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Osaka University

**Enhancing the role of poly(lactic acid) in the circular economy:  
from chain extending polymerization  
to identification of a degrading microorganism**

A Doctoral Thesis

by

**Nobuyuki Kawashima**

Submitted to

The Graduate School of Science

Osaka University

August, 2022

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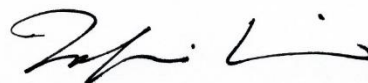
I would like to thank from the bottom of my heart everyone in Mitsui Chemicals who worked on the business development of polylactic acid both in research and marketing for many years.

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August, 2022



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**Nobuyuki Kawashima**

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# Chapter 1

## General Introduction

### 1-1. Background

#### 1-1-1. Social issues of plastics and role of bioplastics

Plastic products contribute to healthy and sound everyday life due to their functionality, convenience and hygiene, and the global community is harnessing their benefits. The annual production of various types of plastics reached 368 million tons in 2019 [1]. With the rise in consumption of plastics society is facing significant challenges from the viewpoints of the risk of resource depletion, elevation of greenhouse gas (GHG) emissions, insufficient treatment of plastic products after use, and pollution flowing into the environment around the world.

To tackle those issues from the viewpoint of a resilient, sustainable and circular economy, along with the 3Rs (Reduce, Reuse, Recycle), bioplastics, which have bio-based and/or biodegradable attributes, are expected to play an important role from two perspectives [2-4]. First, bio-based plastics contribute by reducing GHG emissions and securing fossil resources since they are derived from renewable resources to absorb CO<sub>2</sub> from atmosphere. Next, biodegradable or compostable plastic products such as packaging and single-use tableware can be efficiently composted with organic wastes, which are converted into fertilizer, a valuable product, instead of being landfilled, or in the open environment they can be deployed as agricultural mulch film. Conversion from fossil resources to renewable resources with appropriate treatment methods using biodegradability are being widely studied.

#### 1-1-2. Poly(lactic acid) (PLA) from synthesis to degradation

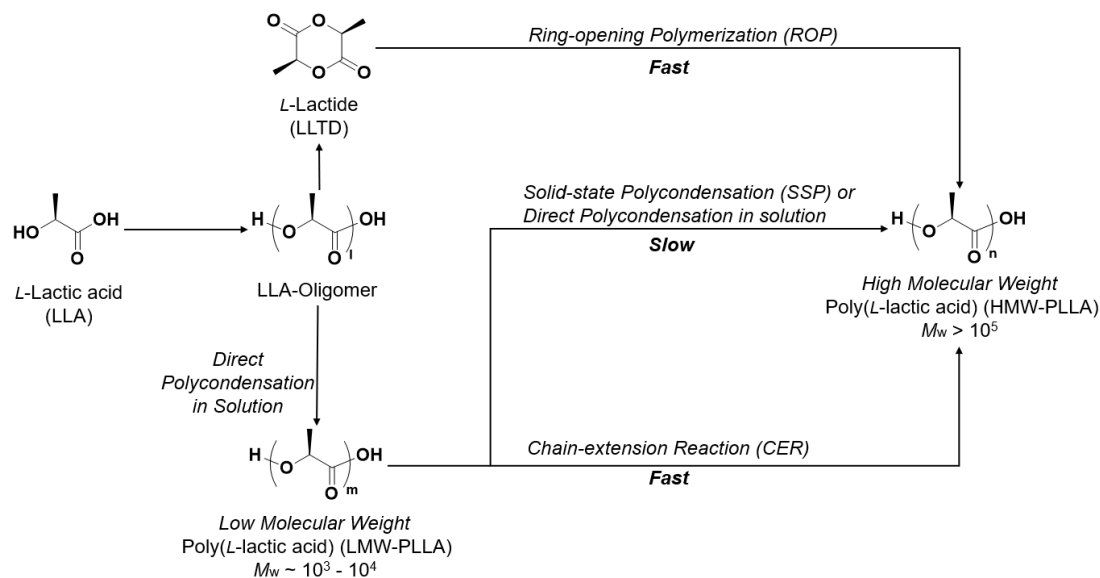
##### 1-1-2-1. Synthesis of PLA and physical properties

PLA is a representative bioplastic that has been socially implemented through large scale production for more than twenty years [5,6]. PLA synthesis methods have been summarized in review papers [7,8].

PLA is a thermoplastic and an aliphatic polyester which consists of lactic acid. Lactic acid has an asymmetric carbon and so several types of PLA exist reflecting chiral forms of lactic acid. Poly(L-lactic acid), poly(D-lactic acid) and poly(DL-lactic acid) are synthesized from L-lactic acid, D-lactic acid and racemic DL-lactic acid, respectively. Due to the high crystallinity and the commercial availability of L-lactic acid, poly(L-lactic acid) is commonly used and shows high transparency and high rigidity. The raw materials for L-lactic acid are obtained from starch and sugar by fermentation which are derived from corn, sugarcane and byproducts of plant resources.

There are three routes to synthesize PLA: ring-opening polymerization (ROP. i.e. lactide method); direct polycondensation (both in solution and solid-state); and chain-extension reaction (CER) as described in Scheme 1-1.

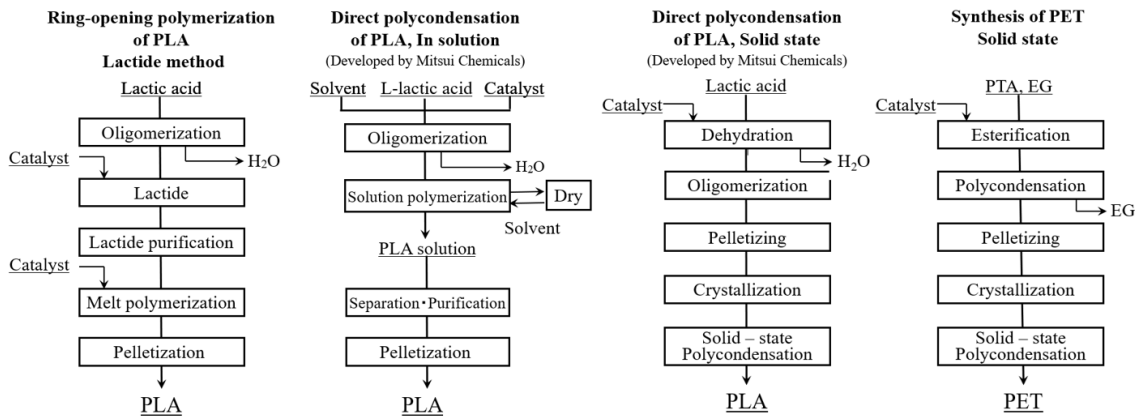
**Scheme 1-1.** Synthesis of PLA by ring-opening polymerization, direct polycondensation and chain-extension reaction.



The Lactide method has been generally used to produce PLA whereby lactic acid is oligomerized to synthesize cyclic dimer lactide by a back-biting reaction, and high molecular weight PLA (HMW-PLA) is obtained by ROP [5-8]. Cargill (currently, NatureWorks) developed this method for their large-scale production in their business development. Both NatureWorks and Total-Corbion continue using this production method to lead PLA commercialization.

The research team in Mitsui Chemicals developed the world's first method for direct polymerization of lactic acid when it was thought to be impossible to synthesize HMW-PLA as an alternative to the conventional polymerization technology. The development of the direct polymerization method has a long history. Dr. W.H. Carothers, who invented the nylon production technology at DuPont, published a paper on the synthesis of aliphatic polyesters by direct polycondensation of hydroxy-carboxylic acids in 1933 [9]. Dr. E. S. Lipinsky mentioned in a 1986 publication [10] that free lactic acid is capable of direct polycondensation, but this equilibrium reaction is difficult to drive to the right to achieve high molecular weight and useful properties. Dr. T. Doi stated in his paper [11] that even if lactic acid is directly dehydrated and polycondensed, PLA with a molecular weight of 10,000 or more cannot be obtained with or without a catalyst. When the temperature is raised too high, depolymerization becomes predominant. Both experts in aliphatic polyesters and bioplastics argued that direct polymerization of aliphatic hydroxycarboxylic acid including lactic acid caused a reverse reaction at equilibrium such that high molecular weight polymers could not be achieved. In 1994, Mitsui Chemicals succeeded in advancing the equilibrium reaction by removing the water generated in the condensation reaction using an azeotropic solvent and returning the solvent (Scheme 1-1) [4,12,13].

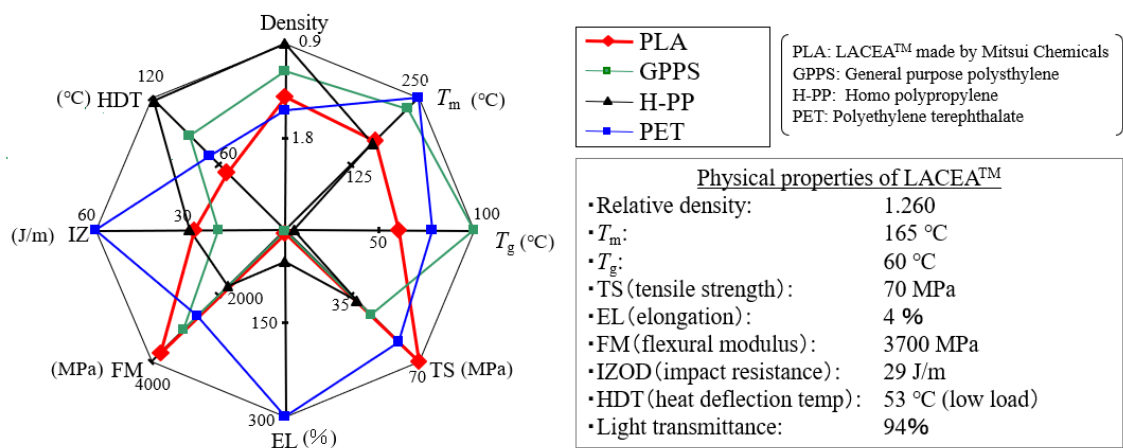
The PLA thus obtained showed higher purity than the PLA produced by the lactide method. A higher L ratio was maintained by controlling temperature during polymerization and no residual lactide was observed while the physical properties of both products were the same. In addition, a solid-state PLA polymerization process based on the direct polymerization of lactic acid, which is similar to the polyethylene terephthalate (PET) production process, was developed by the research team in Mitsui Chemicals (Scheme 1-1 and Figure 1-1) [5,14,15]. This solid-state polymerization process has the potential to use a PET production facility.



**Figure 1-1.** Comparison of polymerization processes.

-Lactide method, direct polycondensation in solution, and solid-state polycondensation of PLA and PET-

As shown in Figure 1-2 [5,13], PLA has excellent transparency and high rigidity. In terms of heat resistance and impact resistance, it was noted to be inferior to PET and polystyrene (PS) such as biaxial oriented polystyrene (OPS) and high impact polystyrene (HIPS). It was also found that

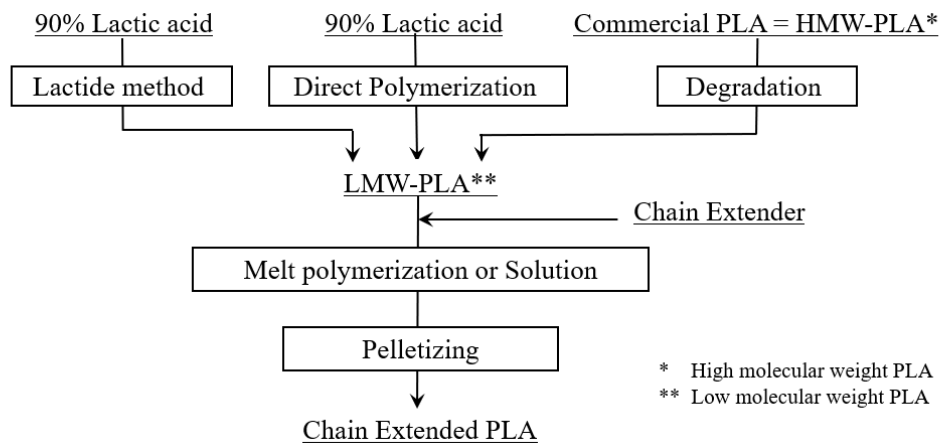


- Advantages: plant origin, high rigidity\*, transparency, biodegradability
  - Shortages: heat resistance\*\*, impact resistance\*\*, high specific gravity, water decomposability
- \* PLA has high FM.  
 \*\*PLA is inferior to PET and PS (OPS: oriented polystyrene and HIPS: high impact polystyrene) in both HDT (heat resistance) and IZOD (impact resistance).

**Figure 1-2.** Comparison of physical properties of PLA and other resins.

although the heat resistance of the biaxially oriented film is high, the rigidity is too high compared to oriented polypropylene (OPP) for packaging film applications. Therefore, PLA cannot be a simple alternative even though the targeted markets of thermoformed products such as PET and PS, and the film such as PP overlap. There are no significant differences of physical properties of PLA even though the synthetic methodologies are different [5,13,14].

It is noted that direct polymerization is considered the least expensive process to produce PLA with molecular weights of several thousands to several tens of thousands [8]. On the other hand, higher molecular weights of more than  $1 \times 10^5$  are required for proper stability and processability. In order to achieve practical levels of molecular weights, CER methods from low molecular weight (LMW-PLA) other than solid-state polymerization have been reported (Figure 1-3). Various chain extenders, which are bifunctional and multifunctional chemicals, were reported to increase the molecular weight of LMW-PLA. The representative chain extenders are dioxazoline, carbodiimide, diisocyanate and Joncryl™: multifunctional epoxy chain extender and are used alone or in combination [16-22]. Among them, diisocyanates, especially hexamethylene diisocyanate (HDI), showed high capability of PLA chain extension [16-18]. The efficient CER method is expected to be a new manufacturing method of PLA and copolymers with new functions. Furthermore, the introduction of the CER method has the potential to contribute to the recycling or repair of PLA, which decreases in molecular weight during use and recycling.



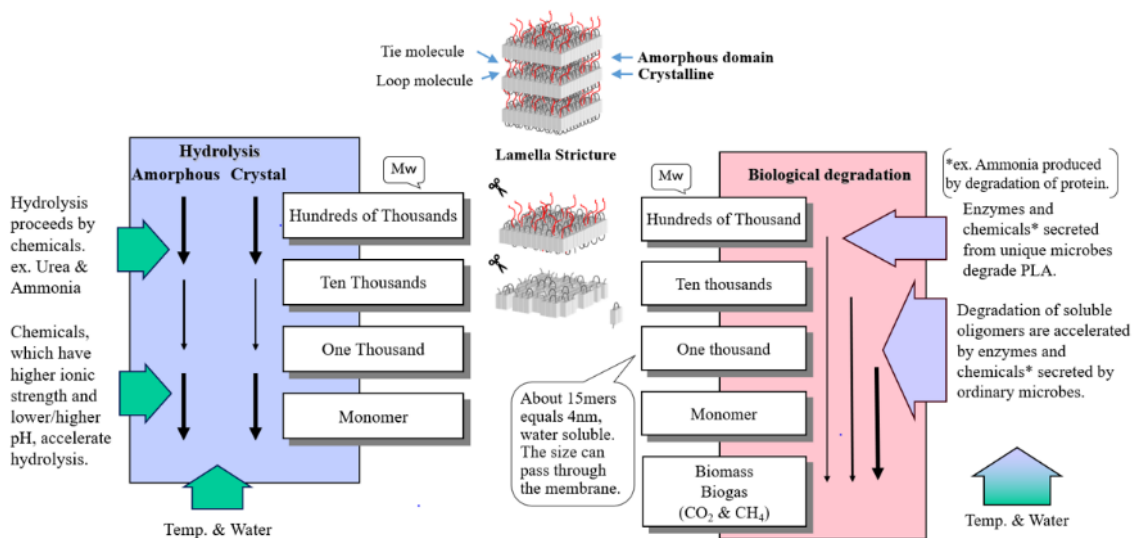
**Figure 1-3.** Chain-extension reaction of PLA.

### 1-1-2-2. Microorganisms and enzymes affecting degradation of PLA

In general, there are two processes for PLA degradation. One is chemical hydrolysis and the other is biological degradation by microorganisms and enzymes (Figure 1-4). It is expected that a combination of the two degradation process models occurs in the environment and during composting. First, PLA is disintegrated and fragmented via chemical hydrolysis, which is accelerated at higher temperatures and pH, or biological hydrolysis, in which microorganisms and



secreted enzymes act. Second, the fragmented PLA is further hydrolyzed and digested by microorganisms. There are various views on the degradation mechanism of PLA. Some studies have reported that PLA degradation is mostly performed chemically with only a limited contribution from microorganisms [23]. A previous study [24] concluded that degradation depended on temperature, not the presence of microbes. PLA sheet decomposition on a laboratory scale using yard waste compost showed that an excessive amount of PLA input produced lactic acid via hydrolysis, resulting in toxicity to microorganisms [25].



**Figure 1-4.** An image of PLA degradation processes by chemical hydrolysis and biological degradation.

Although it seems that the degradation mechanism of PLA has not yet been clearly clarified, PLA-degrading bacteria have been isolated from various environments. After Tokiwa et al., isolated and identified *Amycolatopsis*, a genus of PLA-degrading actinomycetes, in 1997 [26] the same research group isolated a variety of PLA-degrading microorganisms from environmental sources, soil, and compost. Some of these microorganisms belong to the genera of *Rhizobium*, *Bacillus*, and *Tuberibacillus*, identified in 2008 [27]. Others belong to the family *Pseudonocardiaceae* and related genera such as *Amycolatopsis*, *Lentzea*, *Kibdelosporangium*, *Streptoalloteichus*, and *Saccharothrix*, are reported in a review paper published in 2006 [28]. These discoveries were subsequently reviewed in 2017 [29] and 2019 [30].

A study attempted to improve the efficiency of PLA degradation in compost by spraying a mixture of potent PLA-degrading bacterial strains in 2016. In these experiments, a mixture of four strains, classified as species, *Penicillium chrysogenum*, *Cladosporium sphaerospermum*, *Seratia marcescens*, and *Rhodotorula mucilaginosa*, was sprayed onto compost made of vegetable waste, wood chips, and fruit peels. Addition of the bacterial cocktail facilitated PLA degradation and the

results demonstrated the importance of microorganisms in PLA degradation during composting [31]. As reviewed above, it is clear that PLA-degrading microorganisms range from prokaryotes such as actinomycetes to eukaryotes such as fungi (Table 1-1).

**Table 1-1.** Examples of wide range of microorganisms to degrade PLA.

Genus or Genus and Species	Domain /Kingdom	Origin	Ref.
<i>Amycolatopsis</i> sp.	Bacteria	45 soil samples in Tsukuba city, Japan	1997 [26]
<i>Thermomonospora</i>	Bacteria	Mature composts from the processing site in Victoria, Australia	2008 [27]
<i>Thermopolyspora</i>	Bacteria		
<i>Paecilomyces</i>	Fungi		
<i>Penicillium</i> , <i>P. chrysogenum</i>	Fungi	Approximately 300 soil samples from different sources (Tropical rain forest areas of Western Ghats of Kerala, composting grounds in local markets, solid waste treatment plant, mangrove soil, activated sludges and dairy sludge from milk processing plant)	2016 [31]
<i>Cladosporium</i> , <i>C. sphaerospermum</i>	Fungi		
<i>Serratia</i> , <i>S. marcescens</i>	Bacteria		
<i>Rhodotorula</i> , <i>R. mucilaginosa</i>	Fungi		

Microbial protease and lipase are enzymes reported to degrade PLA [32]. These enzymes were subsequently characterized [33-36]. Proteases catalyze proteolysis by breaking down proteins into smaller polypeptides or single amino acids, some can also degrade PLA because they recognize the  $\alpha$ -ester bond of PLA [26, 33, 37, 38]. In Tokiwa's study [28], protease degrades PLA and is secreted by actinomycetes, such as *Amycolatopsis* sp., *Lentzea waywayandensis*, *Kibdelosporangium aridum* and a fungus, *Tritirachium album*. It is also studied that lipases are the family of enzymes that catalyzes the hydrolysis of fats by cleaving the ester bonds in a polymer substrate such as PLA. Lipase is secreted by *Bacillus sinithii* and *Cryptococcus species* [38].

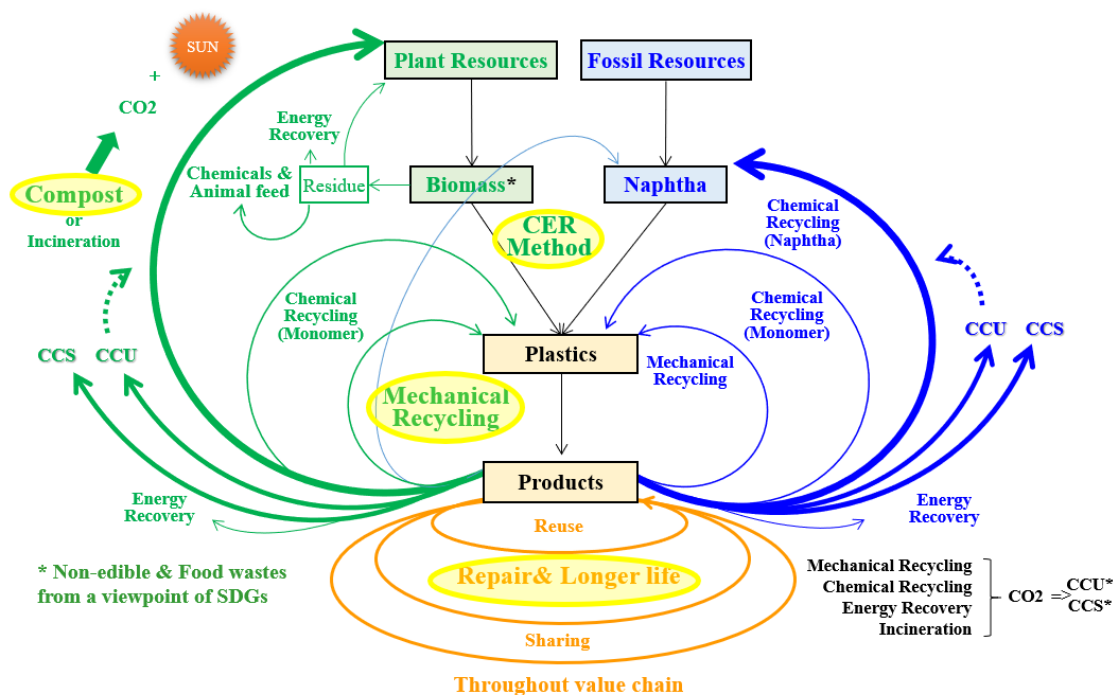
### 1-1-3. Scientific challenges and solutions to enhance a role of PLA in a circular economy

The fact that PLA is derived from renewable resources and has biodegradability does not mean that it will contribute to the construction of a resilient and sustainable society. Without scientific knowledge about PLA and new studies based on it, it will not be possible to solve social issues toward a circular economy.

What is required of materials and products, including plastics, is not the conventional linear business model of mass production, mass consumption, and mass disposal. Along with saving and effective use of resources, it is to shift to a circular economy in which new value is created instead of treating it as waste after use. The Ministry of Economy, Trade and Industry (METI) published comprehensive figure of circular economy in the Circular Economy Vision 2020 [39]. In particular, effective utilization after use is an important issue for PLA. Through long time experiences of bioplastics development, a scheme of the roles that both fossil-based plastics and bioplastics can play toward circular economy is created in Figure 1-5 [4, 40].

There are five important points for establishment of the circular economy for plastics: 1, Aim

for a resilient and sustainable economy.; 2, In addition to reuse and recycling, strengthening through maintenance, repair, longevity, sharing platforms, and products-as-a-service, throughout the value chain.; 3, Digitization, knowledge / information sharing throughout the value chain (environmental impact assessment and tracking data throughout the material / product life cycle).; 4, Reuse waste in other manufacturing processes and after-use.; and 5, Maximize resource value, and minimize waste & environmental impact.



