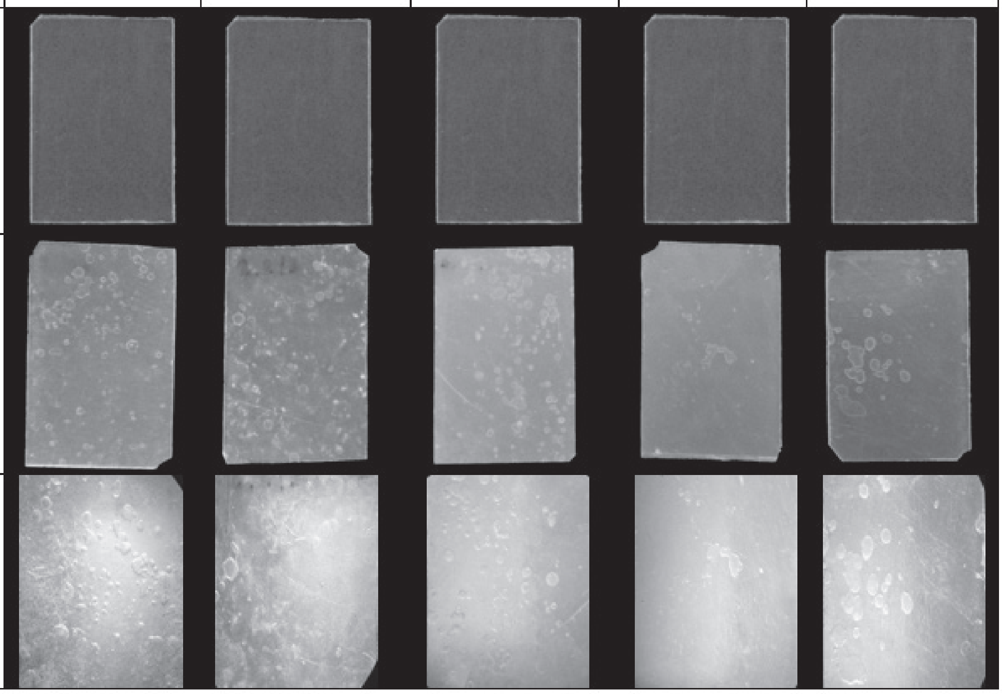


Figure 3 Percentage of the weight loss of PLA sheets in soil mixture supplemented with different nutrient concentrations

The PLA surface of the control treatment (without nutrient addition) 100:16:4 and the treatment added with 100:20:2 changed noticeably (Table 3). The amount of fungal cells in the soil mixtures without biostimultation with nutrients (control treatment) and supplemented with C:N:P at the ratios of 100:20:2, 100:10:2.5, 100:15:5 and 100:10:2 was 6.40, 6.43, 6.52, 6.36 and 6.46 log CFU/g, respectively (Figure 4A). The results showed that the amount of fungal cells was significantly decreased till 45 days. The changes of bacterial cell number in the soil mixture added with different concentration of carbon, nitrogen and phosphorus are presented in Figure 4B. After 30 days, the amounts of bacteria in ratios of soil mixture 100:20:2, 100:10:2.5 and 100:15:5 increased to 9.02, 8.39 and 8.57 log CFU/g, respectively.

Table 3 The digital photographs of the PLA sheets before and after soil burial exposure in different ratios of nutrient addition

Time	Ratio of C:N:P addition				
	Control	100:20:2	100:10:2.5	100:15:5	100:10:2
Day 0					
Day 45					
Light microscope 10X					



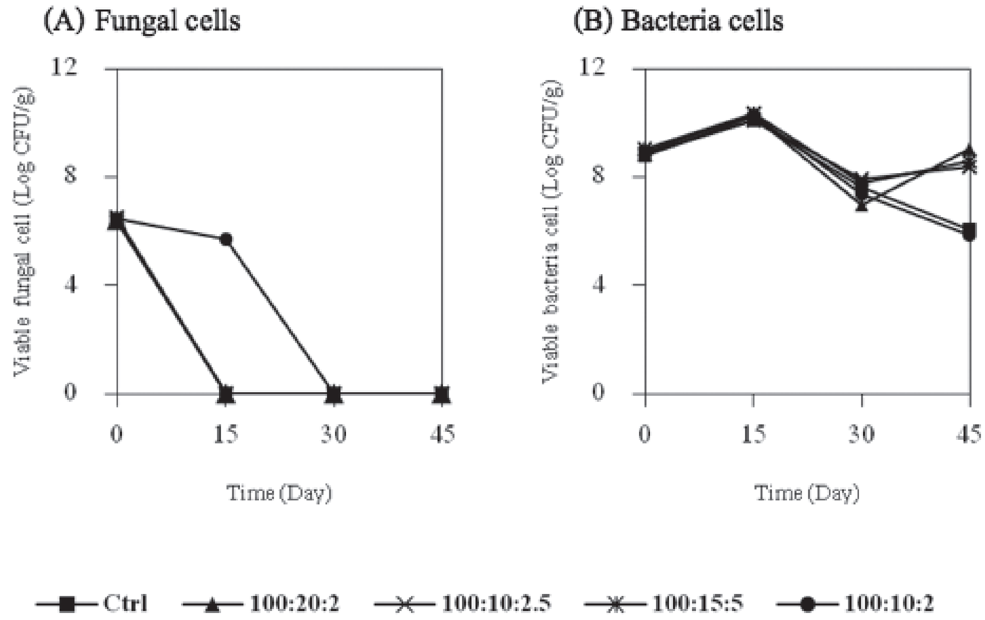


Figure 4 The number of viable cells in the soil mixture with different ratio of C:N:P supplement

CONCLUSION

In this work, PLA biodegradation tests were carried out in different ratios of mixture of landfill soil and wastewater sludge using the soil burial exposure in the laboratory. PLA biodegradation in the soil mixture at the ratio of 25:75 was higher than those at other ratios. The treatment without carbon, nitrogen and phosphorus addition (control treatment) had the highest percentage of PLA biodegradation.

DISCUSSION AND RECOMMENDATION

Biodegradation of PLA sheet in different ratios of mixture of landfill soil and wastewater sludge was characterized using the soil burial exposure in the laboratory at the temperature of 31.7ºC and 59.5% relative humidity. Our results found that PLA biodegradation in the soil mixture at the ratio of 25:75 was higher than those at other ratios. It was due to the higher amount of bacteria and high moisture content in the soil mixture at the ratio of 25:75. Wastewater sludge has moisture content, which hydrolase can enhance the wastewater sludge hydrolysis (Yang et al., 2010). Higher water content which provides optimum condition for bacterial growth may cause a higher degradable rate of PLA sheet. Moreover, higher number of microorganisms may help to increase the degradation rate of PLA sheet. Soil moisture content, soil temperature, soil pH, soil enzymes and microorganisms are factors that affect the rate of naturally occurring biodegradation (Kyrikou and Briassoulis, 2007). At the initial stage of PLA degradation in soil, small holes began to be formed with microorganisms sticking on the film surface. As the degradation proceeds, the microorganisms became very active and films became very brittle because small holes grew in size to penetrate the film. Then, polymer films lost lust and the color changed to yellow, green and finally to black. The film was broken into several small pieces in the end (Kim et al., 2000). In this study, it seems that the soil mixture without nutrient addition has higher PLA biodegradation than that of with nutrient addition. It was probably due to the lower carbon content in this soil mixture without nutrients addition compared to the soil mixture with nutrient addition. Thus, microorganisms tend to use carbon from PLA structure and PLA structure is degraded.

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