**Figure 1-5.** Circular economy model for plastics.

The highlights in yellow in Figure 1-5 indicate the places or factors where each research theme in this thesis can contribute to the circular economy for plastics. First, the study in Chapter 2 (Chain-extension of LMW-PLA with Diisocyanate via Amide Formation to Enhance the Thermal Degradation Stability) contributes to “efficient polymerization”, “recycling” and “repair”. Studies in Chapter 3 (Isolation of a *Norcardiopsis Chromatogenesis* Strain that Degrade PLA in Pig Waste-based Compost), and Chapter 4 (Pilot-Scale Composting Test of PLA for Social Implementation) contribute to “composting” of biodegradable plastics after use. Finally, the study in Chapter 5 (How Do Bioplastics and Fossil-based Plastics Play in a Circular Economy?) created this Figure 1-5 and contributes to consideration of “resources” and “throughout value chain”.

Just because PLA is derived from renewable resources, simple incineration or landfill after use is not appropriate. After using PLA products, recycling is required to continue to make effective use of carbon. However, PLA has a potential hurdle that the molecular weight decreases during processing, utilization and mechanical recycling after use. Therefore, recycling or "repair"

is expected to restore the original molecular weight of PLA by the CER method, which was developed as a new PLA synthesis methodology.

Furthermore, there is a movement to effectively use organic waste such as food residues as fertilizer by composting instead of landfilling or incineration. It is clear that, by replacing packaging containers and tableware, which are not easily separated from food residues and may cause concerns by contamination of non-degradable materials, with biodegradable plastics, it will be possible to compost organic waste in large quantities and in an efficient way. This direction is described in various reports, especially in Europe, and relevant reports are “European policy on bio-based, biodegradable and compostable plastics (2020)” [41] and “Relevance of Biodegradable and Compostable Consumer Plastic Products and Packaging in a Circular Economy (2021)” [42].

In composting facilities animal wastes are widely used as a major component. Isolating and identifying microorganism to degrade PLA in compost followed by future analyses of secreted enzymes and degradation mechanisms will lead to establishing an efficient composting process. In addition, in order to gain the confidence of consumers, facility managers and users of the resulting compost, laboratory scale experimentation is not enough, and pilot scale composting tests are required.

## **1-2. Objective and Outline of This Thesis**

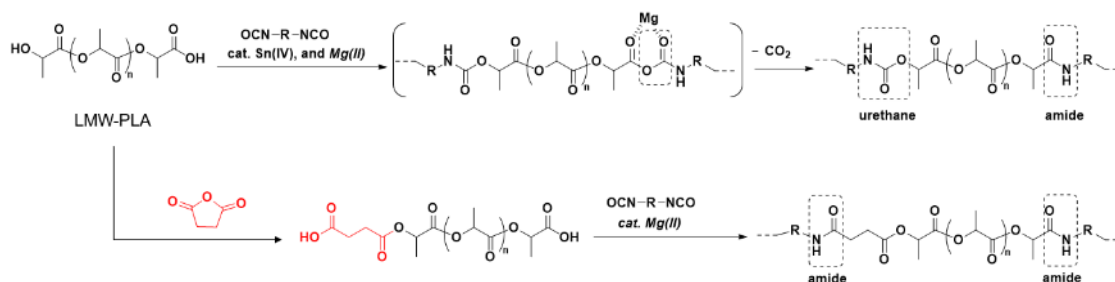
While conducting research to tackle the scientific issues of PLA, it was found that this would lead to the solution of the social issue of constructing an appropriate treatment method for used PLA. The objective and outline of the specific individual themes covered in this thesis are as follows.

### **1-2-1. Chain-extension of low molecular weight poly(lactic acid) with diisocyanate via amide formation**

In the Chapter 2, the CER method was studied as a more efficient polymerization method and as a method for synthesizing new copolymers. As a result of studying CER using various chain extenders having functional groups at both ends, it was found that diisocyanate is the most effective. Among them, HDI can be used to synthesize chain-extended PLA (cePLA) having both a urethane bond derived from an OH group of LA and an isocyanate and an amide bond via a urethane acid anhydride intermediate derived from a COOH group of LA and an isocyanate (Scheme 1-2). A tin-based catalyst was effective for direct polymerization, and a magnesium (Mg)-based catalyst was effective for chain extension to create cePLA. It should be noted that, without Mg-based catalyst, urethane acid anhydride is not transferred into amide bond and results in decomposition by water in the atmosphere. It has been confirmed that the obtained cePLA has the same basic physical properties as the standard PLA. On the other hand, this cePLA was found

to have the lower temperature resistance compared to the standard PLA. In order to improve this heat resistance, after making both ends of the lactic acid oligomer COOH, the chain is extended with HDI to synthesize cePLA which has both amide bonds.

**Scheme 1-2.** Chain extended reaction of PLA with HDI.



Currently, it should be noted that recycling is required after-use even for the bio-based plastics to secure carbon. Although mechanical recycling (MR) is usually applied for PET, PLA may not be suitable for MR because its molecular weight may decrease due to hydrolysis during service life, after use and during recycling process [43]. Therefore, in order to restore the molecular weight of PLA which was degraded by hydrolysis, the above-mentioned CER was applied to perform MR. By converting both ends of LMW-PLA, which was hydrolyzed from standard PLA, to COOH followed by this CER, it was proved that the resulted cePLA showed the same heat resistance, basic physical properties and biodegradability as the standard PLA, and the original PLA was successfully restored, that is, MR was realized for PLA. From the viewpoint of circular economy, this method can be called not only MR but also "repair".

### 1-2-2. Isolation and identification of a microorganism degrading PLA

Identifying a microorganism involved in PLA degradation in compost may lead to the related enzymes and understanding of the degradation mechanism, and result in contributing to optimization of compost processing.

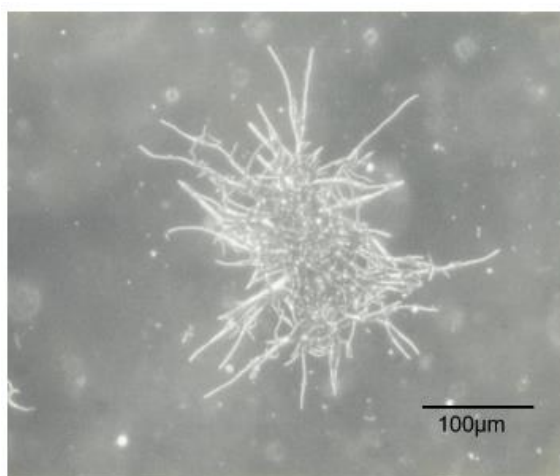
A new *Nocardioopsis* species that degrades PLA was isolated from pig dung-based compost from a municipal composting facility in Japan. Many studies have been carried out at ca. 58°C or higher temperatures and chemical hydrolysis was accelerated (Table 1-2). To obtain strains capable of efficient PLA degradation, the effect of non-enzymatic degradation of PLA was minimized by maintaining the temperature at 37°C or below in this study. After screening a total of 15 animal waste-based compost samples, consisting of pig dung, cow dung, horse dung, or chicken droppings, we found that compost derived from pig dung was most efficient for degradation of PLA film, and used it for isolation of PLA-degrading microorganisms. Screening for PLA-degrading microorganisms in compost was performed using an agar plate-based method;

an emulsifier was omitted to avoid selection of strains that assimilated the emulsifier instead of PLA in the medium.

**Table 1-2.** Main components and temperature of PLA degradation testing in Compost

Main Components of Compost	Temperature	Reference
Mix of animal and plant wastes	58 ± 2 °C	ISO14855 [44]
fiber, fat, and protein in animal fodder	58 °C	2005 [45]
cow manure and wood waste	60–65 °C	2007 [46]
green yard waste	around 60 °C	2007 [47]
		2017 [48]
		2019 [49]
horse manure and plants	70 °C or higher at the beginning	2021 [50]
(15 animal wastes)	(37 °C)	(this study)

After repeated enrichment, six strains were obtained. One strain that exhibited stable PLA degradation on agar plates was subjected to genomic analysis and identified as *Nocardiopsis chromatogenes*, an actinomycete (Figure 1-6).



**Figure 1-6.** Phase-contrast microscopy of strain MT-20147.

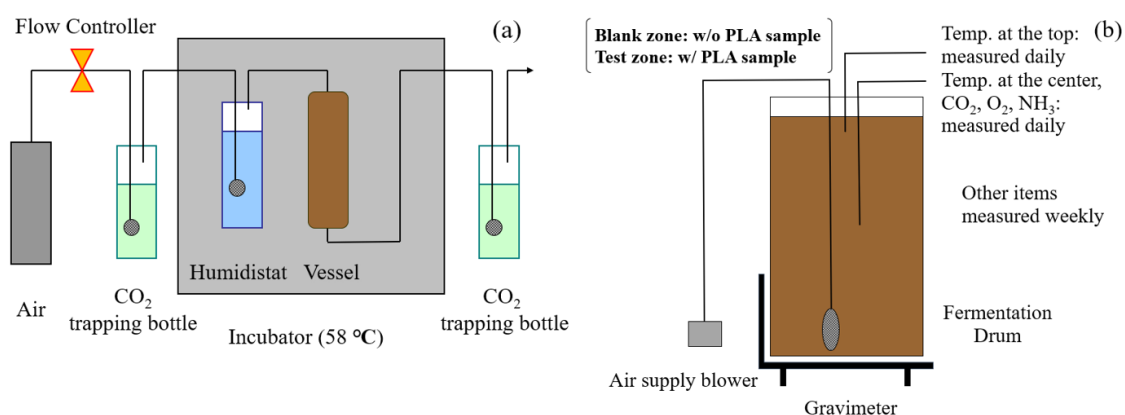
This is the first study focusing on screening animal waste–based composts from farms and facilities leading to isolation of this strain.

Recent studies have examined microorganisms that degrade PET have been conducted. Among them, *Nocardiopsis chromatogenes* was shown to be a PET-degrading species in 2018 [51]. Although PET is aromatic and PLA is aliphatic, and the two compounds have different chemical structures, it is noteworthy that microorganisms from the same genus are involved in the degradation of both polyesters.

### 1-2-3. Pilot scale composting test of PLA for social implementation

It is important to promote the installation of compost facilities as an appropriate treatment after use in order to treat organic wastes efficiently. The challenges of composting biodegradable plastics products are that they need to be completely and safely degraded, cannot affect the composting process, and the resulting compost should not adversely affect the growth of plants such as vegetables. Non-degradable materials such as glass, metal, plastic products, batteries, and stones are often mixed in composting facilities for organic waste.

While it requires resources and time to separate them in advance, there are concerns from consumers, facility management, farmers who use the obtained compost about the introduction of new materials labeled biodegradable. It is important to gain the confidence and trust by them. To realize the implementation of fully approved composting facilities in society, simply evaluating biodegradability in the laboratory such as ISO 14855 [44] is insufficient, in which only CO<sub>2</sub> was measured as shown in Figure 1-7 (a). There have been many studies to demonstrate biodegradability of PLA in compost and safety of the resulted compost [46-49]. However, it appears that only a few studies demonstrate the method and results of a comprehensive and holistic approach on the pilot scale. In this study, a pilot-scale test (100 L or more) using PLA under actual composting conditions shown in Figure 1-7 (b) was conducted in accordance with both international standards ISO 16929 [52] and domestic evaluation methods.



**Figure 1-7.** Schemes of the laboratory-scale compost apparatus according to ISO 14855 (a) and the pilot-scale compost apparatus principle (b).

The results not only confirm its biodegradability and disintegration, but also demonstrate that the presence of a biodegradable plastic product has a negligible impact on the composting process, by measuring various items including temperature, CO<sub>2</sub> concentration, O<sub>2</sub> concentration, ammonia concentration, water content, pH, electrical conductivity, ignition loss, content volume and content weight. All the measured items and specified items of laws and regulations are listed in Table 1-3. The obtained compost did not adversely affect plant germination or growth, demonstrating its safety and high quality. Such a multifaceted perspective makes this study unique and useful for creating a social framework.



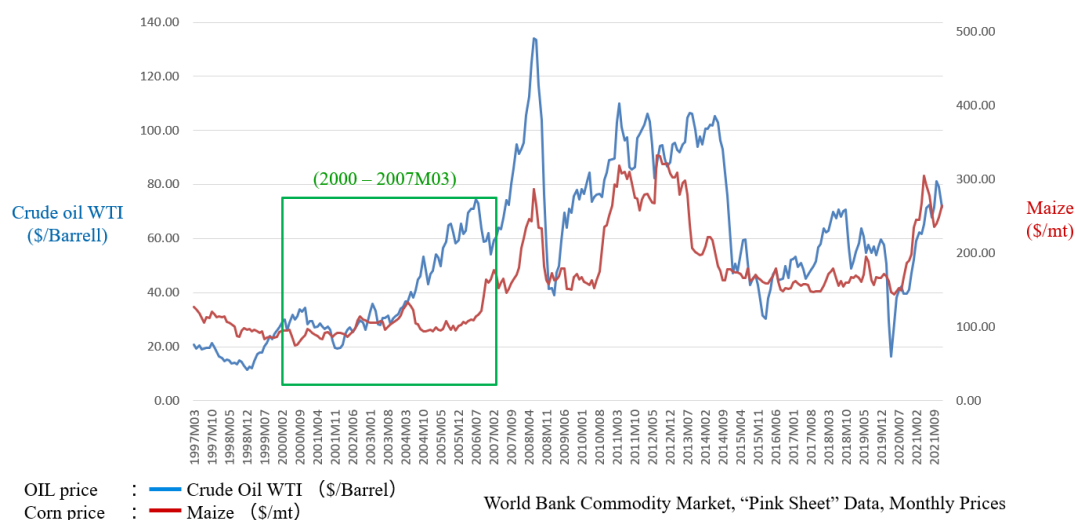
**Table 1-3.** Measured items and specified items of laws and regulations

Items	Contents
Measured	temperature, CO <sub>2</sub> concentration, O <sub>2</sub> concentration, ammonia concentration, water content, pH, electrical conductivity, ignition loss, content volume and content weight
Required in the law & regulation	<ul style="list-style-type: none"> <li>• items specified in the Waste Management and Public Cleaning Act</li> <li>• nutrient items specified in the Fertilizer Control</li> <li>• toxic items specified in the Fertilizer Control Law</li> <li>• plant growth in fertilizer from pilot-scale compost: Cultivation test methods to evaluate harm to plants regulated by the MAFF</li> </ul>

**1-2-4. How do bioplastics and fossil-based plastics play in a circular economy?**

There is growing interest in bioplastics as one of the measures to tackle with climate change and plastic problems, and PLA is the representative. This trend has not started recently. From around 1992 to 2007, we worked on the business development of PLA. The PLA technological and market development began as biodegradable plastics in the early 1990s. This was triggered by insufficient plastics treatment after use as represented by an article with a photograph of fur seal entwined in marine fishing nets in 1989 [53] and an article to convey a heartfelt cry of “Japan will be buried by garbage” in 1994 [54]. As the number of composting facilities required for proper treatment did not increase and the market could not be expected to expand, an interest in PLA as bio-based plastics, that could contribute to the reduction of GHG emissions symbolized by the Kyoto Protocol, elevated.

Lessons learned from experience and knowledge through this transition of business concept are summarized as both bio-based plastics and biodegradable plastics, along with perspectives on recent global movement in circular economy.



**Figure 1-8.** Oil price and Corn price (1997 - 2020).



The essential components required for a circular economy as plastics products, both fossil-derived and bio-based, remains unchanged. For example, it should be noted that it is the regulations and social systems that were in place for the environment that hinder the advancement of environmentally sound products. In order to design a resilient and sustainable business model that is not swayed by fluctuations in raw material prices (Figure 1-8) [55], a holistic approach throughout value chain, from raw material, plastics, application, to the appropriate treatment after use, should be considered [4,40]. Furthermore, what people want is a social system designed with better looking and cooler concept, and products that are more hygienic, convenient, enjoyable and cheaper.

In particular for bioplastics, what needs to be considered with regard to raw materials is whether or not they are edible or non-edible [56], but also a broad perspective of SDGs such as child labor, land and water use and deforestation. Pilot and semi-commercial scale tests are desired rather than laboratory tests for social implementation in order to gain the understanding and trust of consumers and society, pilot scale composting test [50], Kassel project [57], Expo Aichi [58]. In addition, the expected effects and issues of new structures (PLA, polyhydroxyalkanoate (PHA), polytrimethylene terephthalate (PTT) and polyethylene furanoate (PEF)) should not be confused with those of existing structures (Bio PET, Bio PE and Bio PP) [59].

Common issues of bioplastics and fossil-based plastics and issues unique to bioplastics are summarized in Table1- 4. These learnings are expected to lead to guidance and inspiration for the future development to design materials and social system which support the environment.

**Table 1-4.** Common issues of bioplastics and fossil-based plastics and issues unique to bioplastics.

Plastics	Issues
Bioplastics and fossil-based plastics	1. It is the regulations and social systems that were in place for the environment that hinder the advancement of environmentally sound products.
	2. A resilient and sustainable business model should be designed, that is not swayed by fluctuations in raw material prices, considering throughout value chain.
	3. What people want is a social system designed with better looking and cooler concept, and products that are more hygienic, convenient, enjoyable and cheaper.
Bioplastics	1. What needs to be considered with regard to raw materials is whether or not they are edible or non-edible, but also a broad perspective of SDGs.
	2. Pilot scale tests are desired rather than laboratory tests for social implementation
	3. The expected effects and issues of new structures should not be confused with those of existing structures.

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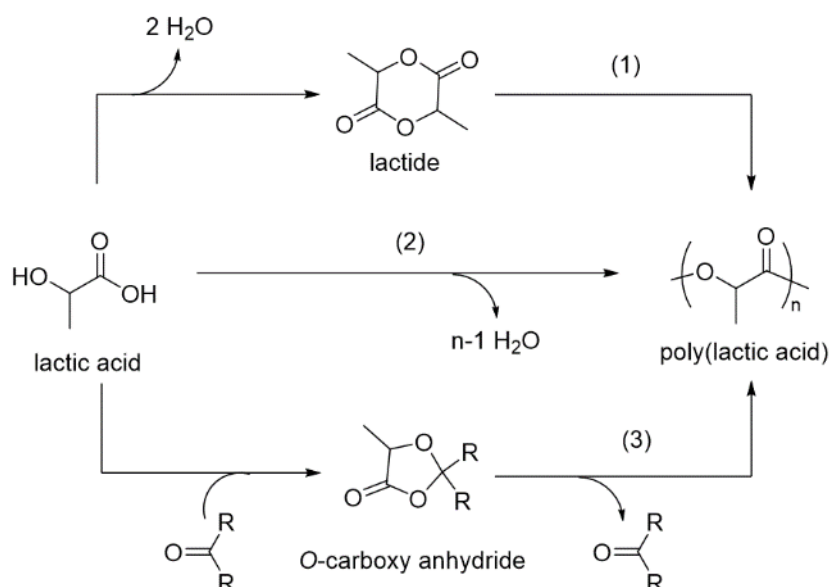
## Chapter 2

# Chain-extension of Low Molecular Weight Poly(lactic acid) (PLA) with Diisocyanate via Amide Formation to Enhance the Thermal Degradation Stability

### 2-1. Introduction

Poly(lactic acid) (PLA), generally used for films, molded products, and fibers, needs a weight-average molecular weight ( $M_w$ ) of over  $10 \times 10^4$  g/mol to give good processability and physical properties. Representative synthetic methods for PLA are (1) ring-opening polymerization (ROP) of lactide, the cyclic dimer of lactic acid (LA) [1], (2) direct polycondensation of LA [2, 3], and more recently, (3) ring-opening polymerization of *O*-carboxy anhydride of LA with aldehyde or ketone elimination [4], as shown Scheme 2-1.

Scheme 2-1. Synthetic methods for PLA.

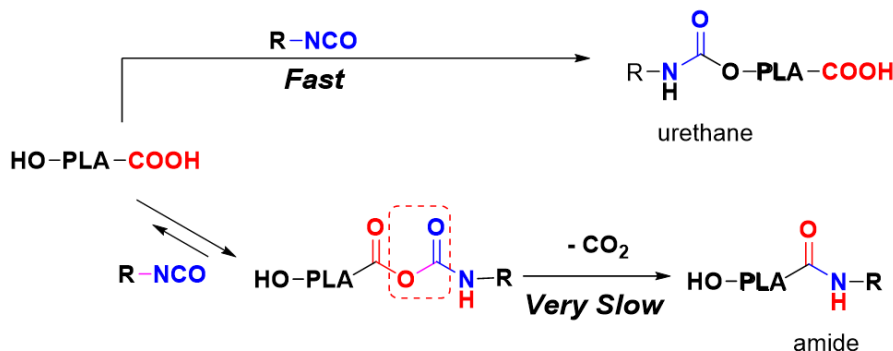


Among them, direct polycondensation is considered advantageous in terms of life cycle assessment [5, 6], but the high molecular weight (HMW) is difficult to achieve. Solid-state polymerization (SSP) [7] and chain extension reaction (CER) following direct polymerization are synthetic methods for HWM-PLA [8-14]. CER is useful not only for easily increasing the molecular weight of low molecular weight PLA (LMW-PLA) but also for expanding the range of polymer design, such as synthesizing block copolymers containing PLA segments by connecting different types of polymers or oligomers [15]. In addition, the CER methodology can potentially be applied to mechanical recycling or repair to restore the molecular weight from LMW-PLA, with decreasing molecular weight resulting from the recycling process [16].

Difunctional compounds such as diisocyanates [8-12], bisoxazolines [9,17,18], diepoxides

[19], diacid chlorides [16,20], acid anhydrides [20], bislactams [17], and carbodiimides [14] are commonly used as chain extenders for polyesters. These functional groups usually react with either hydroxy or carboxylic acid groups of PLA termini. Among them, diisocyanates can react with both the hydroxy and carboxylic acid groups (Scheme 2-2). There are several reports of diisocyanates as chain extenders of PLA. In chain extension with 1,6-hexamethylene diisocyanate (HDI) of PLA, synthesized by ring-open polymerization of lactide catalyzed by tin octanoate, an excess amount of diisocyanates relative to the hydroxy group was required to achieve HMW, where the obtained chain-extended PLA (cePLA) had an insoluble product [12]. It is assumed that the resulted urethane bond with active hydrogen reacted with other diisocyanates and formed branched structures, resulting in HMW-PLA by CER. When telechelic PLA with hydroxy groups at both ends, synthesized by the ROP of lactide with 1,4-butanediol, was used, the HMW-PLA was obtained using 1.0-1.2 equivalents of isocyanates to the hydroxy group of PLA [11]. Seppälä et al. [9] reported that CER via amide formation by the reaction with the carboxylic acid terminus of PLA and isocyanate is very slow, and they achieved HMW-PLA by sequential addition of bisoxazoline after diisocyanate addition to reacting with a terminal carboxylic acid.

**Scheme 2-2.** The reactions of isocyanate with hydroxy and carboxylic acid terminus of PLA.



In this study, the simple and efficient CER method was investigated to produce HMW-PLA from LMW-PLA using only diisocyanate as a chain extender for connecting both hydroxy and carboxylic acid termini. The reaction between isocyanate and a carboxylic acid, which is usually slow, was accelerated by using a catalyst. It is noted that the urethane bond, produced by the reaction of hydroxy group and isocyanate group, has lower thermal stability than the amide bond, produced by the reaction of carboxylic acid and isocyanate. In this study, more durable PLA against thermal degradation in the melting process was achieved by converting the hydroxy terminus to the carboxylic acid terminus to connect PLA chains with amide bonds.

## 2-2. Materials and Methods

### 2-2-1. Materials



An aqueous solution of lactic acid (LA) (90wt%) was purchased from Corbion Japan Co., Ltd. Succinic anhydride (SA) was purchased from Junsei Chemical Co., Ltd.

Magnesium acetylacetonate ( $\text{Mg}(\text{acac})_2$ ), magnesium stearate ( $\text{MgSt}_2$ ), magnesium chloride ( $\text{MgCl}_2$ ), magnesium oxide ( $\text{MgO}$ ), calcium stearate ( $\text{CaSt}_2$ ), tin(II) chloride dihydrate, and dibutyltin(IV) dilaurate (DBTDL) purchased from FUJIFILM Wako Pure Chemicals Corp.

Hexamethylenediamine diisocyanate (HDI), xylylene diisocyanate (XDI), diphenylmethane diisocyanate (MDI), hydrogenated xylylene diisocyanate ( $\text{H}_6\text{XDI}$ ), isophorone diisocyanate (IPDI), dicyclohexylmethane-4,4'-diisocyanate ( $\text{H}_{12}\text{MDI}$ ), and methanesulfonic acid (MSA) (purchased from TCI Co., Ltd.), and other commercially available reagents were used as received.

*Ortho*-dichlorobenzene (ODCB) and xylene (FUJIFILM Wako Pure Chemicals Corp.) were degassed through the nitrogen bubbling overnight to reduce the water content below 10 ppm.

Commercial PLA by ROP (NW 4032D produced by NatureWorks LLC) was stored in  $\text{N}_2$ -filled aluminum bags in a freezer and brought to room temperature prior to use.

## 2-2-2. Characterization

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were collected using  $\text{CDCl}_3$  solutions on a JEOL JNM-ECA500 spectrometer. Size-exclusion chromatography (SEC) was conducted on liquid chromatography (Waters Alliance e2695 Separations Modules) equipped with a Waters 2414 RI detector. Polymer samples were diluted in  $\text{CHCl}_3$  (mobile phase) and passed through SHODEX LF-G and LF-804 columns at 40 °C under a constant volumetric flow rate ( $1 \text{ mL min}^{-1}$ ).  $M_w$  and number average molecular weight ( $M_n$ ) were calculated referenced to polystyrene standards. Differential scanning calorimetry (DSC) was conducted on an SII DSC RDC220 differential scanning calorimeter. Thermogravimetric analysis (TGA) was performed on an SII TG-DTA320 thermogravimetric analyzer at  $10 \text{ }^\circ\text{C min}^{-1}$  to 500 °C under nitrogen atmosphere.

## 2-2-3. Analysis

### 2-2-3-1. Acid value (AV)

AV was defined as the millimolar potassium hydroxide (KOH) required to neutralize 1 g of the PLA. Low molecular weight PLA (0.5 g) was fully dissolved in 20 mL of a mixed solvent  $\text{CHCl}_3/\text{MeOH}$  (7/3 (v/v)). Then two drops of a mixed ethanol solution of bromothymol blue/phenol red as an indicator gave a yellow solution. Titration was carried out with a 0.1N ethanolic potassium hydroxide solution, and the AV of the PLA was determined with the point at which the color changed from yellow to lilac as the endpoint.

### 2-2-3-2. The ratio of terminal carboxylic acid ( $R_{\text{TCA}}$ )

Terminal carboxylic acid ratio ( $R_{\text{TCA}}$ ) was calculated by the amount of hydroxy group by  $^1\text{H}$  NMR and AV.  $^1\text{H}$  NMR spectra of PLA synthesized by post-reaction of SA or PLA obtained from

LA and SA were measured. In these spectra, (A)  $\delta = 2.61$  to  $2.72$  ppm (multiplet) was assigned as the methylene protons derived from succinic acid reacted with the terminal hydroxy group of PLA (4H), and (B)  $\delta = 4.36$  ppm ( $J = 6.92$  (quartet)) was assigned as the methine proton at the  $\alpha$ -position of the hydroxy end group of the PLA (1H). From these two integrated values [A] and [B],  $R_{TCA}$  was calculated using the following equation:

$$R_{TCA} (\%) = \frac{([A]/4) \times 2 + [B]}{([A]/4) \times 2 + [B] \times 2} \times 100$$

## **2-2-4. Preparation of low molecular weight PLA**

### **2-2-4-1. Hydrolytic degradation of commercial PLA**

Commercial PLA (NW 4032D, 50 g) was hydrolytically degraded in the presence of water (10 mL) in xylene (150 mL) at  $150$  °C for 24 h. The resulting PLA was solidified by cooling the solution, and then the white solid was filtrated and dried in vacuo.

### **2-2-4-2. Post-reaction of hydroxy terminus of PLA with SA**

Direct polycondensation of LA was carried out using 0.1 mol% tin(II) chloride dihydrate. The reaction was gradually reduced in vacuo and kept at 50torr for 2 h. After releasing the pressure to atmospheric pressure, xylene (10 wt% to LA) was added to the flask. The Dean-Stark trap was then replaced with a xylene-filled Dean-stark trap. The oil bath temperature was raised to  $180$  °C, and the inlet temperature was maintained at  $150$  °C. The reaction was continued at a reduced pressure (500 torr) for 20 h. After releasing the pressure to atmospheric pressure, Succinic anhydride (1 equiv. to hydroxy group) was added to the flask. The mixture was stirred at an oil bath temperature of  $150$  °C for 2 h to convert the terminal hydroxy group of the PLA into a carboxylic group. The xylene was stripped under a nitrogen stream, and the resulting solid was washed twice with 4 times the weight of 2-propanol containing 1% of 35% hydrochloric acid, filtered, washed several times with 5 times the volume of methanol, dried in vacuo at  $50$  °C, and colorless powder of PLA was obtained.

### **2-2-4-3. Telechilic PLA with carboxylic acid terminus by direct polycondensation in the presence of SA**

Direct polycondensation of 90% LA and 1.5 – 0.33 mol% of SA in 10 wt% xylene in the presence of MSA (0.45 mol%) was performed under reduced pressure at  $140 - 150$  °C for 12 h by azeotropic removal of the produced water. The obtained dry-solidified oligomer was subjected to solid-state polycondensation (SSP) at  $140$  °C for two or three days under a nitrogen stream until sulfur contents derived from MSA decreased to ca. 50 ppm.

### **2-2-5. Chain-extension reaction of LMW-PLA**

To LMW-PLA and Mg(II), and/or DBTDL, dried ODCB (to 50 wt% to the polymer) was

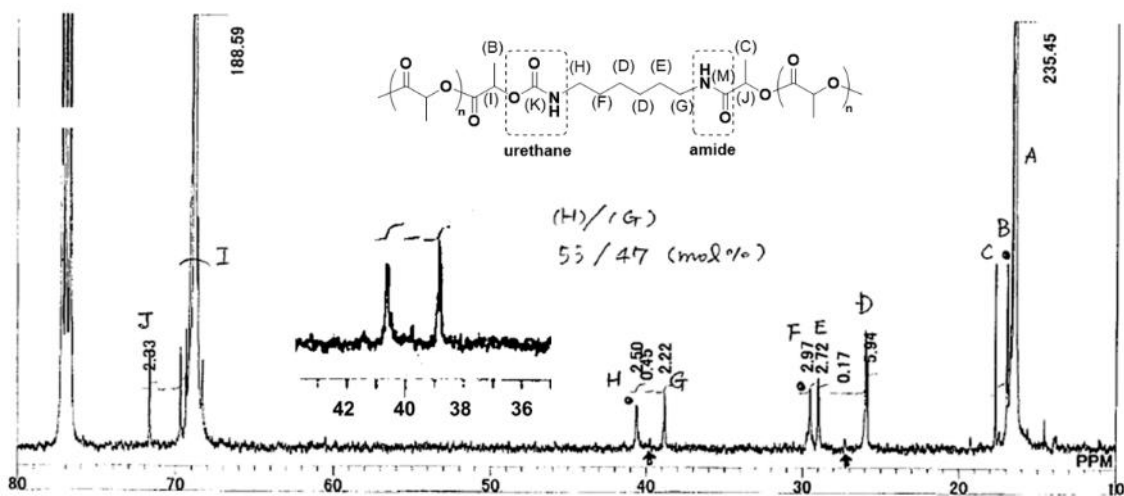
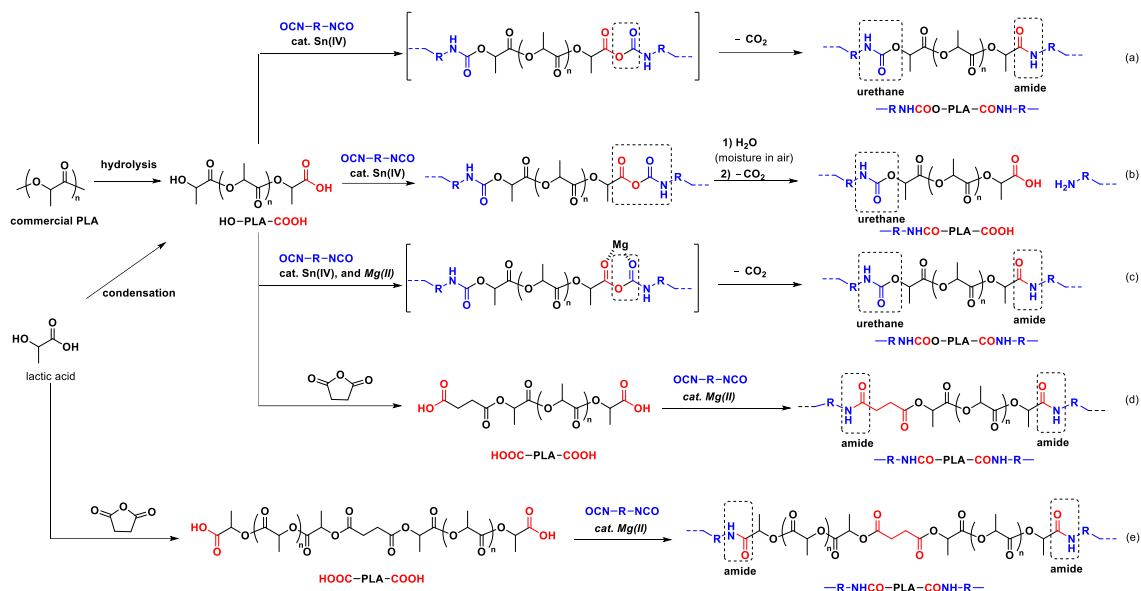
added in a 100 mL glass flask under a nitrogen atmosphere. To set the oil bath temperature at 150 or 190 °C, the LMW-PLA was fully dissolved, then HDI was added dropwise to the solution. After the appropriate reaction time, ODCB/xylene was added to quench the reaction. The resulting cePLA was solidified from the solution by cooling to room temperature, filtered, and dried in vacuo at 50 °C overnight, then dried at 100 °C for another day to give a colorless cePLA.

### 2-3. Results and Discussion

To confirm the potential reactivity of PLA having hydroxy and carboxylic acid terminus with diisocyanate, we prepared a model of LMW-PLA ( $M_w$   $4.1 \times 10^4$  g/mol,  $M_n$   $1.1 \times 10^4$  g/mol,  $AV$   $0.87 \times 10^{-4}$  mol/g) by hydrolysis of commercial PLA ( $M_w$ :  $20 \times 10^4$  g/mol). CER using HDI was investigated in the presence of DBTDL in ODCB at 150 °C. The molecular weights of the resulting PLA were determined by SEC using the aliquots of the reaction mixture at different times. The  $M_w$  increased with time and as the HDI amount increased, and the cePLA with a maximum of  $M_w$   $15 \times 10^4$  g/mol was obtained in 4 h. However, a few weeks of storing the resulting cePLA in the atmosphere at room temperature reduced the  $M_w$  in half ( $8.0 \times 10^4$  g/mol). It was assumed that the hydroxy and carboxylic acid termini would react with diisocyanate to form urethane and amide bonds, respectively, as shown in Scheme 2-3a. It was also assumed that although the reaction between the carboxylic acid termini and isocyanates produced a urethane anhydride intermediate without decarboxylation, resulting in once increasing the  $M_w$ , the urethane anhydride was hydrolyzed by moisture in the atmosphere at room temperature after the isolation as shown in Scheme 2-3b. Gürtler et al. [21] reported that reaction pathways of an isocyanate and an aliphatic carboxylic acid depend on the degree of substitution of the  $\alpha$ -carbon of the aliphatic carboxylic acid. Because the LA has a methyl group at the  $\alpha$ -position of the carboxylic acid, the decarboxylation step to form an amide bond probably did not proceed efficiently, resulting in the remaining urethane anhydrides at a high proportion, which was easily hydrolyzed in the end.

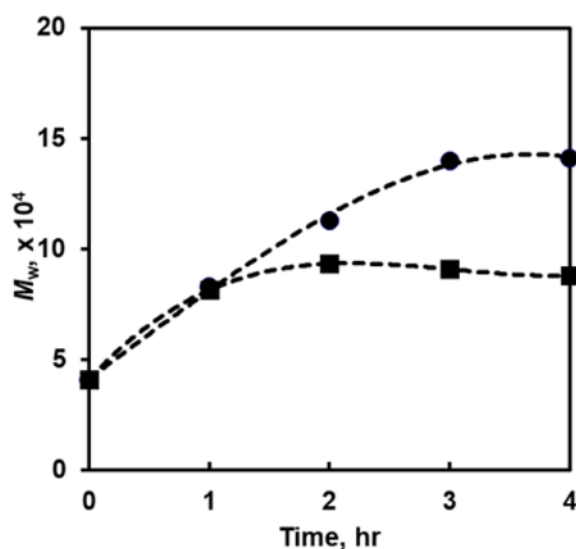
Concerning the desired reaction path to affording a stable amide bond, a catalyst was applied to promote the decarbonylation of urethane anhydride to convert into an amide bond. Gürtler et al. [21] reported the catalysis of several metal salts for the selective reaction of aliphatic isocyanates with carboxylic acids giving amides by effective elimination of carbon dioxide, where magnesium and calcium salts increase reaction rates while improving the selectivity of amide formation through decarbonization when sterically hindered carboxylic acids were used. In this study, DBTDL catalyzed a urethane formation and  $Mg(acac)_2$  catalyzed an amide formation (Scheme 2-3c). The formation of both of amide and urethane bond in the cePLA using the coexistence of the two catalysts was confirmed by  $^{13}C$  NMR, in which the peaks of 39 and 41 ppm were assigned as the  $\alpha$ -carbon of the hexamethylene unit adjacent to the amide and urethane bonds, respectively (Figure 2-1). On the other hand, when only  $Mg(acac)_2$  was used,  $M_w$  increased only up to  $8.0 \times 10^4$  g/mol, suggesting that  $Mg(acac)_2$  did not lead to urethane formation (Figure 2-2).

**Scheme 2-3.** All synthetic routes of CER of PLA with diisocyanate in this chapter.



**Figure 2-1.**  $^{13}\text{C}$  spectrum of cePLA synthesized by CER of LMW-PLA using HDI.

TGA revealed that the temperature of 5% weight loss ( $T_{d5\%}$ ) of the resulting cePLA was 259 °C, which was relatively low compared to commercial PLA by ROP ( $T_{d5\%}$  334 °C). Urethane bonds are known to degrade at ca. 200 - 250 °C [22], suggesting that the presence of urethane bonds led to lower thermal stability than did commercial PLA by ROP.



**Figure 2-2.** CER of PLA ( $M_w 4.1 \times 10^4$ ) with HDI in the presence of  $Mg(acac)_2$  with DBTDL (●), and without DBTDL (■).

To investigate the CER of PLA with carboxylic acid termini in more detail, well-defined telechelic PLA was prepared by direct polycondensation of LA in the presence of 1 mol% SA (Scheme 2-3e). The obtained LMW-PLA had  $M_w$  of  $1.0 \times 10^4$  g/mol,  $M_n$  of 0.72 to  $3.46 \times 10^4$  g/mol and the  $R_{TCA}$  was over 99%, confirmed by  $^1H$  NMR. CER of the carboxylic acid functional telechelic PLA was carried out using  $Mg(acac)_2$  in ODCB by heating with an oil bath thermostatted at 190 °C (Table 1-1, entry 1). A half molar equivalent of HDI was used for the number of polymer termini. (i.e.  $[NCO]_0/[AV]_0 = 1.0$ ,  $[NCO]_0$  stands for an initial isocyanate molar concentration). After the reaction, the polymer solution was highly viscous and diluted by ODCB, solidified by cooling the solution. The resulted cePLA was filtered to yield over 90%. Both  $M_n$  and  $M_w$  increased to about 13 times to the initial LMW-PLA respectively ( $1.02 \times 10^4$  to  $13.0 \times 10^4$  g/mol,  $2.14 \times 10^4$  to  $27.9 \times 10^4$  g/mol).  $T_{d5\%}$  of these cePLA was over 300 °C measured by TGA. These results indicate that the amide bond derived from the carboxylic acid terminus provided high thermal stability than did the urethane bond derived from the hydroxy terminus.

It was confirmed that the CER did not proceed without a catalyst.  $MgSt_2$ ,  $MgCl_2$ ,  $MgO$ , and  $CaSt_2$  also worked for the catalysis of CER via amide formation (Table 1-1, entries 2-5). Based on the availability of the catalyst,  $MgSt_2$  will be more suitable for practical use.

Varying the SA feed from 0.33, 0.5, and 1.5 mol% to LA, various LMW-PLAs having  $M_w$  of 1.5, 3.9,  $6.0 \times 10^4$  g/mol,  $M_n$  of 0.7, 2.3,  $3.5 \times 10^4$  g/mol and  $R_{TCA}$  over 99% were prepared. The CER of these LMW-PLA afforded cePLA of  $M_n > 9 \times 10^4$  g/mol and  $M_w > 20 \times 10^4$  g/mol (Table 2-1, entries 6-8). The melting temperature ( $T_m$ ) of obtained cePLA was correlated by the used LMW-PLA, which means the used initial LMW-PLA of  $T_m$  affects the final  $T_m$  directly. These

thermal properties are summarized in Tables 2-2 and 2-3.

**Table 2-1.** CER of telechelic PLA prepared initial SA feed.<sup>a</sup>

$$\text{HOOC-PLA-COOH} \xrightarrow[\text{ODCB, 190}^\circ\text{C}]{\text{R(NCO)}_2, \text{catalyst}} \left( \text{H-N-R-N-H} \begin{array}{c} \text{O} \\ \parallel \\ \text{PLA} \end{array} \begin{array}{c} \text{O} \\ \parallel \\ \end{array} \right)_n$$

entry	Initial PLA <sup>b</sup>				R(NCO) <sub>2</sub> <sup>f</sup>	catalyst				SEC <sup>c</sup>		
	M <sub>n</sub> , <sup>c</sup> x 10 <sup>4</sup>	M <sub>w</sub> , <sup>c</sup> x 10 <sup>4</sup>	COOH ratio, <sup>d</sup> %	AV <sub>n</sub> , <sup>e</sup> mmol/g		[NCO]/ [COOH]	mol% vs [COOH]	time., hour	M <sub>n</sub> , x 10 <sup>4</sup>	M <sub>w</sub> , x 10 <sup>4</sup>	Đ	
1	1.0 <sub>2</sub>	2.1 <sub>4</sub>	>99	0.287	HDI	1.01	Mg(acac) <sub>2</sub>	1.0	2	13.0	27.9	2.1 <sub>5</sub>
2						1.03	MgSt <sub>2</sub>	1.0	2	17.0	37.0	2.1 <sub>8</sub>
3						1.03	MgCl <sub>2</sub>	1.0	2	12.9	27.4	2.1 <sub>2</sub>
4						1.00	MgO	2.0	3	12.3	25.6	2.0 <sub>8</sub>
5						1.03	CaSt <sub>2</sub>	1.1	3	14.8	29.1	1.9 <sub>7</sub>
6	0.7 <sub>2</sub>	1.4 <sub>5</sub>	>99	0.403		1.00	MgSt <sub>2</sub>	0.33	2	10.7	22.0	2.0 <sub>6</sub>
7	2.3 <sub>4</sub>	3.9 <sub>4</sub>	>99	0.162		1.00		1.0	2	13.2	30.0	2.2 <sub>7</sub>
8	3.4 <sub>6</sub>	5.7 <sub>8</sub>	>99	0.122		1.02		1.1	1	9.3	21.6	2.3 <sub>2</sub>
9	1.0 <sub>5</sub>	23.0	>99	0.272	HDI	1.00	MgSt <sub>2</sub>	0.45	4	12.3	29.0	2.3 <sub>6</sub>
10					XDI	1.00		0.46	1	17.6	37.4	2.1 <sub>3</sub>
11					MDI	1.01		0.45	4	13.7	29.6	2.1 <sub>6</sub>
12					H <sub>6</sub> XDI	1.00		0.46	4	8.3	17.1	2.0 <sub>6</sub>
13					IPDI	1.01		0.45	4	5.5	10.6	1.9 <sub>1</sub>
14					H <sub>12</sub> MDI	1.01		0.45	4	4.9	9.3	1.9 <sub>0</sub>

<sup>a</sup> CER was carried out in ODCB at 190°C (oil bath temperature) under N<sub>2</sub>. See materials and method section in detail. <sup>b</sup> The initial PLA (LMW-PLA) was prepared by direct polycondensation of LA with SA following solid-state polymerization. The amount of SA feed controlled the molecular weight. <sup>c</sup> Measured by SEC (CHCl<sub>3</sub> eluent, 1 mL/min., polystyrene standard). <sup>d</sup> Calculated by <sup>1</sup>H NMR. <sup>e</sup> Estimated by titration. <sup>f</sup> Diisocyanate.

The versatility of common diisocyanate species, including HDI, XDI, MDI, H<sub>6</sub>XDI, IPDI, and H<sub>12</sub>MDI was examined (Table 1-1, entries 9-14). The molecular weight increased with respect to reaction time (Figure 2-3), and all of the listed diisocyanates increased M<sub>n</sub> more than four times. Among them, when XDI was used, the molecular weight was increased fastest, which reached M<sub>n</sub> of 17.6 × 10<sup>4</sup> and M<sub>w</sub> of 37.0 × 10<sup>4</sup> g/mol in 1 h. It was noted that all the cePLAs listed in Table 2-1 was fully dissolved in CHCl<sub>3</sub>, and did not found any insoluble parts.

**Table 2-2.** LMW telechelic PLA synthesized by SSP.<sup>a</sup>

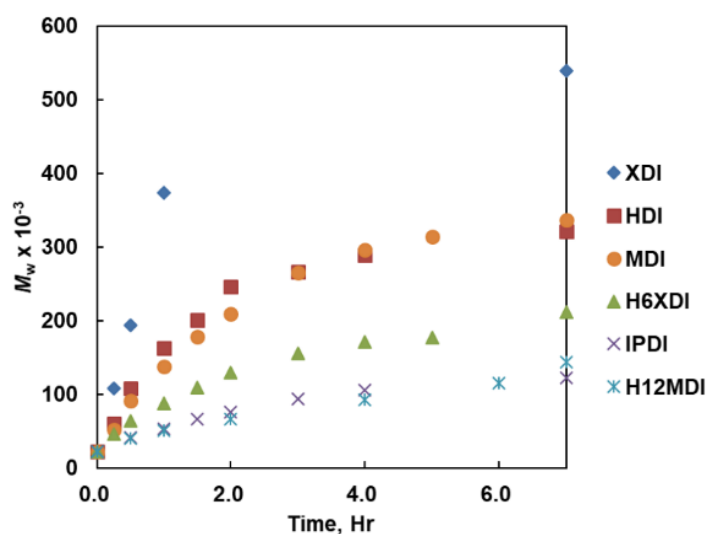
entry	PLA <sup>b</sup> Initial PLA in Table1	SA, <sup>c</sup> mol%	M <sub>n</sub> , <sup>d</sup> x10 <sup>4</sup>	M <sub>w</sub> , <sup>d</sup> x10 <sup>4</sup>	Đ <sup>d</sup>	1 <sup>st</sup> heating <sup>e</sup>		2 <sup>nd</sup> heating <sup>e</sup>					
						T <sub>m</sub> , <sup>f</sup> °C	ΔH <sub>m</sub> , <sup>g</sup> J/g	T <sub>g</sub> , <sup>h</sup> °C	T <sub>c</sub> , <sup>i</sup> °C	ΔH <sub>c</sub> , <sup>j</sup> J/g	T <sub>m1</sub> , <sup>f</sup> °C	T <sub>m2</sub> , <sup>f</sup> °C	ΔH <sub>m</sub> , <sup>g</sup> J/g
1	entry 6	1.5	0.72	1.45	2.0 <sub>3</sub>	155	48	55	113	42	143	154	40
2	others <sup>k</sup>	1	1.08	2.20	2.0 <sub>4</sub>	160	58	58	113	41	151	159	41
3	entry 7	0.5	2.34	3.94	1.6 <sub>9</sub>	166	66	59	109	38	159	165	47
4	entry 8	0.33	3.46	5.78	1.6 <sub>7</sub>	168	70	60	111	45	162	nd	48

<sup>a</sup> See experimental in detail for the polymerization conditions. <sup>b</sup> Corresponding to initial PLA in Table 1. <sup>c</sup> SA: succinic anhydride, based on LA feed. <sup>d</sup> Measured by SEC (CHCl<sub>3</sub> as eluent, PS standard). <sup>e</sup> Measured by DSC (heating ratio 10°C/min). <sup>f</sup> Melting temperature. nd: not detected <sup>g</sup> Melting enthalpy. <sup>h</sup> Glass transition temperature. <sup>i</sup> Crystallization temperature. <sup>j</sup> Crystallization enthalpy. <sup>k</sup> Corresponding to the initial PLA in Table 1, entry 1-5 and 9-14.

**Table 2-3.** Thermal properties of cePLA from LMW-PLA prepared by copolymerization with SA.<sup>a</sup>

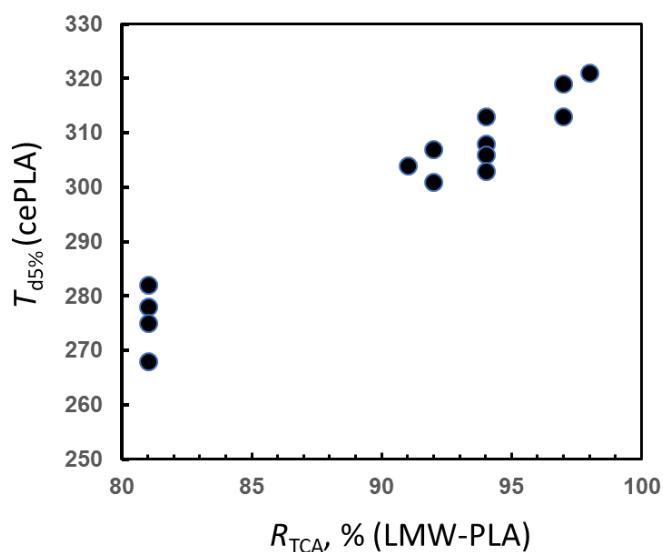
entry <sup>c</sup>	1 <sup>st</sup> heating <sup>b</sup>		2 <sup>nd</sup> heating <sup>b</sup>				
	$T_m$ , <sup>d</sup> °C	$\Delta H_m$ , <sup>e</sup> J/g	$T_g$ , <sup>e</sup> °C	$T_c$ , <sup>f</sup> °C	$\Delta H_c$ , <sup>g</sup> J/g	$T_m$ , <sup>d</sup> °C	$\Delta H_m$ , <sup>e</sup> J/g
6	146	45	59	nd	0	nd	0
2	150	46	59	nd	0	151	1
7	157	49	60	131	15	157	11
8	160	55	60	128	26	161	28
10	153	52	57	nd	1.4	150	2
11	152	52	60	nd	0	nd	0
12	153	53	57	nd	0.6	151	1
13	154	56	58	nd	3.6	154	4
14	152	51	57	nd	0.7	151	3

<sup>a</sup> See experimental in detail for the polymerization conditions. <sup>b</sup> DSC conditions: 1st heating; rt to 250 °C, 10 °C/min, cooling: rapid cooling, 2nd heating; 25 to 250 °C. nd: not detected. <sup>c</sup> Corresponding to Table 1. <sup>d</sup> Melting temperature. <sup>e</sup> Melting enthalpy. <sup>f</sup> Glass transition temperature. <sup>g</sup> Crystallization temperature. <sup>h</sup> Crystallization enthalpy.

**Figure 2-3.** CER of telechelic PLA having carboxylic termini with various diisocyanate using MgSt<sub>2</sub> catalyst.

Thinking of a practical approach to applying hydrolytically degraded LMW-PLA for mechanical recycling, the difunctional telechelic PLA with carboxylic acids at both ends was prepared by post-reaction of LMW-PLA with a hydroxy terminus with SA. As a model of hydrolytically degraded PLA, after the polymerization of LMW-PLA with  $M_w$  of about  $2.0 \times 10^4$  g/mol using tin(II) chloride, the post-reaction using one equivalent of SA to the molar concentration of hydroxy terminus reached around 90% conversion ( $R_{TCA}$  94%), which was confirmed by <sup>1</sup>H NMR. CER of the dried LMW-PLA ( $M_w$   $2.0 \times 10^4$  g/mol, AV 0.227 mol/g) was

performed using 1.06 equiv. (to  $[AV]_0$ ) of HDI in the presence of  $Mg(acac)_2$  (30 ppm as Mg) in ODCB at 130 °C as shown in Scheme 2-3d. HMW-cePLA having  $18.0 \times 10^4$  g/mol of  $M_w$  was obtained after 1h. The resulting cePLA was not decreased its molecular weight under the atmosphere. The thermal degradation of the cePLA was evaluated by TGA. The relationship between  $T_{d5\%}$  of cePLA and the  $R_{TCA}$  of LMW-PLA used to CER was shown in Figure 2-4.  $T_{d5\%}$  was up to 321 °C for the cePLA from carboxylic acid group enriched PLA, although  $T_{d5\%}$  was 259 °C for the cePLA from unmodified low molecular weight PLA.



**Figure 2-4.** Relationship between  $R_{TCA}$  of LMW PLA used to CER. and thermal degradation temperature of cePLA.

Although thermal degradation should be affected by several factors, such as the types and concentration of terminal groups, catalytic residues, the figure shows that the  $T_{d5\%}$  increases as the  $R_{TCA}$  of LMW-PLA used for CER increases, indicating that the amide bond contributes to the increased resistance to thermal degradation. Thermal and mechanical properties of cePLAs, which were synthesized by CER of LMW-PLA at the hydroxy termini with or without SA, respectively, and commercial PLA were compared in Table 2-4. The cePLA, derived from a reaction of the hydroxy terminus of LMW-PLA with SA, showed a similar heat resistance compared to the commercial PLA. The physical properties of the representative cePLA were measured by the compression-molded films. The tensile modulus (TM) and tensile strengths (TS) were 3.5 GPa and 70 MPa, respectively. These were comparable to commercial PLA by ROP (3.6 GPa and 75 MPa, respectively).

The biodegradability of the cePLA was also confirmed based on ISO 14855-1 (JIS K6953-1:2000), in which the cePLA showed almost identical degradability to commercial PLA (Figure

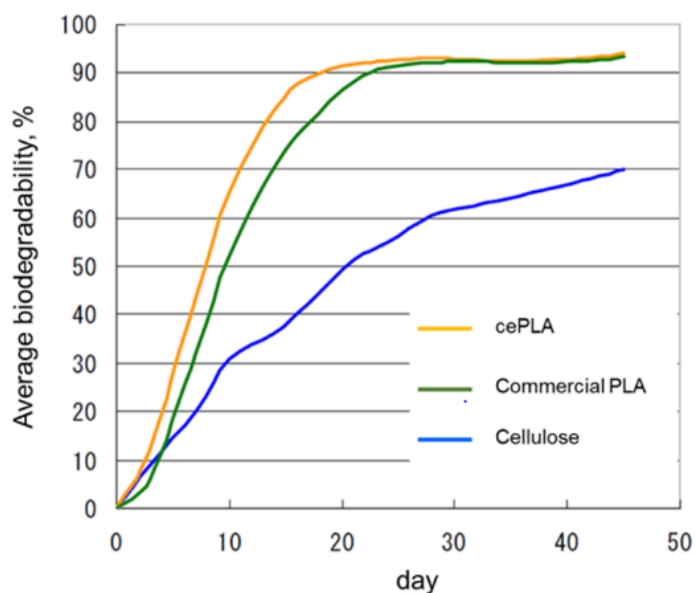


2-5).

**Table 2-4.** Comparison of thermal and mechanical properties of cePLA and commercial PLA.

		cePLA		Commercial PLA
Synthetic methods		CER by HDI		ROP of Lactide
Enchainment type		Urethane/Amide	Amide/Amide	-
LMW-PLA	$M_w, \times 10^4$	4.1	2.0	-
	$M_n, \times 10^4$	1.1	1.0	-
	$R_{TCA}, \%$	50	94	-
HMW-PLA	$M_w, \times 10^4$	14	20	20
	$M_n, \times 10^4$	7	7	10
Thermal Properties	$T_g, ^\circ\text{C}$	60	60	61
	$T_m, ^\circ\text{C}$	159	156	166
	$T_{d5\%}, ^\circ\text{C}$	259 <sup>a</sup>	321 <sup>a</sup>	339
Mechanical Properties	TM, <sup>b</sup> GPa	N/A <sup>d</sup>	3.5	3.6
	TS, <sup>c</sup> MPa	N/A <sup>d</sup>	70	75

<sup>a</sup> Stabilizer was not added. <sup>b</sup> TM: Tensile Modulus. <sup>c</sup> TS: Tensile Strength. <sup>d</sup> N/A: Not available.



**Figure 2-5.** Biodegradability test based on JIS K6953-1:2000.

Materials: cePLA ( $M_n$ ;  $7.15 \times 10^4$ ,  $M_w$ ;  $22.6 \times 10^4$  g/mol)

Commercial PLA: NW 4032D

Cellulose: TLC grade, particle size < 20mm

## 2-4. Conclusion

The CER method was expected to be a more efficient polymerization method and a method for synthesizing new copolymers.

In this study, the new CER method of producing HMW-PLA using diisocyanate as a chain extender was developed, and the findings are summarized here.

- i. Although the reaction between the carboxylic acid termini of PLA and isocyanate is slower than the urethane bond formation between the hydroxy termini of PLA and diisocyanate, Mg(II) catalyst effectively catalyzed the amide bond formation between carboxylic acid termini of PLA and diisocyanate.
- ii. Telechelic PLA with carboxylic acid terminus was prepared by the following two methods: reaction of the hydroxy termini of LMW-PLA prepared by direct polycondensation with SA, or direct polycondensation of LA in the presence of SA. These LMW-PLA samples gave HMW-PLA via amide formation by CER.
- iii. The cePLA obtained via amide formation had a higher thermal decomposition resistance than did the cePLA with urethane bond, which reflected the difference in their thermal stability.
- iv. The mechanical properties and biodegradability of cePLA from the CER of LMW-PLA converted the hydroxy terminus to carboxylic acid by SA were comparable to commercially available PLA.

The CER via amide bond formation is expected to efficiently convert LMW-PLA to HMW-PLA with durable to thermal degradation through converting the hydroxy terminus into a carboxylic acid. This methodology will be helpful and meaningful for the mechanical recycling of degraded commercial PLA and from a perspective of “product repair” in the circular economy business model. Furthermore, the efficient CER method is expected to be a new manufacturing method of PLA and copolymers with new functions.

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## Chapter 3

# Isolation of a *Nocardiopsis Chromatogenes* Strain that Degrades PLA in Pig Waste-based Compost

### 3-1. Introduction

To construct a feasible framework for PLA waste management, further studies on the factors and mechanisms influencing biodegradability are necessary. The factors to consider include types of compost, microorganisms, enzymes involved in degradation, physical properties of PLA, and treatment conditions such as temperature, pH, and time. The composition of compost depends on the types of waste and the composting facility. Furthermore, even at the same composting facility, the degradability of PLA varies under different composting conditions, including the microbiota.

This study investigated the composition of 15 different composts based on animal wastes. The compost consisted of pig dung, cow dung, horse dung, or chicken droppings together with organic house wastes. The efficiency of PLA degradation was screened at a relatively low temperature (e.g., 37 °C). On-Farm Composting Methods, which contains guidance published by the Food and Agriculture Organization, states that in the aerobic composting process, microorganisms decompose organic matter to generate heat, which accelerates the breakdown of proteins [1]. The resulting ammonia accelerates hydrolysis of PLA at higher temperatures. To focus on the isolation of microorganisms capable of degrading PLA, the temperature was regulated at 37 °C to avoid promoting chemical decomposition. Emulsifiers such as those developed for PLA by Pranamuda et al. [2] are often used to isolate PLA-degrading microorganisms. In the current study, emulsifiers were removed afterwards to avoid the possibility of selecting microorganisms that assimilate these compounds. In this study a microorganism strain was isolated from the most efficient pig-dung compost that was 99.2% homologous to *Nocardiopsis chromatogenes* (hereinafter referred to as *N. chromatogenes*) by 16S rRNA gene analysis. Furthermore, our strain exhibited physiological characteristics of a *Nocardiopsis* species that was reported previously [3]. This is the first study focusing on screening animal waste-based composts from farms and facilities, leading to the isolation of a strain of *N. chromatogenes*.

### 3-2. Materials and Methods

#### 3-2-1. Materials

##### 3-2-1-1. Compost

Fifteen different types of matured compost were collected. They originated from nine operating municipal composting facilities in Japan. Among them, eight were based on pig dung, four on cow dung, one on horse dung, one on chicken droppings, and one on a mixture of cow dung and chicken droppings along with organic house wastes.

### 3-2-1-2. PLA

A PLA cast film was used in the compost screening test. The film measured  $3 \times 5$  cm with a thickness of 20–30  $\mu\text{m}$ . The film was produced by laboratory-sale T-die extrusion using the PLA resin LACEA™ (Mitsui Chemicals). LACEA™ is a BPS-certified Green Plastic (PL#40701). The PLA resin in our study was derived from our pilot facility using a direct polymerization process of lactic acid [4,5] with a  $M_w$  of  $1.34 \times 10^5$ ,  $M_w/M_n = 3.0$ , D ratio of 1.2%, and residual lactide  $> 0.01\%$ . It showed  $T_m$ : 165 °C and  $T_g$ : 58 °C. These properties are very similar to commercially available PLA produced by NatureWorks (Plymouth, MN, USA). PLA powder (40–50  $\mu\text{m}$  radius) was used to prepare PLA microspheres (1–5  $\mu\text{m}$  radius).

### 3-2-1-3. Reagents

Glycerol, L-asparagine,  $\text{K}_2\text{HPO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , NaCl,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , and  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  were purchased from FUJIFILM Wako Pure Chemical Corp. (Osaka, Japan). Bacto agar, which was produced by Becton, Dickinson and Company (NJ, USA), was added to a glycerol/ asparagine-based medium.

Plysurf A 210G, which was produced by DKS, Co. Ltd. (Kyoto, Japan), was used as an emulsifier to disperse PLA microspheres.

TE buffer (TE: Tris and EDTA, Tris: 2-Amino-2-(hydroxymethyl) propane-1,3-diol; EDTA: 2,2',2'',2'''-(ethane-1,2-diyl)dinitrilo) tetraacetic acid) was also purchased from FUJIFILM Wako Pure Chemical Corp. (Osaka, Japan).

## 3-2-2. Methods

### 3-2-2-1. Screening of composts for PLA film degradation (primary screening)

Fifteen composts from nine municipal composting facilities were placed in separate jars with PLA films. The jars, which had a dimension of  $10 \times 10 \times 11.5$   $\text{cm}^3$  and contained PLA films, were placed in an incubator at 37 °C for three months to suppress non-enzymatic degradation of PLA. Two-thirds of the 5 cm PLA film was inserted vertically into the jar with the compost (Figure 3-1).



**Figure 3-1.** PLA film degradation test with the compost in a jar.

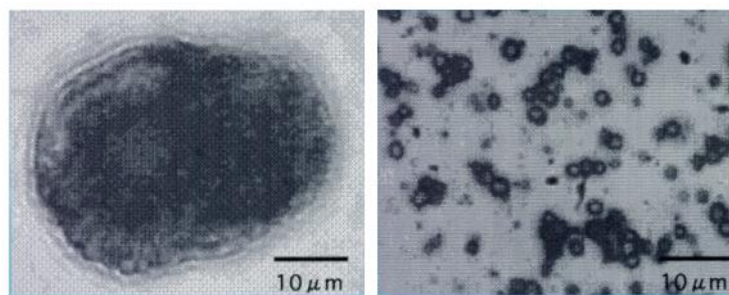
### 3-2-2-2. Further evaluation of selected composts and collection of microorganisms (secondary screening)

One compost selected from the primary screen was subject to PLA film degradation at 37 °C for 6 months. During the test period, the sample was occasionally sprayed with water to avoid drying.

### 3-2-2-3. Single-colony isolation of PLA-degrading microorganisms on agar plates

Strain screening used a glycerol/asparagine-based medium, which was previously reported in 1993 [6]. Two-layer agar plates were prepared. The bottom layer was prepared using a glycerol/asparagine-based medium with a composition of 0.5 g/L glycerol, 0.5 g/L L-asparagine, 0.5 g/L K<sub>2</sub>HPO<sub>4</sub>, 0.5 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g/L NaCl, 1 mg/mL FeSO<sub>4</sub>·7H<sub>2</sub>O, 1 mg/mL MnCl<sub>2</sub>·4H<sub>2</sub>O, and 1 mg/mL ZnSO<sub>4</sub>·7H<sub>2</sub>O at a pH of 7.0. To the medium was added 20 g of Bacto agar. The resultant mixture was autoclaved at 120 °C for 20 min and poured into petri dishes.

The top layer was prepared using PLA powder (40–50 μm radius). As shown in Figure 3-2, 1 g of PLA powder was dissolved in 40 mL of methylene chloride. The resultant solution was emulsified with 1 L of 100 mg/L of emulsifier Plysurf A 210G. The emulsion was subjected to a warm bath and an evaporator to evaporate the methylene chloride. The resultant PLA microsphere particles were collected by filtration followed by repeated washing and filtration to remove the emulsifier. The collected PLA microsphere particles were mixed with 1 L of the same glycerol/asparagine broth as for the bottom layer, and following the addition of 20 g of Bacto agar, the PLA-containing medium was autoclaved and poured onto the bottom layer of the petri dishes.



**Figure 3-2.** Photos of the PLA powder and microspheres. Average size of PLA powder (left) is 40–50 μm and that of a PLA microsphere (right) is 1–5 μm.

### 3-2-2-4. Isolation of strains from the agar plates

After six months of secondary screening, degradation of PLA film and growth of microorganisms on the degraded portion was observed. The microorganisms were collected with an inoculation loop and streaked on a two-layer agar plate of which the top layer contained PLA. The plates were incubated at 37 °C for about two weeks until clear zones were visible. The clear

zone was scraped with an inoculation loop and streaked onto new agar plates to enrich and isolate single colonies. This process was repeated four times.

At the end, a surface of a PLA film, which was placed in the medium described in 3-3 together with the isolated microorganism, was observed by Scanning Electron Microscope, JSM-840A, provided by JEOL Ltd. (Tokyo, Japan).

### **3-2-2-5. Characterization of isolated strains and phylogenetic analysis**

The cell wall chemotype [7,8,9] were analyzed by Japan Food Research Laboratories (Tokyo, Japan). The colonies on each plate were collected and resuspended in TE buffer (TE: Tris and EDTA, Tris: 2-Amino-2-(hydroxymethyl) propane-1,3-diol; EDTA: 2,2',2'',2'''-(ethane-1,2-diyldinitrilo) tetraacetic acid) to extract the total genomic DNA. The cells were disrupted by a vortex with glass beads. Then a clear lysate was prepared via centrifugation (7,000 g) to remove the debris. The lysate was subsequently subject to PCR (polymerase chain reaction) amplification using the primers 27f (5'-GAGTTTGATCCTGGCTCAG-3') and 1525r (5'-AGGAGGTGATCCAGCC-3'). The portion coded for 16S rRNA was obtained with an initial denaturation at 95 °C for 5 min and 30 cycles at 95 °C for 1 min, annealing at 54 °C for 1 min, a primer extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The PCR product was purified using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The 16S rRNA gene sequences were obtained using the dRhodamine Dye Terminator Cycle Sequencing Kit on an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA) with Genetyx software. The 16S rRNA gene sequences were manually assembled from a combination of separate fragments generated with the following primers: 27f (5'-GAGTTTGATCCTGGCTCAG-3'), 357f (5'-CTCCTGCGGGAGGCAGCAG-3'), 536f (5'-CCAGCAGCCGCGGTAATAC-3'), 803f (5'-GATTAGATACCTGGTAGT-3'), 1114f (5'-GCAACGAGCGCAACCC-3'), 519r (5'-GTATTACCGCGGCTGCTGGC-3'), 907r (5'-CCGTCAATTCCTTTGAGTTT-3'), 1385r (5'-CGGTGTGTACAAGGCC-3'), and 1525r (5'-AGGAGGTGATCCAGCC-3'). The complete sequence was identified using the BLAST (Basic Local Alignment Tool) program (NCBI; <http://www.ncbi.nlm.nih.gov/>) and aligned against reference strain sequences. Phylogenetic analysis was performed using the neighbor-joining algorithm of MEGA5 software [10] with bootstrap analysis based on 1000 re-samplings of the dataset. The obtained sequence data was submitted to the DNA Data Bank of Japan (DDBJ).

## **3-3. Results and Discussion**

### **3-3-1. Screening of composts for PLA film degradation (primary screening)**

The films were inspected visually every 2 weeks during the screening for PLA film degradation in the 15 composts. The pig dung-based composts No. 2, 3, 9, 12, 13, and 15 degraded the PLA film, whereas two composts of the same type, No. 11 or 14, did not. The cow dung-based



composts (No. 1, 5, 7, 8), the horse dung-based compost (No. 4), the chicken dropping-based compost (No. 6), or the mixture (No. 10) did not degrade PLA film. The PLA film degraded in six composts derived from pig dung after three months of composting in an incubator at 37 °C (Tables 3-1 and 3-2).

**Table 3-1.** Origin, main component, and ability to degrade PLA films.

Municipal composting facility <sup>a</sup> in Japan		Main compost component	Degradation <sup>b</sup>
Hokkaido A (Hayakita)	#1	cow dung-based	N
Hokkaido B (Kita-Hiroshima)	#2	pig dung-based	Y
Aomori	#3	pig dung-based	Y
Aomori	#4	horse dung-based	N
Yamagata	#5	cow dung-based	N
Yamagata	#6	chicken dropping-based	N
Niigata	#7	A cow dung-based	N
Niigata	#8	B cow dung-based	N
Niigata	#9	pig dung-based	Y
Chiba	#10	cow dung- and chicken dropping-based	N
Nagano	#11	pig dung-based	N
Nagano	#12	pig dung-based	Y
Aiichi	#13	pig dung-based	Y
Aiichi	#14	pig dung-based	N
Kagoshima	#15	pig dung-based	Y

<sup>a</sup>Composting facilities provided the compost at the authors' request.

<sup>b</sup>Y: degraded, N: not degraded.

**Table 3-2.** Number of compost facilities and ID number of samples capable of degrading PLA films by the main compost component.

Main compost component	Number of composting facilities	Numbers of compost samples and the ID numbers of samples capable of degrading PLA films
Pig dung	8	6 (#2, #3, #9, #12, #13, #15)
Cow dung	4	0
Chicken droppings	1	0
Horse dung	1	0
Cow dung and chicken droppings	1	0

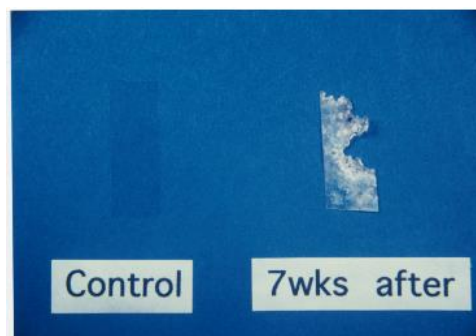
Since the temperature was controlled at 37 °C and ammonia generation by the protein decomposition was suppressed, the hydrolysis of PLA was not accelerated. It is speculated that degradation of PLA occurs only if the microorganisms to degrade PLA exist in the composts. In this experiment, no microorganisms capable of degrading PLA at 37 °C were present in compost derived from cow dung, chicken droppings, or horse dung, but such organisms were present in compost derived from pig dung.

A previous report examined pig dung- and cow dung-based composts for degradation of food

wastes in 2009 [11]. That study compared the process of composting between cow dung/food waste and pig manure/food waste. The results revealed that the number of bacteria was about two orders of magnitude higher in the pig dung system than in the cow dung system, and the pH was also lower.

The standard test method for evaluating biodegradable plastic with compost (ISO14855) is performed at  $58 \pm 2$  °C. Many studies have evaluated the degradability of PLA products in compost on pilot or commercial scales. Examples include compost with fiber, fat, and protein in animal fodder at 58 °C in 2005 [12]; compost with cow manure and wood waste at 60–65 °C in 2006 and 2007 [13,14]; and compost with green yard waste as a main component around 60 °C [15,16,17] in 2007, 2017 and 2019 respectively. These studies employed temperatures of 58 °C or higher. In addition, a pilot-scale PLA degradation test employed compost containing horse manure and plants [18] in 2021. Because the sample was fresh and had a total weight of 110 kg, the internal temperature was 70 °C or higher at the beginning of the composting process. Around 10 days, the pH exceeded 9.0 as the ammonia concentration increased. Degradation proceeded because hydrolysis was promoted at the early stage. Controlling the temperature to avoid hydrolysis was critical to identify microorganisms capable of degrading PLA.

The compost derived from pig dung (No. 2) provided by a municipal composting facility in Kita-hiroshima in Hokkaido, Japan showed the highest PLA degradation activity. This was a mature compost with a pH of 7. Accordingly, pig dung (No. 2) was selected for further study, which involved evaluation of selected composts and collection of microorganisms (secondary screening). The selected compost (No. 2) was subject to further evaluation of PLA degradation. PLA film was placed in the compost, and degradation was monitored. After incubating at 37 °C for six months, films placed in the selected compost (No. 2) were degraded (Figure 3-3).



**Figure 3-3.** A photograph of the PLA film before and after the degradation test. (left) Control before testing and (right) after seven weeks in the compost

### 3-3-2. Isolation of the strain

A cluster of microorganisms was observed on the degraded portions of the films. The cluster was collected with an inoculation loop to isolate the strain responsible for the degradation of PLA

film. The cluster was streaked on agar film, and clear zones were spotted after 1.5 months. To avoid isolating microorganisms that assimilate and grow on the emulsifier, the emulsifier was removed during plate prep. As described in the Methods, after PLA powder was dissolved in  $\text{CH}_2\text{Cl}_2$ , the emulsifier was added, and the resultant emulsified solution was warmed in order to evaporate the  $\text{CH}_2\text{Cl}_2$ . PLA microspheres were collected by repeated filtration and washing to remove the emulsifier. Conversion of PLA powder to microspheres was predicted to increase contact with the microorganisms.

Previous studies have used emulsifiers to generate microspheres [2,20,19,21] in 1997, 2001, 2006, and 2016 respectively. However, microspheres can also be generated without emulsifiers. For example, after dissolving PLA pellets in chloroform, methanol [22,23] in 2004 and 2019 or ethanol [24] in 2014 can be added for homogenization. Then microspheres are obtained via filtration.

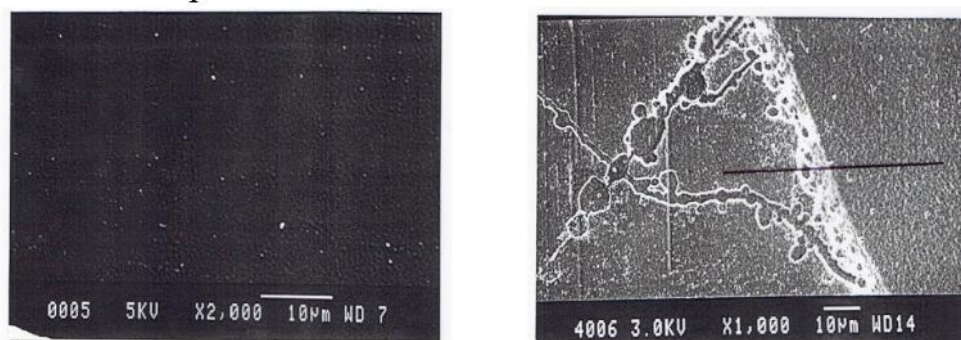
To further purify the microorganisms, the clear zone was scraped and streaked onto a new plate. This process was repeated four times. Ultimately, six colonies were isolated that produced a clear zone on the plate. The large halo formed (Figure 3-4) suggested that enzymes were secreted. One strain, MT-24107, was selected for further analysis and identification. Phase-contrast microscopy showed that a halo formed on the edge of the white fungus as shown in Figure 1-6 in Chapter 1. The thread-like spreading pattern is characteristic of actinomycetes.



**Figure. 3-4.** A photograph of halo formation on a PLA agar plate by strain MT-20147. It took 2–3 weeks for strain MT-20147 to form a halo on a PLA agar plate. This was acquired 1.5 months after cultivation started.

The reason for using the two-layer screening system in this experiment is as follows. Because it takes about one month to decompose PLA, it is difficult to detect the decomposition halo in a one-layer system in which PLA microspheres are dispersed over the entire agar medium. By adopting a two-layer system, when the upper microspheres decompose and halos are generated, it is easier for light to pass through, and detection is easier. PLA microspheres were employed in our plate screening for PLA-degrading microorganisms in order to shorten the overall time required. Although PLA powder (40–50  $\mu\text{m}$  radius) took four weeks to form a clear zone (i.e., halo), the process took only two weeks when PLA microspheres (1–5  $\mu\text{m}$  radius) were used.

MT-20147 was suspended in medium with the same composition as during agar plate testing after secondary screening, except for the addition of Bacto agar. The suspended liquid was poured onto a PLA film placed in a Petri dish. After incubation for several days, the film surface was washed and observed by electron microscopy. A characteristic degradation path was observed on the film surface, as shown in Figure 3-5.



**Figure 3-5.** Photographs of PLA film degradation by suspended media of strain MT-20147. Surface of PLA film before the suspension (left) and after the suspension (right).

### 3-3-3. Identification of the strain

As a result of physiological characterization shown in Table 3-3, the strain was presumed to be *Nocardiosis* and was classified as an actinomycete because of its cell wall and quinone types [25].

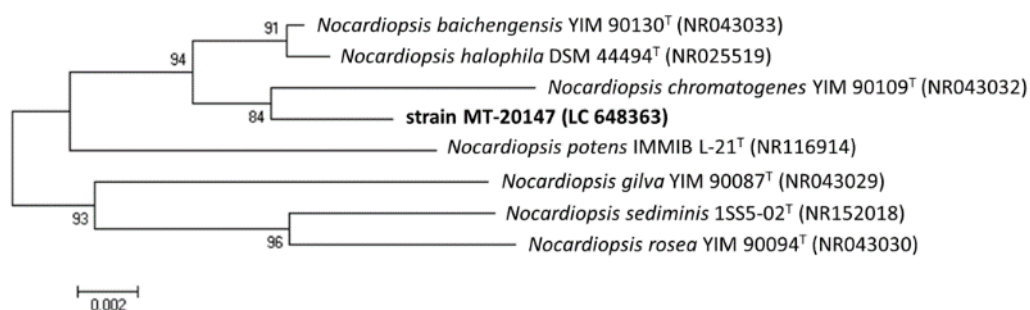
**Table 3-3.** Physiological characterization of strain MT-20147

Items	Results
Cell wall composition	Type III
Quinone system	MK-10(H <sub>6</sub> ), -10(H <sub>8</sub> )
Substrate mycelium	+
Aerial mycelium	+
Colony color	Grayish orange
Production of water-soluble pigments	+ Brownish orange
Nitrate reduction	+
Growth at 30 °C/40 °C/50 °C	+ / + / -
Carbon source utilization	
Aesculin	+
Casein	+
DNA	-
Gelatin	-
Guanine	-
Hypoxanthine	-
Starch	+
Testosterone	-
Tyrosine	-
Xanthine	-
DNA GC content (mol%)*	73

\* According to the HPLC method.

However, spore formation was not observed, and the genus could not be confirmed from this result alone. Colonies of isolated strain MT-20147 were scraped and collected. The harvested cells were physically disrupted by glass beads and the genomic DNA was extracted.

Sequencing started with the primer 27f. Using the resultant sequence data, the new primers 519r, 357f, and 536f were designed. Sequence analysis was performed for the region read by these primers, and subsequent primers were designed in the same manner to cover a 1,489-bp fragment in the 16S rRNA gene by assembling contigs. The obtained sequence data was registered and published at the DDBJ with the accession number LC648363. The sequence of the assembled contigs was analyzed using BLAST. The contigs showed a similarity with *N. chromatogenes* (99.2%), *N. baichengensis* (98.8%), and *N. halophila* (98.7%). A phylogenetic tree was created with the MEGA5 software as shown in Figure 3-6.



**Figure 3-6.** Phylogenetic tree analysis of strain MT-20147.

### 3-3-4. Isolation of *N. chromatogenes*

This study isolated and identified PLA-degrading microorganisms using animal-based composts (pig, cow, horse, and chicken). Multiple reports have described the isolation of PLA-degrading microorganisms, but to date no study has identified *N. chromatogenes* as a PLA-degrading microbe. In the 1990's, actinomycetes isolated from the natural environment (e.g., soil) were identified as PLA-degrading microorganisms, and multiple actinomycetes have been described not only in soil but also in compost. Furthermore, *Bacillus subtilis* and fungi capable of degrading PLA have also been reported.

PLA-degrading microorganisms have been reviewed based on the classification of the cited references. The species identified in this study has yet to be reported. Tokiwa et al. (1997) isolated and identified *Amycolatopsis*, a genus of actinomycete whose members are capable of degrading PLA, from 45 types of soil samples in Tsukuba City, Japan in 1997 [2]. This bacterium also degraded silk (1999) [26]. Using actinomycetes obtained from public institutions, *Kibdelosporangium aridum* (2003) [27] and *Saccharothrix waywayandensis* (2003) [28] degraded PLA, and *Amycolatopsis orientalis* produced PLA-degrading enzymes (2006) [19]. Furthermore, Tokiwa et al. reviewed the importance of actinomycetes as PLA-degrading microorganisms in

conjunction with active enzymes in 2004 [29]. The same type of microorganism, *Amycolatopsis sp.*, was isolated from 300 soil samples in 2001 [20].

In addition to actinomycetes, *Laceyella sacchari* isolated from forest soil in 2014 [30] and strains of *Pseudomonas* and *Bacillus*, both isolated from sludge in 2017 [31], were capable of PLA degradation. One study in 2016 showed that three out of four microbes isolated from 300 soil samples from various sources were fungi: *Penicillium chrysogenum sp.*, *Cladosporium sphaerospermum sp.*, and *Rhodotorula mucilaginosa sp.*; the exception was *Serratia marcescens* [21].

PLA-degrading microorganisms in compost are diverse. The raw materials used in the compost test for isolation and identification of PLA-degrading microorganisms are mainly animal feed, food waste, and plant residue, and animal manure has been used in only a few cases. *Bacillus smithii* of the order Bacillales was obtained from a garbage fermenter, and the PLA-degrading enzyme was identified as a serine protease in 2001 [32]. *Bacillus licheniformis* of the order Bacillales was isolated from compost made from animal fodder and identified as a PLA-degrading microorganism in 2008 [33].

Microorganisms that form biofilms were shown to degrade PLA in compost (2015) [34]. These organisms were of the genera *Acidovorax*, *Aeromonas*, *Arthrobacter*, and *Chryseobacterium*. All four are bacteria, and *Arthrobacter* is a type of actinomycete. *Thermopolyspora flexuosa*, another actinomycete, was identified from lab-scale compost in 2014 [24]. As reviewed above, it is clear that PLA-degrading microorganisms range from prokaryotes such as actinomycetes to eukaryotes such as fungi.

Butbunchu and Pathom-Aree [35] published a comprehensive review on PLA-degrading microorganisms in 2019. That paper described five families, eleven genera, and twenty-five species for which enzyme classification was also available. Furthermore, a unique review article on microorganisms, which degrade fossil-based plastics and biodegradable plastics, including PLA, was published in 2017 [36].

In the long history of research on PLA-degrading microorganisms, *N. chromatogenes* has not been identified. Recent studies in 2018 have examined microorganisms that degrade PET have been conducted. Among them, *N. chromatogenes* was shown to be a PET-degrading species [37]. Although PET is aromatic and PLA is aliphatic, and the two compounds have different chemical structures, it is noteworthy that microorganisms from the same genus are involved in the degradation of both polyesters.

As previously described, the PLA degradation mechanism consists of hydrolysis, enzymatic degradation, microbial degradation, and combinations thereof. However, the enzymes secreted from the isolated microorganism in this study, *N. chromatogenes*, have yet to be identified. A future direction includes identifying and evaluating its degradation mechanism. To carry out the degradation in compost more efficiently and economically, future work should elucidate the

mechanisms, microbes, and enzymes secreted during the process of degradation.

### 3-4. Conclusion

Identifying a microorganism involved in PLA degradation in compost may lead to identifying the enzymes involved and understanding the degradation mechanism, which will result in the optimization of the compost processing.

In this study, a new microorganism that degrades PLA was isolated by controlling the isolation and screening conditions.

- i. 15 different composts based on animal wastes including pig dung, cow dung, horse dung, or chicken droppings were used together with organic house wastes. Under a condition of controlled temperature, only pig dung-based compost degraded a PLA film.
- ii. Two distinct strategies to isolate PLA-specific degrading microorganisms were employed. The first strategy was to eliminate PLA degradation via hydrolysis. Specifically, the temperature of the compost, which was derived from various types of livestock dung, was kept below 37 °C because ammonia generation or a higher pH due to proteolysis at high temperatures may promote hydrolysis. Keeping the temperature lower inhibited hydrolysis in the initial stage of degradation. Consequently, non-enzymatic degradation was avoided.
- iii. The second strategy was to avoid the possibility of selecting microbes that assimilate the emulsifier by removing it after the screening process.
- iv. As a result, a microorganism capable of degrading PLA was isolated from pig dung-based compost. Based on the properties of the microorganism and a genetic analysis, it was identified as *N. chromatogenes*, an actinomycete of order Streptosporangiales. This is the first time that *N. chromatogenes* has been shown to degrade PLA.

Future studies will characterize the PLA degradation, identify the enzymes involved, and elucidate the degradation mechanism. Since recent studies examining microorganisms that degrade PET, including *N. chromatogenes*, have been conducted, a new wholistic approach can lead to the design of a unique degradation system for used plastics.

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## **Chapter 4**

### **Pilot-scale Composting Test of PLA for Social Implementation**

#### **4-1. Introduction**

Composting is expected to play an important role in disposing of PLA products because of its biodegradability. Although many papers were published from the early 1990s to 2019 relating to the biodegradability of PLA in compost as well as the quality and safety of the resulting compost, few papers reported a comprehensive and holistic approach on the pilot scale that included an evaluation of plant growth.

The purpose of this study was to demonstrate the importance of a pilot-scale composting test, as opposed to a laboratory test, to show that composting is a viable treatment method for social implementation for packaging made of PLA contaminated with food waste. The degradability of the product, impact on the composting process, and quality and safety of the obtained compost were examined. The results showed that both biodegradability and disintegration were demonstrable under actual composting conditions on a pilot scale, indicating that the presence or absence of PLA products did not affect the composting process. Furthermore, the obtained compost is both of high quality and safe, and it does not adversely affect plant growth. This type of comprehensive pilot-scale compost test should be useful to design a socially acceptable system to treat organic waste containing biodegradable products after use.

#### **4-2. Materials and Methods**

##### **4-2-1. PLA sample and compost raw materials**

###### **4-2-1-1. Standard biodegradability test of PLA in compost**

According to ISO 14855 [1], in the basic test, which indicates the biodegradability of PLA, PLA powder and mature compost from a compost facility in Hayakita-cho, Hokkaido used at  $58 \pm 2$  °C. ISO 14755 [1] was later modified into ISO 14855-1 [2] and ISO 14855-2 [3], although the basic principles remained the same.

###### **4-2-1-2. Biodegradability and disintegration of PLA products in the pilot-scale compost**

PLA film-laminated paper plates, which are a Biodegradable Plastics Society (BPS) certified Green Plastic #216 provided by Tohcello, were used in the pilot-scale compost test. The plates were composed of a 40  $\mu\text{m}$ -thick PLA film made from LACEA™ (Mitsui Chemicals) and a paper part made from 100% pulp paper with a weight of 320  $\text{g}/\text{m}^2$  (Figure 4-1).

In accordance with ISO 16929 [4], the raw material for compost was wood chips as an auxiliary material, horse and animal waste as return compost, and plant-based waste as a substitute

for fermentation with artificial garbage. Table 4-1 shows the composition ratios of these compost raw materials.

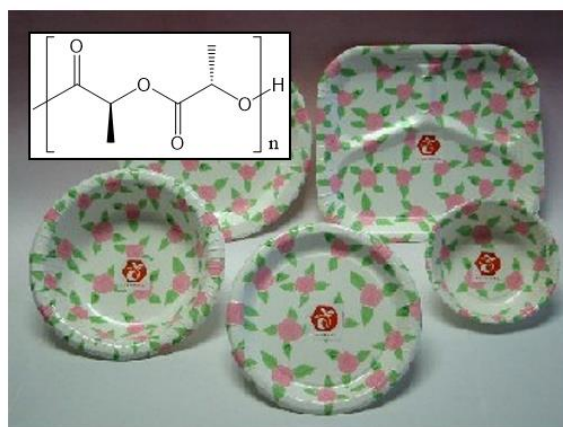


Figure 4-1. PLA film-laminated paper plates as a test sample.

Table 4-1. Formation of the composting material

Component	Description	Weight (wet, kg)
	Feed for rabbits (solid compounded feed)	9
Plant-based wastes	Cabbage	28
	Potato	5.4
	Onion	5
	Tomato	4.6
	Banana	3.2
	Carrot	1.8
	Cucumber	1.7
	Grapefruit	1.5
Supplied compost	Horse dung compost product	40
Auxiliary raw material	Larch	10
Total		110.2

#### 4-2-2. Test equipment and processing conditions

##### 4-2-2-1. Biodegradability standard test of PLA in compost

In the standard model of ISO 14855 [1], about 100 g of a sample is put into about 600 g of compost. A newly developed small-scale model is shown in Figure 1-7 (a) in Chapter 1. In this model, about 5 g of a sample was put into 100-150 g of compost. The temperature was kept at  $58 \pm 2$  °C. CO<sub>2</sub> generated by biodegradation was absorbed by the alkaline solution and measured by titration.

##### 4-2-2-2. Biodegradability and disintegration of the PLA product in pilot-scale compost

Each fermenter was a 180 L metal columnar drum (without a stirring mechanism) equipped with a blower with an airflow meter, non-dispersive infrared absorption (NDIR) - type CO<sub>2</sub> concentration meter, thermometer, and other measurement equipment as shown in Figure 1-7 (b) in Chapter 1.

Table 4-2 summarizes the list of auxiliary apparatus.

**Table 4-2.** List of auxiliary apparatus used in the pilot-scale compost experiment.

Apparatus	Device	Description
Air supply	Blower	Roots-type blower
	Air flow meter	Flow-rate control type airflow meter with a needle valve
	Air supply pipe	PVC loop-type air supply pipe
Gas meters	CO <sub>2</sub> meter	NDIR gas meter
	Collection device	Diffused gas collector made of PET
	Selection switch	Electromagnetic valve
	Mist separator	Liquid displacement type
	Recorder	Intermittent recorder (also used for temperature)
Thermometer	Temperature sensor	JIS Pt100 platinum temperature measurement resistor
	Recorder	Intermittent recorder (also used for gas)

Fermenters, which contained 170 L of compost raw materials, were prepared and divided into two zones (Figure 4-2). In the test zone, PLA samples (1.1 kg) were added to the fermenter. In the blank zone, the fermenter did not contain PLA samples. To confirm the validity, each zone was duplicated. After the test was completed, the contents of the fermenter in each treatment area were sieved to separate particles of non-disintegrated PLA samples with a diameter greater than 2 mm, and as a result the residual rate of PLA samples was measured. The maximum test treatment period was 80 days. However, biodegradation was completed within about 60 days. The test ended after confirming that fermentation had stopped, which was determined when fermentation conditions such as CO<sub>2</sub> concentration and temperature no longer changed over time.



**Figure 4-2.** A photograph of the pilot-scale compost apparatus. (left) Two drums are the blank zone without PLA samples and (right) two drums are the test zone with PLA samples. All drums were equipped with an air blower, gas concentration meter, and thermometer. Drums were placed on a gravimeter.

The temperature depended on the heat generated by biodegradation of the compost. External heat was not added. Table 4-3 summarizes the measured items. PLA film-laminated paper plates were put into the compost after cutting into  $5 \times 5 \text{ cm}^2$  pieces as a test sample.

The test zone contained the PLA sample, whereas the blank zone did not. Table 4-1 shows the compost raw materials. The PLA sample was a PLA film-laminated paper plate cut into  $5 \times 5 \text{ cm}^2$  pieces. Table 4-4 shows the conditions.

**Table 4-3.** Measured items.

Item	Method	Measurement frequency and remarks
Temperature	JIS Pt100	Daily, continuous measurement Top: 15 cm from the surface Center: 40 cm from the surface
CO <sub>2</sub> concentration	Gas detector, NDIR	Daily, continuous measurement 40 cm from the surface
O <sub>2</sub> concentration	Gas detector	Daily, frequency varies with the change in the measurement value 40 cm from the surface
NH <sub>3</sub> concentration	Gas detector	Daily, frequency varies with the change in the measurement value 40 cm from the surface
Water content	Weight method	Weekly, at the time of turning
Ignition loss	Weight method	Weekly, at the time of turning
pH	Glass electrode	Weekly, at the time of turning
EC	Glass electrode	Weekly, at the time of turning
C/N <sub>2</sub> ratio	C/N meter	Weekly, at the time of turning
Contents volume	Calculated value	Weekly, at the time of turning
Contents weight	Direct weighing	Weekly, at the time of turning
Amount of water added	Direct weighing	At the time of water addition

**Table 4-4.** Operating conditions (according to ISO 16929 [13]).

Item	Operation Condition
Agitation/turning	Drum contents were spread on the sheet, stirred manually, and returned to the drums each week.
Air supply	5 to 25 L/min.-m <sup>3</sup> (Value was set such that the oxygen concentration in the compost was above 5%.)
Water addition	Water was added such that the water content of the compost would not fall below 50% when turning the compost.
Nitrogen control	Urea solution was added to compensate for the drop in the nitrogen concentration due to the addition of water.

### 4-2-3. Measurement items

#### 4-2-3-1. Biodegradability standard test of PLA in compost

The CO<sub>2</sub> generated by biodegradation was measured according to ISO 14855 [1].

#### 4-2-3-2. Biodegradability and disintegration of PLA products in the pilot-scale compost

The tests measured the following: temperature, CO<sub>2</sub>, O<sub>2</sub>, NH<sub>3</sub>, water, content, loss on ignition, pH, electrical conductivity (EC), carbon/nitrogen ratio, content volume, content weight, and added water weight. At the end of the test, the residual PLA fraction was measured. For the

compost input, the elution test described in the Waste Management and Public Cleaning Act was conducted [5]. For the treated compost, the water, nitrogen, and heavy metal contents were analyzed according to the items described in the Fertilizer Regulation Act in Japan [6]. The blank zone and test zone were each measured twice and graphed with their average values. They appear on the graph as an error bar and a circle of the average value. Items were measured to confirm whether composting progressed smoothly. For example, the ignition loss and EC indicated whether or not the compost was suitable as fertilizer by evaluating the degree of decomposition of organic matter and salt concentration. On the other hand, the carbon/nitrogen ratio determined the balance between carbon and nitrogen as a fertilizer.

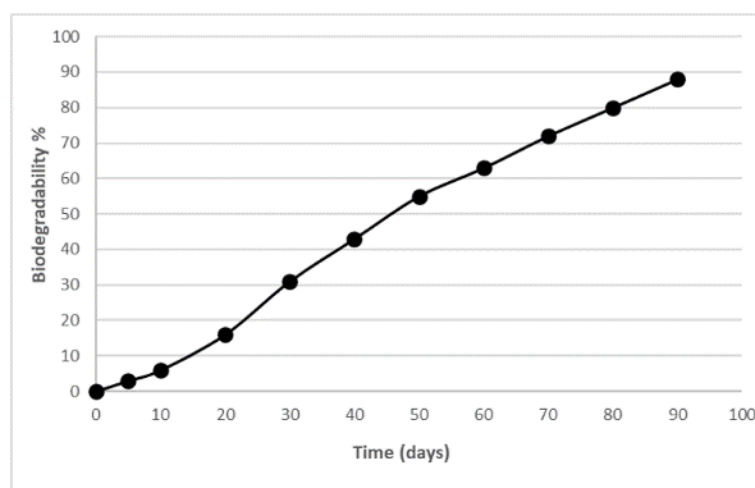
#### 4-2-4. Plant growth

Finally, the effect of the resulting compost on the germination and growth of Japanese komatsuna (Japanese mustard spinach) was evaluated. The resulting compost was used as a fertilizer. B-1 and B-2 were obtained from compost without the PLA sample. T-1 and T-2 were grown in the compost with the PLA sample based on the test method [7] of plant growth. This method measures toxicity against plants and is regulated by the Ministry of Agriculture, Forestry and Fisheries in Japan No. 1943 (18 April 1984). Komatsuna seeds were buried in each type of fertilizer to observe the effects of the presence of the PLA sample.

### 4-3. Results and Discussion

#### 4-3-1. Biodegradability standard test of PLA in compost

Tracing the biodegradability of PLA powder using the amount of CO<sub>2</sub> generated in the mature compost confirmed that about 88% of the PLA decomposed in 90 days (Figure 4-3).

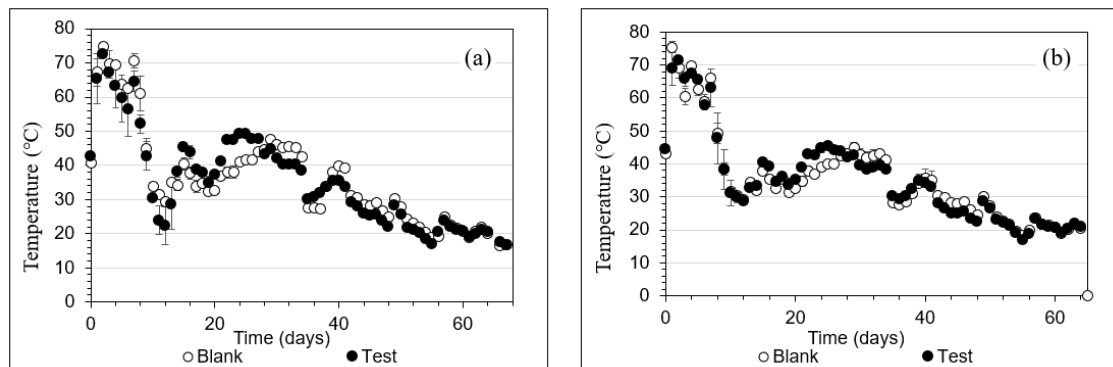


**Figure 4-3.** Biodegradability index of the PLA powder measured by the amount of CO<sub>2</sub> emission according to ISO 14855.

## 4-3-2. Biodegradability and disintegration of PLA products in the pilot-scale compost

### 4-3-2-1. Changes in the temperature and gas concentration in the fermenter

The temperature during the test period was measured at the top (Figure 4-4 (a)) and center of the fermenter (Figure 4-4 (b)). The blank and test zones did not differ significantly as both showed a temperature of 65 °C or higher for a minimum of 48 h at the early stage of fermentation. Hence, the compost meets the test standards of ISO 16929 and the epidemiological conditions of Japan.



**Figure 4-4.** Changes in the average temperature at the top (15 cm from surface) of the fermentation drum (a) and at the center (40 cm from the surface) of the fermentation drum (b).

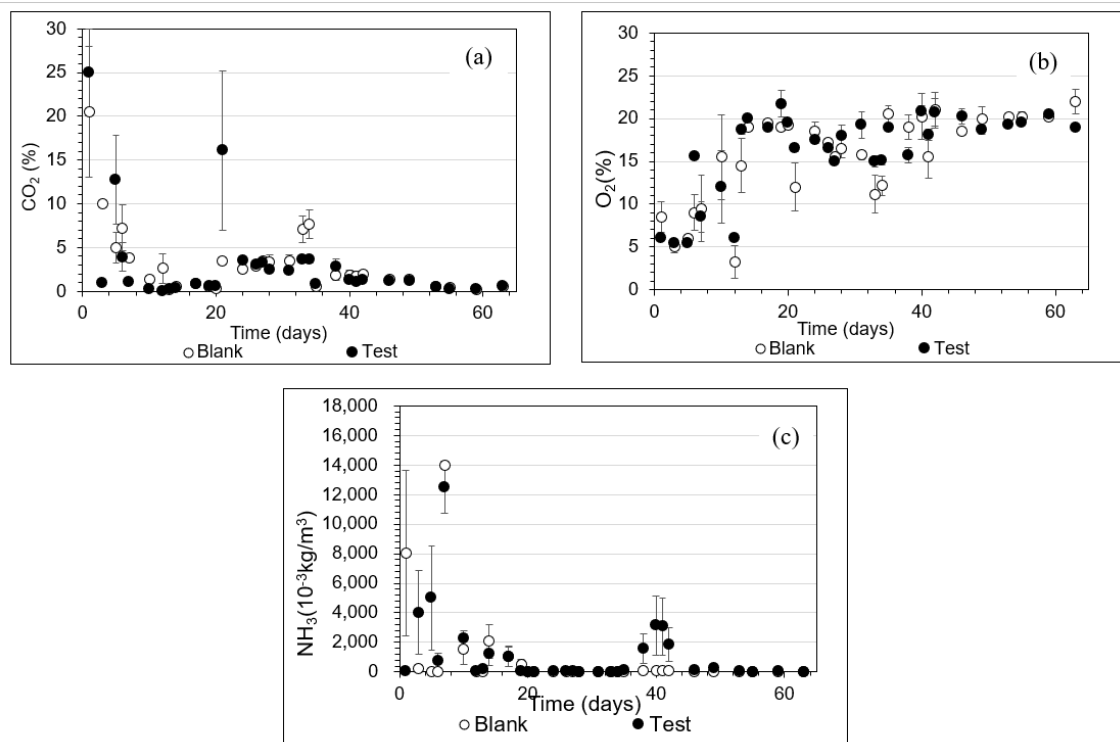
Figure 4-5 (a) shows the daily change in CO<sub>2</sub> content in both the blank and test conditions. The content initially increased, but then decreased rapidly. From 10–50 days, the content was 0–5%, but it settled at around 1% or less after 50 days. There are some points that do not overlap in the data of the blank zone and the test zone, but when comparing the behavior before and after, there is no significant difference between the two zones.

Figure 4-5 (b) plots the daily change in O<sub>2</sub> content. As O<sub>2</sub> consumption was intense at the beginning of fermentation, it dropped to less than 10%. After 10 days, it stabilized at over 10% of the O<sub>2</sub> content by adjusting the air supply rate. Similar to the trends of CO<sub>2</sub> content, although there are some irregular points, the blank and test zones did not differ significantly.

The NH<sub>3</sub> concentration in the fermenter was initially high but decreased after 20 days (Figure 4-5 (c)). The data from the large error bar at around the 40 days mark may appear irregular, but when comparing it to the decomposition behavior of the two zones at other periods, there were no significant differences.

The similar trends in the temperature, CO<sub>2</sub>, O<sub>2</sub>, and NH<sub>3</sub> concentrations in the blank and test zones indicate that the presence of the PLA film did not affect the composting process.

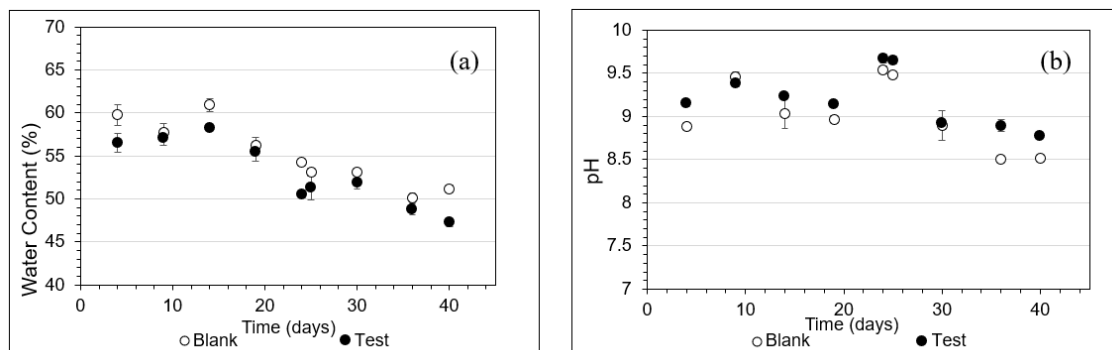




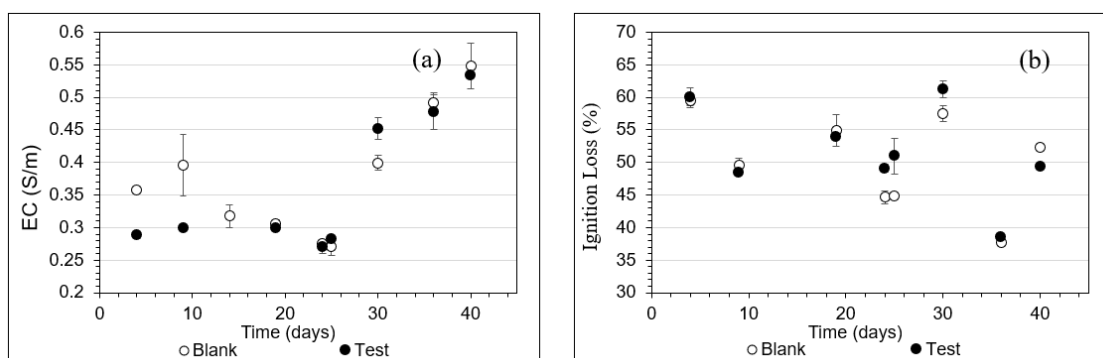
**Figure 4-5.** Changes in the average CO<sub>2</sub> content (a), O<sub>2</sub> content (b) and NH<sub>3</sub> concentration (c) with time.

#### 4-3-2-2. Property changes to the fermentation mass in the fermenter

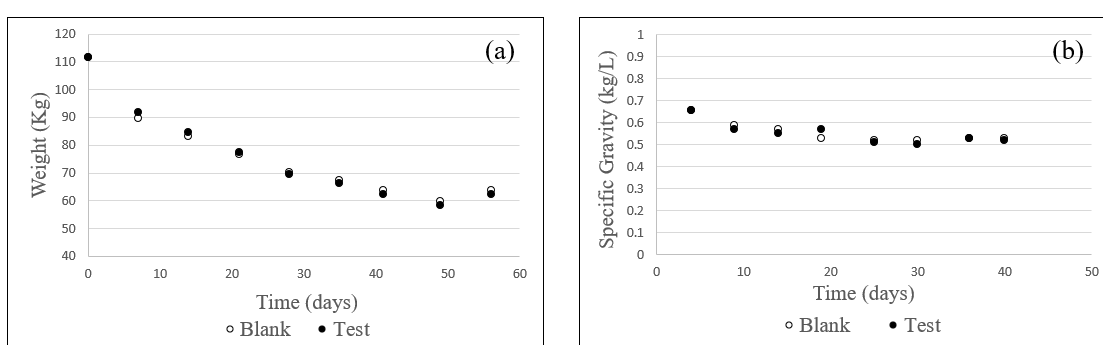
The test (treated) and blank (untreated) zones did not significantly differ with regard to water content, pH, EC, and residual amount of ignition during the test period (Figures 4-6 (a) and (b), 4-7 (a) and (b), and 4-8 (a) and (b)) show the weight per container and bulk specific gravity measured during the test period, respectively. The test and blank zones showed similar results. These results further demonstrate that the presence of PLA film-laminated paper did not affect the composting process.



**Figure 4-6.** Changes in water content (a) and pH (b) with time.



**Figure 4-7.** Changes in electrical conductivity (a) and ignition loss (b) with time.



**Figure 4-8.** Changes in weight (a) and bulk specific gravity (b) with time.

#### 4-3-2-3. Residual rate of the PLA sample at the end of test

To investigate the residual rate of PLA samples, the compost after the test was passed through a 2 mm sieve. The PLA sample remaining in the compost with a diameter of 2 mm or more was 0.054 kg, and the residual rate was 5.23% compared with the input PLA sample by dry weight. Table 4-5 shows the average weight of each zone at the end of the test. Overall, 94.8% of the PLA sample, which weighed 1.031 kg at the time of input, decomposed. Furthermore, fermentation did not vary significantly between the blank and test zones. These results provide additional evidence that the presence of PLA film-laminated paper does not affect the composting process.

**Table 4-5.** Weights before and after the test.

	Non-treated zone	Treated zone
Av. Weight of the compost (dry weight)	59.95 kg (29.26 kg)	60.05 kg (31.67 kg)
Av. weight of the test specimen		
Before charge (water content)		1.1 kg (6.3%) = 1.031 kg as dry
After test (water content)		0.067 kg (24.82%) = 0.054 kg as dry
Residual rate of the test specimen		5.23% (compared in dry weight)

#### 4-3-2-4. Quality of inputs and composted products

The elution test of the input materials was conducted based on the Waste Management and Public Cleaning Act [5] in Japan. As shown in Table 4-6, the results were all below the requirements for heavy metals, organophosphorus compounds, and other regulated compounds.

**Table 4-6.** Analysis results of items specified in the Waste Management and Public Cleaning Act (unit:  $10^{-3}$  kg/m<sup>3</sup>).

Analysis Item	Blank 1	Blank 2	Test 1	Test 2	Standard Value
alkyl mercury compounds	<0.0005	<0.0005	<0.0005	<0.0005	not detect
mercury or its compounds	<0.0005	<0.0005	<0.0005	<0.0005	$\leq 0.0005$
cadmium or its compounds	<0.002	<0.002	<0.002	<0.002	$\leq 0.3$
lead or its compounds	<0.01	<0.01	<0.01	<0.01	$\leq 0.3$
organophosphorus compounds	<0.001	<0.001	<0.001	<0.001	$\leq 1$
chromium (VI) compounds	<0.05	<0.05	<0.05	<0.05	$\leq 1.5$
arsenic or its compounds	0.031	0.030	0.031	0.030	$\leq 0.3$
cyan compounds	<0.1	<0.1	<0.1	<0.1	$\leq 1$
polychlorinated biphenyls	<0.0005	<0.0005	<0.0005	<0.0005	$\leq 0.003$
trichloroethylene	<0.001	<0.001	<0.001	<0.001	$\leq 0.3$
tetrachloroethylene	<0.001	<0.001	<0.001	<0.001	$\leq 0.1$
dichloromethane	<0.001	<0.001	<0.001	<0.001	$\leq 0.2$
carbon tetrachloride	<0.001	<0.001	<0.001	<0.001	$\leq 0.02$
1,2-dichloroethane	<0.001	<0.001	<0.001	<0.001	$\leq 0.04$
1,1-dichloroethylene	<0.001	<0.001	<0.001	<0.001	$\leq 0.2$
cis-1,2-dichloroethylene	0.004	<0.001	<0.001	<0.001	$\leq 0.4$
1,1,1-trichloroethane	<0.001	<0.001	<0.001	<0.001	$\leq 3$
1,1,2-trichloroethane	<0.001	<0.001	<0.001	<0.001	$\leq 0.06$
1,3-dichlorobenzene	<0.001	<0.001	<0.001	<0.001	$\leq 0.02$
benzene	<0.001	<0.001	<0.001	<0.001	$\leq 0.1$
thiuram	<0.006	<0.006	<0.006	<0.006	$\leq 0.06$
simazine	<0.003	<0.003	<0.003	<0.003	$\leq 0.03$
thiobencarb	<0.01	<0.01	<0.01	<0.01	$\leq 0.2$
selenium or its compounds	<0.002	<0.002	<0.002	<0.002	$\leq 0.3$

The composted products were analyzed by the test method based on the “Fertilizer Regulation Act” [6]. Table 4-7 shows the nutrient contents (e.g., moisture content, total nitrogen, total phosphoric acid). Table 4-8 shows the level of toxic items regulated by the Fertilizer Control Law (e.g., arsenic, cadmium, mercury). The results confirm that the compost is suitable for use as fertilizer.

**Table 4-7.** Analysis results of nutrient items specified in the Fertilizer Control Law.

Analysis item	Unit	Blank 1	Blank 2	Test 1	Test 2
Water content	%	44.5	44.2	43.8	44.0
Total nitrogen amount	%	1.54	1.57	1.36	1.44
Total phosphoric amount	%	0.96	0.99	0.96	0.96
Total potassium amount	%	1.58	1.64	1.67	1.66
Total copper amount	mg/kg	23.1	22.4	23.9	23.9
Total zinc amount	mg/kg	77.3	80.0	78.8	75.0
Total lime amount	%	0.88	0.86	0.91	0.88
Organic carbon	%	11.4	11.4	10.8	11.2
Carbon/Nitrogen ratio		7.4	7.3	7.9	7.8

**Table 4-8.** Analysis results of toxic items specified in the Fertilizer Control Law (Unit: %).

Analysis item	Blank 1	Blank 2	Test 1	Test 2	Standard Value
Arsenic	0.0010	0.0007	0.0009	0.0009	<0.005
Cadmium	<0.000005	<0.000005	<0.000005	<0.000005	<0.0005
Mercury	0.00003	<0.000002	0.00003	<0.000002	<0.0002
Nickel	0.003	0.003	0.008	0.007	<0.03
Chromium	<0.005	<0.005	<0.005	<0.005	<0.05
Lead	<0.001	<0.001	<0.001	<0.001	<0.01

#### 4-3-2-5. Confirmation of effects on plant germination

Finally, based on the practical test method [7] of plant growth, which measures the toxicity against plants and is related to the scope of ISO16929 [4], we confirmed the effect of the PLA sample-treated compost on plant germination. There were two test groups to grow komatsuna or Japanese mustard spinach. The control samples were grown in fertilizer from the pilot-scale compost without the PLA sample (B-1 and B-2), while the tests were grown in fertilizer from the pilot-scale compost with the PLA sample (T-1 and T-2). The komatsuna seeds were buried in each type of fertilizer. After three weeks, good growth was observed in both groups.

Additionally, neither group showed abnormal growth (Table 4-9). Hence, the compost-treated product with the PLA sample did not induce harmful effect on plants, demonstrating its suitability as a good compost product.

**Table 4-9.** Test results of plant growth in fertilizer from pilot-scale compost.

Zone	Rate of Germination			Results of Growth			Abnormality
	1 week (%)	2 weeks (%)	3 weeks (%)	Leaf length 2 Weeks (cm)	Leaf length 3 Weeks (cm)	Weight (g/pot)	
B-1	100.0	100.0	100.0	5.0	7.7	16.5	None
B-2	98.3	98.3	98.3	5.2	8.2	18.7	None
T-1	98.3	98.3	98.3	4.8	7.5	15.3	None
T-2	98.3	98.3	98.3	5.7	7.6	16.2	None

#### 4-3-2-6. Insight from the history of PLA degradation in compost

Prior studies were analyzed by surveying the literature from 1992 to 2019 on pilot-scale biodegradability and safety of the resulting compost. Many papers published from the 1990s to the early 2000s were related to the promotion of PLA polymers' biodegradability [8–16]. Some studies also worked to improve the mechanical properties by producing copolymers [9] or blending with other biodegradable polymers [11,15] and additives [13]. Other studies reported direct and simple biodegradability testing of PLA [17,18], and socially implementable biodegradability experiments using compost [19–21]. However, full-scale studies on the

biodegradability and disintegration results utilizing existing real composting facilities for commercially available PLA products only began to appear around 2005.

In 2006 and 2007, papers were published demonstrating the degradability of a variety of commercial PLA products in compost such as cheese packaging [22], carpet and fiber products [23], bottles, trays and deli-containers [24], and knives and packaging [25]. In one study [24], three commercially available forms of PLA packaging and containers were exposed to real composting conditions at Michigan State University under ambient exposure conditions. Degradation in a real composting facility was monitored by visual inspections, SEC, DSC, and TGA. It should be noted the need to address the compostability of these packages under real composting conditions for social implementation since the standard methodology of evaluating biodegradability in simulated composting conditions has limitations. They also showed that the compostability of the complete package in real composting conditions may take longer than a simple piece of polymer.

Professor Narayan, who is a leader in research on biodegradable polymers, and colleagues have published many papers regarding biodegradability. Among these, two papers compared ASTM/ISO tests with realistic composting conditions in 2007 [26] and 2009 [27]. Based on their disintegration tests on real compost (fresh compost) using PLA bottles, they pointed out that standard test methods such as ISO 14855 only answer whether a plastic is biodegradable [1]. Standard methods do not answer the question of whether it is fully biodegradable in an actual compost facility. This follows on from ISO 17088, which was announced in 2008 and revised in 2019 [28].

From industrial and business perspectives, major consumer goods companies have documented [29] that it is pointless to use PLA and other biodegradable plastic products unless they are properly processed in a composting plant. Without proper composting, they will not contribute to waste reduction.

In 2017, a paper was published that questioned the decomposability of compost in actual facilities, even if decomposition is demonstrated on the laboratory scale [30]. They argued that compost facilities vary in operational methods, and the actual degradability depends on the form. As the above studies show, it is important to confirm biodegradability in actual composting facilities as social implementation of bioplastics with biodegradable properties such as PLA progresses. All the messages delivered by the above papers are similar to the one that this paper tries to convey.

There are few examples demonstrating the safety of PLA by assessing plant growth in the literature. A paper in 2019 questioned the safety of micro and nanoparticles, which is a concern during decomposition of PLA products, including their additives [31]. In many practical available resins, various additives are used in the manufacturing process. Hence, it is necessary to investigate whether such additives decompose in real compost. Additionally, the safety of

decomposed materials and resulting compost should be evaluated. However, it appears that only a few studies demonstrate the method and results of such a comprehensive and holistic approach on the pilot scale.

#### **4-3-2-7. Influencing elements affecting the biodegradability time in compost**

To date, the biodegradability of PLA in compost has been demonstrated on the laboratory scale according to ISO 14855 [1]. Consistent with our previous paper [32], this study shows that more than 80% of PLA biodegrades in 80 days using mature compost (Figures 1-7 (a) in Chapter 1 and 4-3). According to the paper by M. Kunioka et al. on the Biodegradability Evaluation of Polymers by ISO 14855-2 [3], they found that 80% or more of the powder was biodegraded after 50 days [33]. In contrast, when a sample is shaped into PLA cups, it takes 100 days or more to reach a minimum of 80% decomposition. Hence, the sample shape affects decomposition time.

ISO 14855 tracks the biodegradability by the amount of CO<sub>2</sub> generated [1]. On the other hand, this study used ISO 16929, which assesses the contents of the fermenter after testing and sieving [1]. The decomposition rate was subsequently calculated by comparing the dry weight of the remaining PLA sample and the original PLA sample. Such a comparison is challenging. Here, if degradation is considered to be complete at the time when the temperature, CO<sub>2</sub> concentration, and weight in the container stopped changing, it means that 94.8% of the PLA sample was degraded in 50–60 days. Comparing the above results in laboratory-scale and pilot-scale studies, the degradability on the laboratory scale and the pilot scale was at least equivalent when considering the sample shape, and suggest a slightly faster degradation time on the pilot scale.

#### **4-3-2-8. Holistic approach and social implementation**

This study investigated both the biodegradability and disintegration property of the product in the compost. The PLA samples did not affect the composting process adversely. The quality and safety of the obtained compost were maintained. Such results are difficult to obtain in a laboratory-scale test. However, the pilot-scale test provided results suitable as a proof of concept for social implementation. That is, a pilot-scale test was used as a holistic approach to demonstrate the feasibility compost facilities to process PLA products.

PLA products have been used in two major global events for social implementation: the Kassel project [34–37] and the Aichi EXPO [38]. The Kassel project ran from 2001 to 2002 in the city of Kassel, Germany. PLA film-laminated paper plates were used in a supermarket. After use, they were collected, sorted, and safely composted. During the Aichi EXPO in Japan, single-use and compostable tableware made from PLA was used in the food court and composted after use. In both cases, vegetables were grown in the resulting compost. Beside the global events described above, garbage bags made of PLA were introduced in Kosaka Town, Akita Prefecture, and Furano City [39,40]. Organic waste was also collected from homes in Hokkaido and treated

in composting facilities. The resulting compost was used as fertilizer in domestic farms. Additionally, some companies have focused on the closed system, which is beyond the involvement adopted in business models based on related holistic approaches and is drawing attention from the viewpoint of a circular economy. In Colorado, USA, a hamburger chain minimized the use of packaging and containers, and introduced bio-based and biodegradable packaging and cups made of paper and PLA [41]. The environmental contribution was not made through use of the material itself but rather the recovery and composting treatment implemented after use.

#### **4-4. Conclusion**

It is important to promote compost facilities as an appropriate after use treatment of organic waste. The challenges for composting biodegradable plastics products are that the process and the resulting compost must be safe.

In this study, a pilot-scale test (100 L or more) using PLA film-laminated paper under actual composting conditions was conducted in accordance with both international standards and domestic evaluation methods.

- i. The results demonstrated that the presence of a biodegradable plastic product has a negligible impact on the composting process, by measuring temperature, concentrations of CO<sub>2</sub>/O<sub>2</sub>/ammonia, water content, pH, electrical conductivity, ignition loss, and content volume/weight.
- ii. Composting PLA satisfied the various conditions described in the “Plastic disintegration under composting conditions” in ISO 16929. In addition, the elution test of the input materials met the requirements specified in “Waste Management and Public Cleaning Act”, and an analysis of the obtained compost based on the “Fertilizer Regulation Act” indicated that it did not suffer from quality problems.
- iii. The obtained compost did not adversely affect plant germination or growth, demonstrating its safety and high quality, even if the compost contained residual PLA.

This comprehensive and holistic pilot-scale demonstration test shows that composting PLA products after use is effective and can be integrated into society. Consequently, composting treatment of organic waste together with biodegradable plastics will contribute to a circular economy by adding value to waste.

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## **Chapter 5**

# **How Do Bioplastics and Fossil-based Plastics Play in a Circular Economy?**

### **5-1. Introduction**

Plastics contribute socially and environmentally to a healthy and sound everyday life, and their usage will increase globally including in developing countries. This trend is well documented in “The Future of Petrochemicals” by the International Energy Agency (IEA) report [1] and “Plastics - the Facts 2020” by the Plastics Europe [2]. The challenge with plastics lies in efficiently managing and utilizing used plastics, avoiding litter into both marine and terrestrial environments, not depleting resources and resource conversion, which lead to discussion on how plastics should move forward in a sustainable society and a circular economy. Bioplastics, both bio-based and biodegradable, have reemerged as the potential solutions. To clarify the role of bio-based, biodegradable, together with fossil-based plastics, it is meaningful to assess lessons learned from experiences in the 1990s and 2000s [3-5], when I was responsible for a PLA project, which representing the bioplastics, along with my perspectives on recent global movement in circular economy.

Reflecting on the lessons learned, it is essential to accommodate the value chain, collection and recycling systems, and government regulatory agencies when developing environmentally sound products. Additionally, environmental issues are sometimes clouded by public perceptions and the original purpose of creating environmentally sound products can be lost.

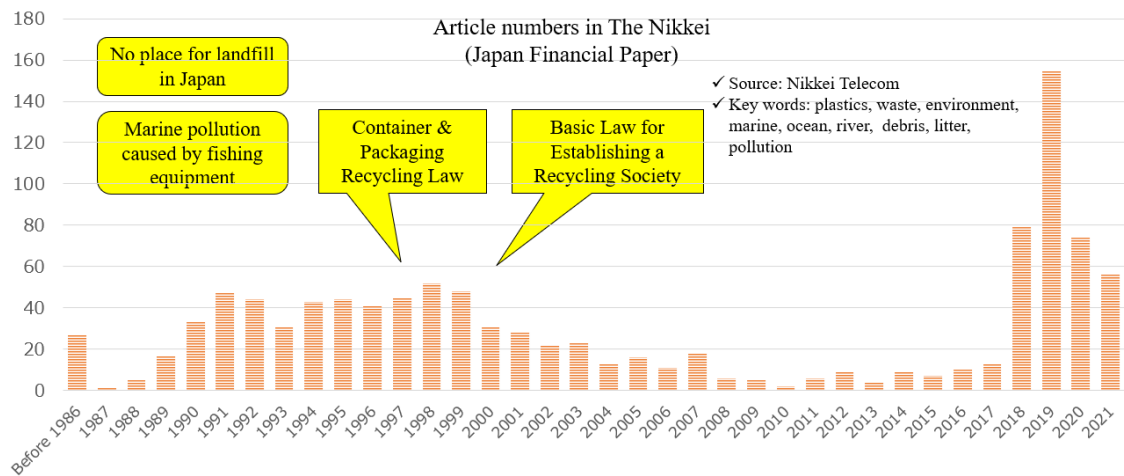
The issues with plastics have attracted the attention not only of industry and polymer scientists, but also the media, and the general public. Now is the time to reflect on the role of fossil-based plastics and bioplastics and establish a responsible future from the viewpoint of a circular economy [6] instead of a linear economy model [7], which focuses only on resource circulation.

### **5-2. What Is the True Nature of a Circular Economy?**

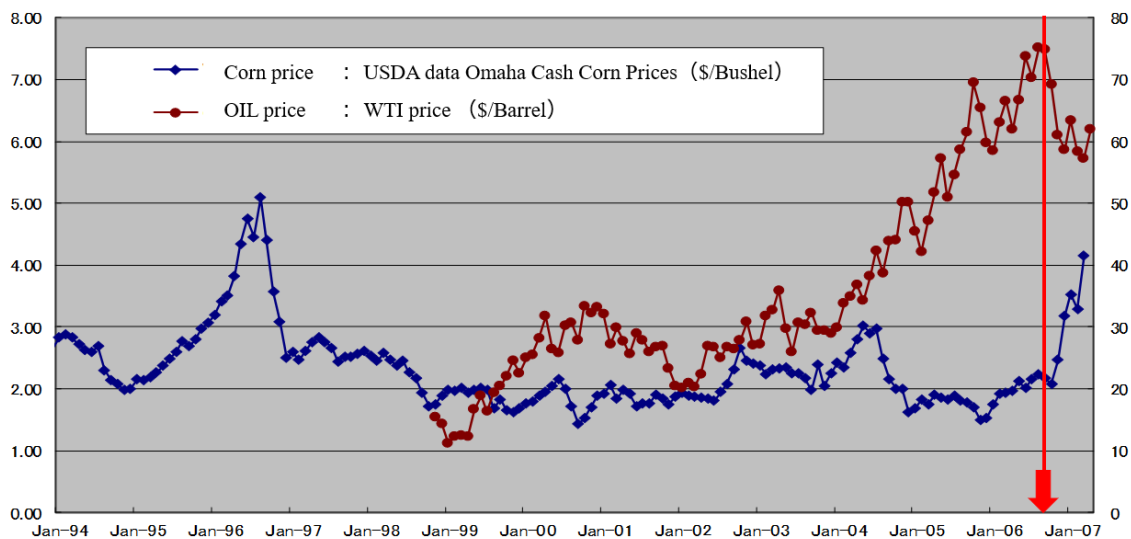
Figure 5-1 overviews the number of articles in newspapers related to plastics waste and ocean debris in Japan according to the Nikkei Telecom database. Consistent with the global trend, the numbers sharply and drastically increased further from 2018 after 10 years calm periods. Due to growing concerns over environmental and resource issues, a resource circulation society, a closed loop society, and 3R (Reduce, Reuse, and Recycle) have been in the spotlight. These initiatives led to the circular economy concept [6,7].

If only resource circulation is addressed, society becomes vulnerable to resource security threats and resource price fluctuations. For instance, if resource prices fall, the values of biomass resources and recycled resources would be lost. Consequently, circulation will not be achieved.

Figure 5-2 shows the trends of oil and corn prices from 1994 to 2007. Until 2006, corn prices remained low, while oil prices kept increasing. It was speculated that this cost advantage would be realized in big business for bio-based products compared to petroleum-based products. However, at the end of 2006, oil prices began to decline while corn prices began to increase dramatically. These changes must have been a reflection of the huge increase of bio-ethanol consumption. PLA products were forced to bear the cost increase, resulting in an uncertain market with a fragile future. This incident highlights that resource circulation itself is insufficient to realize sustainable business. Additional aspects and a new business model were needed.

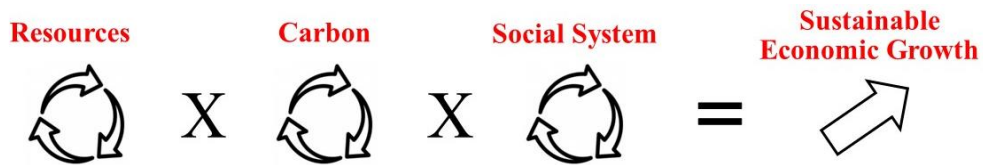


**Figure 5-1.** Number of newspaper articles about plastics wastes in Japan.  
–The problem of plastic waste hasn't happened recently. –



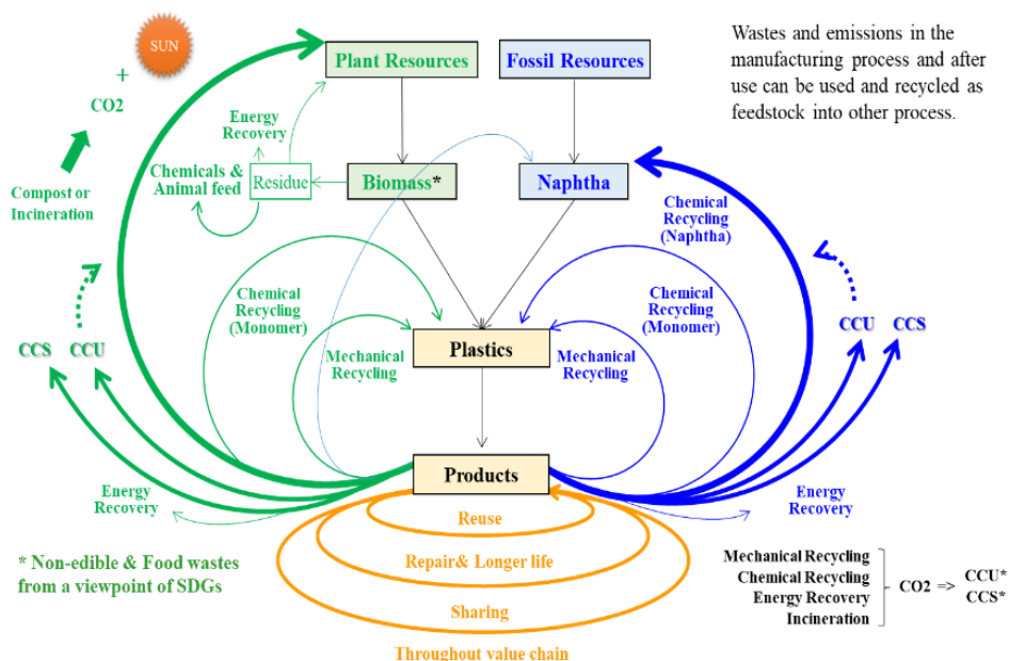
**Figure 5-2.** Trends of oil and corn prices from 1994 to 2007. In 2006, the oil price dropped while the corn price increased drastically.

A circular economy is a business model to achieve economic sustainability by adopting resource circulation and realizing economic growth that is decoupled from resource limitations. The era of business models that only consider materials is over. Merely pursuing cost and performance improvements of materials will not solve impending social, waste management, and environmental issues. A holistic approach, which includes resource circulation, carbon neutrality, and social systems, is necessary for resilient and sustainable economic growth (Figure 5-3).



**Figure 5-3.** A holistic approach for resilient and sustainable economic growth.

Designing future models of fossil-based plastics and bioplastics must encompass raw materials, plastic product, applications, and waste/byproduct management, which will be enhanced not only by reuse and recycling but also by maintenance, repair and life extension, and sharing throughout the value chain, which is summarized in a chart of a circular model of plastics [8] (Figure 5-4). This transition will be accelerated by digitalization and knowledge sharing such as artificial intelligence and robotics.



**Figure 5-4.** Circular economy model for plastics created to express both fossil-based plastics and bioplastics.

Even with recycling, an economical competitive business model should be constructed considering the three elements described in Figure 5-3. In other words, sustainable economic growth should be designed by maximizing resource value while minimizing wastes/emissions and environmental impact. In addition, wastes and emissions in the manufacturing process and after use can be used and recycled as feedstock into the other process.

How can a circular economy model for plastics be strengthened? One way is to introduce new perspectives such as digital technologies to enhance a traceability, and collaborating with other industries such as agriculture and policy framework, and incorporating an ambitious and innovative carbon dioxide capture and utilization (CCU) or storage (CCS) [9].

### 5-3. Bio-Plastics Business Development from the 1990s to the Early 2000s

The United States, Europe, and Japan began biodegradable plastics business development at the end of the 1980s to address waste management problems, particularly plastic litter in land and oceans. In the early 1990s, the media began to focus on plastics and the environment. Examples of published articles included “No place for landfills in Japan; Japan will be buried by garbage” and “To prevent marine pollution caused by fishing equipment, biodegradable plastics need to be developed that degrade naturally in the ocean.”

Although extensive development was conducted, market growth was much slower than biodegradable plastics manufacturers expected. One factor for slow growth was that the social system and infrastructure to collect, sort, and treat compost waste and biodegradable plastics products had not been established appropriately. Another factor was cost and property improvement. In Japan, environmentally sound incineration technology had improved just as the Container and Packing Recycling Law was passed [10]. These improvements seemed to solve the landfill issues.

Initially, biodegradability attracted attention as a novel concept [11,12]. Customers were attracted to plant-based or renewable resources due to the Kyoto Protocol and increasing oil prices (Table 5-1).

**Table 5-1.** Changes in concepts from biodegradability to bio-based.

Period	Early 90's	Late 90's	2000's
Concept of PLA	Biodegradability	Plant origin Biodegradability	Plant origin
Target applications	Fishing line & net, Compostables, Farming mulch	Packaging, Ag. Materials, Compostables	Packaging, Durables, Fiber
Social topics & keywords	Life of landfill Plastics disposal in environment		Kyoto protocol (GWG) Oil price, Biomass

Figure 5-5 classifies bio-based plastics and biodegradable plastics [11,12]. The horizontal circle summarizes biodegradable plastics, which include bio-based and fossil-based. The vertical circle shows bio-based plastics, which include biodegradables and non-biodegradables. Bio-based plastics contribute on the front end, while bio-degradable plastics contribute on the back end. Even currently, these terms are still misunderstood, and the difference needs to be explained to stakeholders.

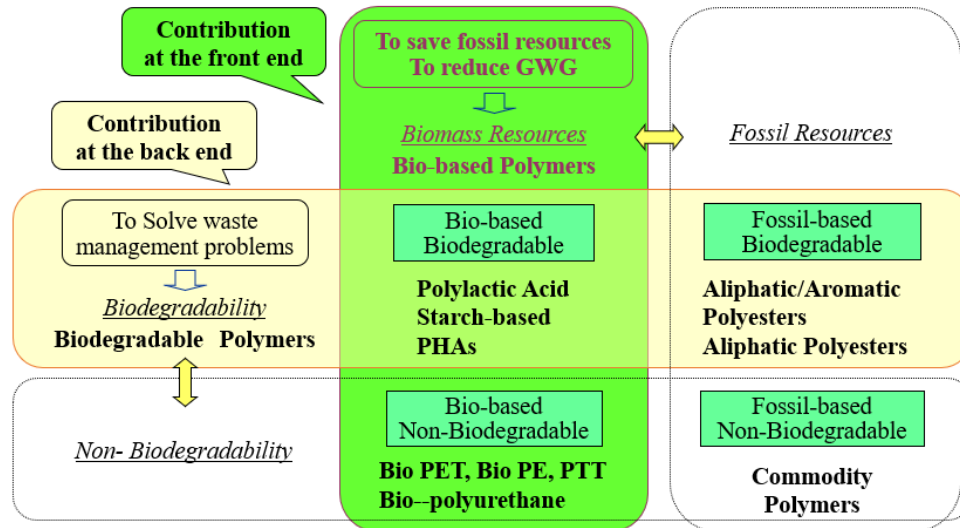


Figure 5-5. Positioning of bio-based polymers and biodegradable polymers.

In the case of PLA, the market also shifted [11,12] (Table 5-1). The concept in the late 1990s evolved from biodegradable to bio-based. In particular, expanding applications from compostable to bio-based containers, packaging, and durables became a priority (Table 5-2).

Table 5-2. Characteristics and applications of LACEA™, PLA

Field	Appeal to the customers & Reasons		Applications
Packaging & Container	Plant origin Transparency	Saving fossil resources, Reduction of GWG	Thermoformed container, Films (Shrink, Overlap), Paper lamination, Bottles
	Plant origin Low heat of combustion	Product design for disposal which does not require separation	Cutting blade for wrapping film, Envelop with window
Compost	Degradable in compost & soil Plant origin	Labor saving (ex. Garbage bags which does not require separation), Bio-recycling	Compostable Bags, Kitchen Net
	Alkaline-degradable	Labor saving	Mulch film, Nonwoven, Pot, Drainage device
Agri. & Civil Eng'g	Plant origin	Saving fossil resources, Reduction of GWG	Automobile & electrical appliances
	Plant origin Silk touch	Product design with natural material	Fibers

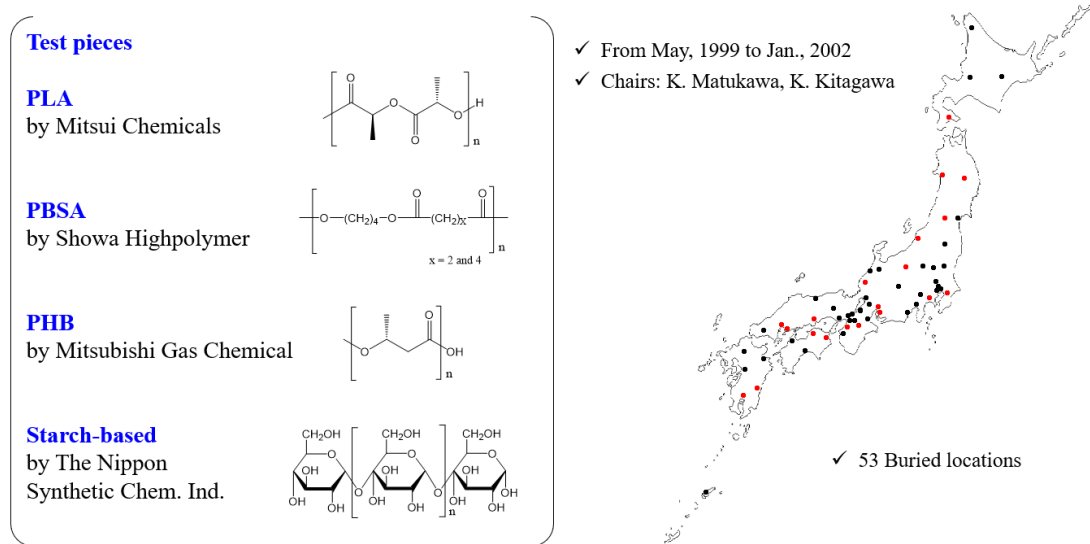


#### 5-4. Three Social Implementation Projects

Although the bioplastics market shifted to highlight renewable resources, fundamental studies to implement biodegradable products in society were conducted from the late 1990s to the early 2000s. These studies were national and international collaborations involving researchers, manufacturers, retailers, waste disposal services, and governments. Here three projects are introduced, in which I led to participate proactively.

##### 5-4-1. Field test of soil biodegradability of biodegradable plastics in Japan

“Field Test of Soil Biodegradability of Biodegradable Plastics in Japan” [13], which was conducted by the working groups for polymer, cooperative committee of materials and chemistry, was a challenging two-year test involving 53 local research centers from Hokkaido to Kagoshima in Japan (Figure 5-6).



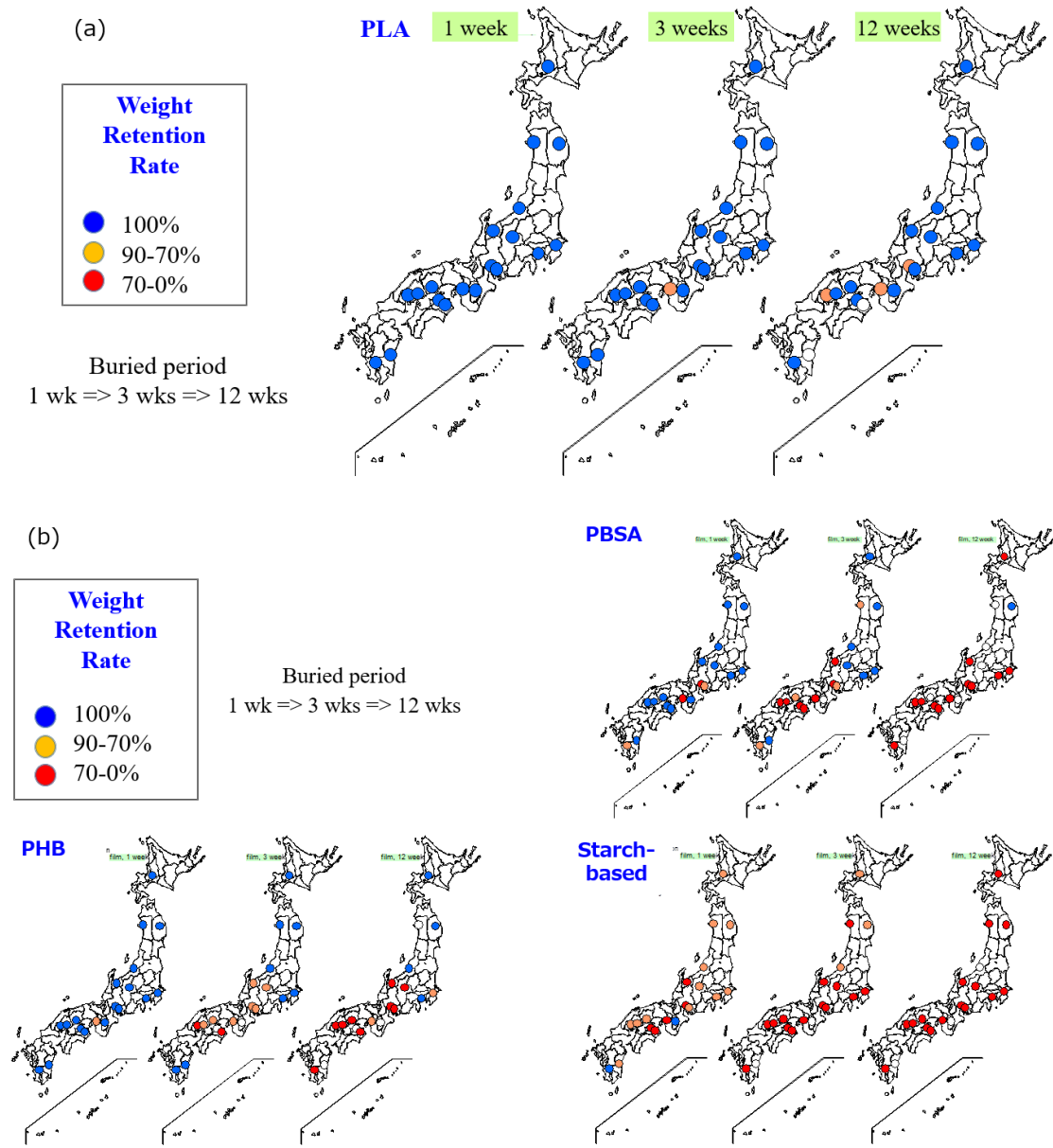
**Figure 5-6.** Field test of soil biodegradability in Japan

At that time, various biodegradable plastics were developed. Among them, PLA [4,14,15], polybutylene succinate-co-adipate (PBSA) [16], polyhydroxyalkanoates (PHAs) [17-19], and starch-based plastics [20] (starch + polycaprolactone [PCL]) were widely introduced in the market. In the field tests, these biodegradable plastic films and injection molded test pieces were buried in the soil to demonstrate degradation ability by measuring the changes in weights and other physical properties. Although many biodegradability tests were conducted around the world, this was one of the first comprehensive and systematic field tests of soil biodegradability.

Figure 5-7 (a) and (b) summarizes the results of the weight changes of the plastic films measured during the test period. Blue indicates a 100% weight retention rate, which means that degradation did not occur. Red denotes that more than 70% degradation occurred, and yellow

shows in-between values. Starch-based plastic films lost weight fastest and degraded within one week. PBS and PHB also degraded by not as fast as starch-based films. PLA maintained its weight the longest (about 12 weeks).

The biodegradabilities obviously varied by the chemical structures of the polymers. Although the same materials were tested, soil conditions such as temperature, pH, and water content also affect biodegradability. In the case of PLA, it degraded into oligomers by hydrolysis and subsequently biodegraded into CO<sub>2</sub> and H<sub>2</sub>O [4].



**Figure 5-7.** Results of the field test soil biodegradability of biodegradable plastics in Japan using films. At each location, the buried periods are 1, 3, and 12 weeks (left to right). Small circles represent the weight retention rate. Blue, yellow, and red indicate no degradation, 70–90% weight retention, and 0–70% weight retention, respectively. These roughly observed rates reflect the difficulty of removing the soil during the measurement. (a) PLA, and (b) PBSA, PHB, and Starch-based.

### 5-4-2. Kassel project

In Germany, a society implementation project was organized from 2001 to 2002 in the city of Kassel. Figure 5-8 summarizes the Kassel project [21-23]. Over a 1.5 years period, 15 biodegradable products, which equaled 31 tons, were sold at 80 shops and returned to a composting facility called Biobins by customers, via the shops. Some of the products were treated in backyard composting facilities of consumers.

The city-wide project aimed to address concerns over complete biodegradability, compost safety, and contamination of non-biodegradable materials mistakenly thrown into biobins as well as improving consumer and public acceptance. Prior to the project, the compostability and safety of compost products were examined on a pilot scale in laboratory tests. As a matter of course, a pilot scale compostability experiment and safety demonstration of grown plants were conducted [24] before PLA-laminated paper plates were sent to the project.

After the project, a market survey, which targeted consumers who participated in the project through their daily shopping activities, was conducted. Biodegradable plastics are supported as environmentally friendly because they are made from renewable resources and are compostable. and consumers recognized that biodegradable packaging is to be composted through either a biobin or backyard composting.

Upon completion, many participants and stakeholders evaluated the project highly, and the results were disseminated widely. Martin Lichtl, who led the project, reported in “Learnings from the Kassel Project” that if the infrastructure exists, biodegradability and compostability are important. In addition, renewable resource-based plastics are also important because the composting infrastructure is not required since greenhouse gas (GHG) emissions are reduced even though they are incinerated.

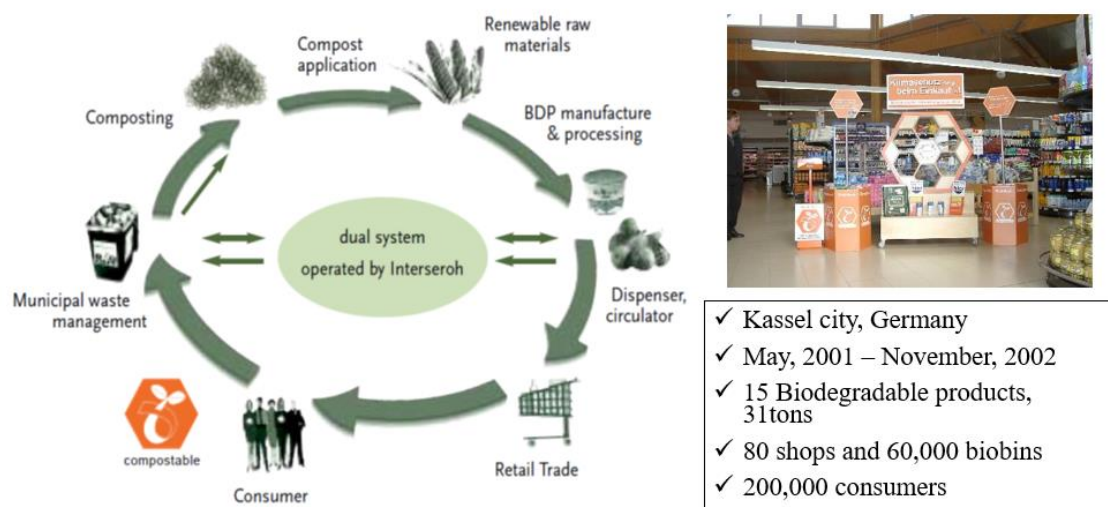


Figure 5-8. Outline of the Kassel Project in Germany.

Dr. Harald Kaeb, Secretary General of the International German Biodegradable Polymers Association and Working Group (IBAW), which is currently called European Bioplastics, published the Basic Principles of IBAW after the project. This document indicates that it is important to comply with Nature Circularity, which consists of renewable resources, reduction of GHG emission, and conservation of fossil resources. Fossil-based biodegradable plastics have meaning as supporting materials for renewable resource-based biodegradable plastics to maintain their physical properties. In addition, to avoid contamination of non-biodegradable or unsafe materials, a closed loop system should be established to demonstrate the role of biodegradable products.

In parallel with technology and market development of bioplastics, ISO test methods [25] and specifications for biodegradability and compostability were developed and harmonized globally from the late 1990s to mid-2000s (Table 5-3).

**Table 5-3.** ISO test methods of compostability and biodegradability.

ISO	Microbes	Detection	Feature	Issue year First edition/Current version
14855-1	Compost	CO <sub>2</sub>	Flask Scale	2005/2012
14855-2	Compost	CO <sub>2</sub>	Lab Scale	2007
17556	Soil	CO <sub>2</sub>	Flask Scale	2003/2012
14851	Sludge	BOD	Flask Scale	1999
14852	Sludge	CO <sub>2</sub>	Flask Scale	1999/2018
16929	Compost	disintegration	Pilot Scale (140L<)	2002/2013
20200	Compost	disintegration	Lab Scale (5-20L)	2004/2015

### 5-4-3. Aichi Expo

In 2005, 2005 EXPO Aichi Japan was held from Mar. 25 to Sept. 25. Their main theme was “Nature Wisdom” and highlighted “Circular Society”. I communicated with the cabinet office and the related ministry offices. We decided to cooperate with the Japanese government in the Aichi Expo by providing two types of tableware. One-way and compostable tableware and returnable tableware were made from PLA and its compounds. The returnable tableware was repeatedly used for 6 months without serious damage. Mechanical or chemical recyclability was assessed experimentally. The compostable tableware was collected after use, composted with food waste, and the compost was used to grow vegetables that were consumed in the food court. Figure 5-9 summarizes the results [26].

This study showed that to promote new materials such as PLA, it is not enough to introduce the products. As shown in these three examples, it is necessary to design and execute a society implementation project to gain social and market acceptance.



Figure 5-9. PLA Tableware Projects, both returnable and compostable, in 2005 EXPO Aichi Japan.

## 5-5. Roles, Issues, and Future Expectations for Bioplastics

Here the roles, issues, and future expectations of bioplastics for both biodegradable and bio-based plastics are summarized based on our rollercoaster experience of PLA business and technology development [11,12,27,28].

### 5-5-1. Roles and issues of biodegradable plastics

The role of biodegradable plastics is in waste treatment due to aerobic degradation into CO<sub>2</sub> and H<sub>2</sub>O or anaerobic degradation to CH<sub>4</sub>. Hence, they contribute at the back end.

Lessons which I learned while marketing PLA as biodegradable plastics are as follows. First, each after-use treatment has its own issues. For example, there are three main issues with composting: collection and sorting of biodegradable materials, quality of the final compost to meet public acceptance, and the number of facilities to accommodate the waste. (In the late 1990s, only 30 composting facilities existed in Japan.) Second, there is a concern that the word biodegradability may cause a moral hazard risk such as littering in the environment. Third, the balance between stability during use and storage and the degradability after use needs to be adjusted in each application. Although appropriate physical properties, including stability, are required during storage and use, biodegradable products need to degrade after use. For example, applications for fishing nets and lines were anticipated as a solution to marine pollution in the early 1990s, but the physical properties of such products did not meet the requirements. Expectations for biodegradable plastics can be summarized in the following messages. Only providing biodegradable materials is not a viable solution. A business model, social “closed system,” and materials for waste treatment such as composting for each use must be taken into consideration. Second, education, cultural, and behavioral changes are important to promote sorting and prevent moral hazards such as littering. In addition, if bio-based resources are used, they contribute to GHG emission reduction.

Technology and market developments of biodegradable plastics have continued after the

completion of social implementation projects using biodegradable plastics such as the ones shown in Section 4. Such initiatives include changes in raw materials and production process development [29] to further reduce the environmental burden as well as a biodegradability study [30,31] from the viewpoint of sustainability. Additionally, business activities to demonstrate a “closed loop system” have been implemented in a fast-food restaurant chain [32] in which all cups, forks, knives, and packaging are compostable. These materials, along with leftover foods, are gathered, transported to a municipal composting facility, and composted. I believe that large-scale and successful businesses will not be realized without accumulating this type of niche business model.

### **5-5-2. Lessons learned for bio-based plastics**

In the case of PLA marketing as bio-based plastics, we faced and learned from the following issues. First, regulations and social systems developed for environmental protection hinder advances in innovative environmentally sound products. For example, Eco Mark in Japan is the environmental label that is attached to products recognized to help the environment. It was originally applied to products containing mechanically recycled materials. Since the life cycle assessment (LCA) advantage was not considered in the early 2000s, Eco Mark was not granted to PLA products. PLA-based products were not approved until 2016, which was 10 years after the initial review.

Since 2017, discussion has continued from the viewpoint of LCA. In addition to PLA, Bio polyethylene (Bio PE), Bio polyethylene terephthalate (Bio PET) (only ethylene glycol can be bio-based), and Bio polypropylene terephthalate (Bio PTT) (only trimethyleneglycol can be bio-based), are listed [33] in approved categories such as clothing, household commodity, refill containers, and resource-saving containers.

Another example, which is applicable to the present day globally, occurred in egg-carton recycling. PLA-based egg cartons were introduced in the market. In the beginning, they were welcomed by retailers and consumers. However, complaints from PET-packaging recyclers citing cloudiness of the recycled PET became louder as the distribution increased, and the market slowed down. If Bio-PET was used in this application instead of PLA, this kind of problem may have been avoided.

It is difficult to sort plastic packaging for mechanical recycling unless it can be easily distinguished by consumers such as PET bottles or foamed polystyrene. Removing food is also problematic. In this regard, PLA products can meet various waste treatment methods: mechanical recycling, chemical recycling and composting, and incineration. These methods can help reduce GHG emission even though it is not sorted with other wastes.

In addition to PLA, Bio-PE, and Bio-PET, a new variety of bioplastics have recently been developed and commercialized such as bio-polyurethane [34-36] from bio-origin polyols and bio-

polyamide [37,38] from bio-origin diamines. Furthermore, a new production process of bio polypropylene (BIO PP) using isopropyl alcohol has been introduced [39]. It is important to continue development not by emphasizing the term “bio-based” but by prioritizing how these bio-based plastics can contribute to GHG emission reductions, changes in raw materials, and minimizing environmental burden.

### 5-5-3. Expectations for bioplastics

I developed and defined a positioning of bioplastics in terms of innovations and raw materials. Table 5-4 summarizes the positioning [40], which clarifies the differences for stakeholders committed to bioplastics technology and business development. Bioplastics can be classified in two categories: new and existing structured plastics. Each category product has its own issues. In the case of new structured plastics such as PLA and PHAs, efforts to create a new market reflecting the properties and functionalities need to be prioritized. Simply replacing existing plastics is not sufficient to induce lasting change. On the other hand, for existing structured plastics such as bio-PE, bio-PP, and bio-PET, cost competitiveness, securing raw materials, and impact assessment of lifecycle should be emphasized. These should not be confused with research and business development.

For bioplastics to become a core component of society over conventional plastics, the advantage in lifecycle analysis [15,41-45] especially issues of raw material and resource usage need to be clarified. Namely, efficient utilization of nonedible resources and food wastes, and land and water must be showcased.

**Table 5-4.** Positioning of bioplastics from the viewpoints of innovations and resources.

	Product Innovation	Process Innovation
		Conversion from existing process to bioprocess Examples: amino acids, acrylamide
<b>Fossil fuel</b>	General purpose ~ functional materials	<b>Expected effects</b> 1. Reduction of energy in the manufacturing process 2. Reduction of CO2 emissions
	<b>Bioplastics (new structure)</b> Example: PLA, PHAs, PEF, PTT	<b>Bioplastics (existing structure)</b> Example: Bio PET, Bio PE, Bio PP
	<b>Expected effects</b> 1. New features and functions (biodegradability is also a function) 2. Reduction of fossil fuel and CO2	<b>Expected effects</b> - Reduction of fossil fuel and CO2
<b>Biomass</b>	<b>Challenges</b> 1. Application development utilizing new features & functions 2. Securing sustainable raw materials (SDGs) 3. 3. Legislation and proper treatment system after use (especially for biodegradable plastics)	<b>Challenges</b> 1. Cost competitiveness (adding environmental value) 2. Securing sustainable raw materials (SDGs) 3. LCA assessment 4. Introduction of reuse system

Edible / non-edible, land use, water use, human rights / working environment, etc.

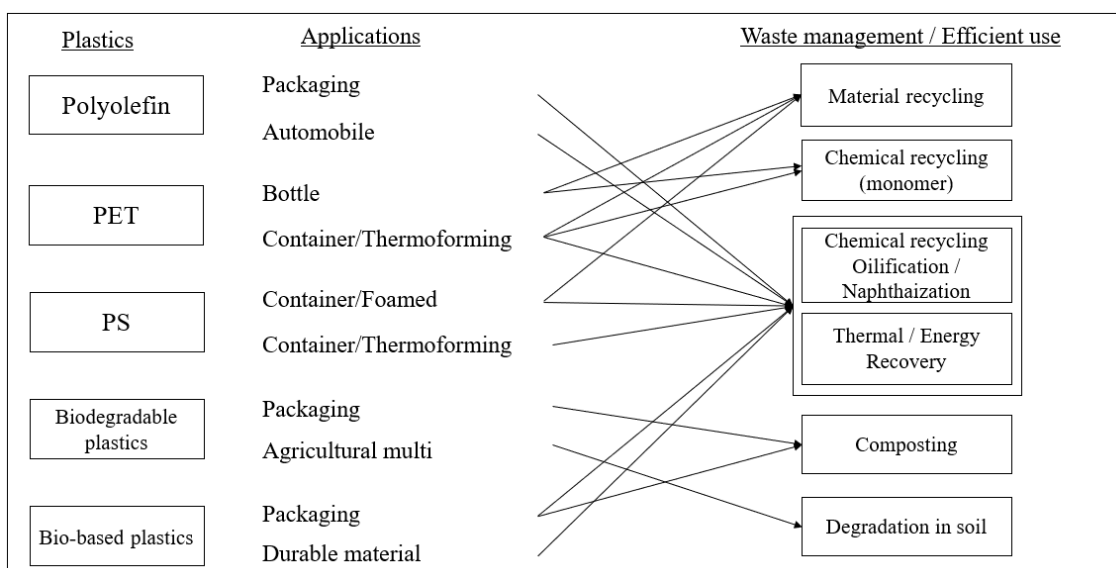


### 5-6. Efficient Treatment of Used Plastics

Although I have focused on the role of bioplastics toward a circular economy herein, the lessons learned from bioplastics can be extended to all plastics categories, including fossil-based plastics. Here the idea of efficient utilization of used plastics is discussed.

A material flow spanning from plastics production through collection to the recovery and efficient utilization for 2020 was reported in Japan [46]. This detailed report was prepared by Plastic Waste Management Institute, Japan. They have been accumulating data since 2000. In 2000, only 46% of the collected used plastics were efficiently utilized. However, 86% of the collected used plastics were efficiently utilized in 2019. Efficient utilization consists of “mechanical recycling,” “liquefaction, gasification, blast furnace,” “densified-reuse derived fuel and cement material/fuel,” “incineration with power generation,” and “incineration with heat utilization facility.” In Japan, the collection and sorting of used plastics were promoted by the framework of the Containers and Packaging Recycling Law [10], which has mitigated littering on land and into the ocean. I anticipate that utilization technologies and efficient use rate should improve both in the mid- and long-terms.

To enhance the efficient utilization of used plastics and to reduce the environmental burden, Figure 5-10 diagrams efficient waste management for different plastics and applications. PET is a good example. A PET bottle is suitable for mechanical recycling and chemical recycling (monomer recycling) since collection and sorting used plastics is relatively easy while maintaining cleanliness. On the other hand, a PET thermo-formed container may be contaminated with food waste as well as other plastic containers and packaging such as a polystyrene (PS) thermo-formed container. Hence, it is not suitable for mechanical and monomer recycling even though it is collected. In addition, thermal recovery and chemical recycling are considered to be appropriate solutions.



**Figure 5-10.** Efficient waste management for each plastic and application.



When CCU and CCS are further developed in practical ways, recovery and recycling will be improved to reduce GHG emissions even though CO<sub>2</sub> is emitted.

Depending on the application, biodegradable plastic products should also be treated appropriately for waste treatment. Biodegradable packaging can be composted if the closed system is established from use to collection and sorting to avoid any contamination of non-biodegradable materials. Biodegradable multifilm can be degraded in soil if contaminating non-degradable films are absent. Under a similar consideration as car-to-car recycling, bio-based plastics can be efficiently used by chemical recycling and thermal/energy recovery to contribute to carbon neutralization even though it is not well sorted after collection.

The era of business models only considering materials is over. Business models must take a holistic approach. To realize a sustainable society, they have to cover raw materials, plastics, applications, and efficient waste management as this will realize a circular economy.

## **5-7. Conclusion**

This chapter reviewed the roles and issues of biodegradable and bio-based plastics, which were learned through experiences of technology and market development of PLA. By applying personal knowledge and experience, the mid- and long-term approaches for resilient and sustainable development of plastics, including fossil-based, can be discussed from the viewpoint of a circular economy, as opposed to allowing short-term approaches and reputational needs to dictate the future of plastics. Important considerations for the development of bioplastics and fossil-based plastics include:

- i. Three aspects need to be observed: zero-emission of used plastics leaked into the environment (land and ocean), improved utilization rate and utilization technologies for used plastic products, and reduction in CO<sub>2</sub> emissions.
- ii. Business models both for bioplastics and fossil-based plastics should be designed throughout the value chain considering raw materials, plastic materials, applications, and waste management using a firsthand understanding of the real challenges to society and an appropriate evaluation of life cycle assessment. It should be noted that existing regulations and systems to achieve an environmentally sound society are often obstacles when developing environmentally sound products.
- iii. Mechanical recycling and chemical recycling as well as thermal and energy recovery will play important roles in the efficient utilization of used plastics for both bioplastics and fossil-based plastics. This will be strengthened by reuse, maintenance, repair and life extension, and sharing, together with an innovative development of CCU and CCS.
- iv. To enhance the recognition of bio-based plastics, issues of efficient utilization of nonedible resources and food wastes as well as issues of land and water and child labor need to be solved from the viewpoint of life cycle assessments and SDGs.

- v. Biodegradable plastics will have social and environmental impacts if a closed system is established to treat them after use in compost along with organic wastes.

Taking a holistic approach that considers each step of the value chain and implementing integrated approaches that highlight innovation should achieve meaningful progress in addressing the challenges and issues of bioplastics and fossil-based plastics.

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## Chapter 6

### Summary and Conclusion

In this thesis, various aspects of PLA were studied from basic research to social implementation. Basic research or science will have an opportunity to contribute to future daily life as a disruptive innovation to provide solutions for life or social pain. In this sense this study should have the potential to enhance the role of PLA in the circular economy.

In Chapter 2, it is reported that the simple and efficient CER method to produce HMW-PLA from LMW-PLA using diisocyanate as a chain extender for connecting the hydroxy and carboxylic termini. The reaction between isocyanate and carboxylic acid, which is usually slow, was accelerated by using a catalyst. It is noted that the urethane bond, produced by the reaction of hydroxy group and isocyanate, is relatively lower thermal stability than the amide bond, produced by the reaction of carboxylic acid and isocyanate. In this study, more durable PLA against thermal degradation in the melting process was achieved by converting the hydroxy terminus to the carboxylic acid terminus to connect the PLA chain with amide bonds. This method can be applied to expand the range of polymer design such as synthesizing PLA block copolymer, and to mechanical recycling or repair to restore the molecular weight from LMW PLA, which results during the recycling process.

In Chapter 3, screening animal waste-based composts from farms and facilities to degrade PLA was tested, and this is the first study to lead to the isolation and identification of a strain of *Nocardiosis chromatogenes*. This study employed distinct approaches to isolate PLA-specific degrading microorganisms. The one of approaches attempted to eliminate PLA degradation via hydrolysis. Specifically, the compost temperature, which was derived from various types of livestock dung, was kept below 37 °C because ammonia generation or a higher pH due to proteolysis at high temperatures may promote hydrolysis. Keeping the temperature lower inhibited hydrolysis in the initial stage of degradation. Consequently, non-enzymatic degradation was avoided. Future studies are expected to develop characterizing the PLA degradation, identifying the enzymes involved, and elucidating the degradation mechanism, and results in creating and designing efficient treatment system of PLA product after use.

The purpose of the study in Chapter 4 was to demonstrate the importance of a pilot-scale composting test, rather than a laboratory test, to show that composting is the proper treatment method for social implementation after using packaging made of PLA contaminated with food waste. As a result, it was confirmed that both biodegradability and disintegration are demonstrated under actual composting conditions on the pilot scale, and the presence or absence of PLA products does not affect the composting process. Furthermore, the obtained compost is high quality and safe and does not show adverse effects on plant growth. This type of comprehensive

pilot-scale compost test should be useful to design a social system to treat organic wastes together with biodegradable products after use.

In Chapter 5, lessons learned from experiences in PLA business development are summarized along with perspectives on recent global movement in circular economy. For medium- and long-term goals of plastics development, three aspects need to be observed: zero-emission of used plastics leaked into the environment (land and ocean), improved utilization rate and utilization technologies for used plastic products, and reduction in CO<sub>2</sub> emissions. Business models both for bioplastics and fossil-based plastics are common and should be designed throughout the value chain considering raw materials, plastic materials, applications, and waste management. A unique circular economy model for plastics was designed, in which the places and factors where each research theme in this thesis can contribute are highlighted in a comprehensive way.

Holistic research approaches of PLA representing bioplastics in this thesis will lead to guidance and inspiration for the future development of materials and social systems that support environment protection and resource conversion.



## List of Publications

1. Kawashima, N.; Usugi, S.; Ogawa, R. Chain-extension of Low Molecular Weight Poly(lactic acid) with Diisocyanate via Amide Formation to Enhance the Thermal Degradation Stability. In preparation.
2. Kawashima, N.; Tokuda, J.; Yagi, T.; Takahashi K. Isolation of a *Norcardiopsis chromatogenesis* Strain that Degrade Poly(lactic acid) in Pig Waste-based Compost. *Arch. Microbiol.* **2022**, In press.
3. Kawashima, N.; Yagi, T.; Kojima, K. Pilot-scale Composting Test of Poly(lactic acid) for Social Implementation. *Sustainability* **2021**, *13*, 1654-1672. DOI: 10.3390/su13041654
4. Kawashima, N.; Yagi, T.; Kojima, K. How Do Bioplastics and Fossil-Based Plastics Play in a Circular Economy? *Macromol. Mater. Eng.* **2019**, *304*, 1900383. DOI: 10.1002/mame.201900383
5. Kawashima, N.; Matsuo, M.; Sugi, M. Role of Bio-based Polymers toward Building a Sustainable Society -Through a Business Development of Poly(lactic acid), LACEA -. *Kobunshi Ronbunshu* **2005**, *62*, 233-241. DOI:10.1295/KORON.62.233

Current status of the publications, dated September 12, 2023

1. Kawashima, N.; Usugi, S.; Ogawa, R. Diisocyanate-based chain extension via Mg(II) catalyzed amide formation to high-molecular-weight poly(lactic acid). *J. Polym. Sci.* **2023**, 1-8. DOI: 10.1002/pol.20230284
2. Kawashima, N.; Tokuda, J.; Yagi, T.; Takahashi K. Isolation of a *Norcardiopsis chromatogenesis* Strain that Degrade Poly(lactic acid) in Pig Waste-based Compost. *Arch. Microbiol.* **2022**, *204*, 599. DOI: 10.1007/s00203-022-03144-w