New Method of Rapid and Simple Colorimetric Assay for Detecting the Enzymatic Degradation of Poly Lactic Acid Plastic Films

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***Abstract*—Biodegradable plastics (BPs), such as Poly Lactic Acid (PLA), has received much attention from the viewpoint of environmental protection and solid-waste management in agriculture field. Various microorganisms, including bacteria, fungi, and yeast, have been reported to degrade PLA by measuring the decrease in the turbidity of PLA dispersions. However, the results indicated that there are many microorganisms that can degrade PLA dispersions but not solid BPs. The objective in this study was developed a rapid and simple methods that more suitable for evaluating the degradation of solid PLA films. Evaluation of the relative PLA-degradation activity using dye-containing PLA films (dye-PLA assay) indicated Optical Density (OD600), and Total Organic Carbon (TOC). It can conclude that two methods are suitable for evaluation of the relative PLA-degradation activity using dye-containing PLA films (dye-PLA assay). Moreover, enzyme solution from *Aneurinibacillus migulanus* had similar ability to proteinase K from *Engyodontium* album within 24 hrs at 30 ℃.**

***Index Terms*—biodegradation, Poly Lactic Acid (PLA), *Aneurinibacillus migulanus*.**

I. Introduction

The use of biodegradable plastics (BPs), such as Poly Lactic Acid (PLA), poly (ε-caprolactone) (PCL), and poly (butylene succinate-coadipate) (PBSA), has received much attention from the viewpoint of environmental protection and solid-waste management [1]. In natural environment, microorganisms can degrade and assimilate BPs as a carbon source and release CO_2; thus, the international standard for the evaluation of their biodegradability is based on measuring the amount of CO_2 released under the specified conditions for 180 days or less [1], [2]. Various BPs degrading microorganisms including bacteria, fungi, and yeast have been reported [3]-[13]. In many cases, the abilities of these microorganisms to degrade BPs have been assayed easily by measuring the decrease in the turbidity of BP dispersions. This assay can be used as an initial screening for BPs degrading microorganisms. However, preliminary results indicated that there are many microorganisms that can degrade BP dispersions but not solid BPs [12]. Therefore, a second round of screening is needed to identify the microorganisms or enzymes that can degrade solid BPs. The degradation of solid BPs could be evaluated by measuring the weight loss of BP films following enzymatic degradation, but this method needs a relatively long time (100 days or less) to confirm [5], [11].

Alternatively, the degradation could be evaluated by measuring the increase in solubilized monomers or oligomers released from the BP films into the reaction solution. For Biodegradable Plastics (BPs) such as Poly(Butylene Succinate (PBS), PLA and Poly Urethanes (PU) which are difficult to recover from glass slides this assay technique performed on a glass slide as described above would be more suitable [13].

The applications for PLA are thermoformed products such as drink cups, take-away food trays, containers and planter boxes. The material has good rigidity characteristics, allowing it to replace polystryene and PET in some applications. PLA is fully biodegradable when composted in a large-scale operation with temperatures of 60℃ and above. The first stage of degradation of PLA (two weeks) is via hydrolysis to water-soluble compounds and lactic acid. Rapid metabolisation of these products converted into CO_2, water and biomass by a variety of microorganisms. There have been reports on the degradation of PLA oligomers (molecular weight ~1000) by Fusarium moniliforme and Penicillium roquefort [14] and the degradation of PLA by Amycolatopsis sp. [3], [15] and by Bacillus brevis [16]. In addition, enzymatic degradation of low molecular weight PLA (molecular weight~2000) has been shown using esterase-type enzymes such as Rhizopus delemer lipase [17]. McCarthy [18] showed that APLA presents a soil degradation rate much slower compared to PBSA [19].

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Bacillus migulanus sp. nov. In 1996, using gene sequence alignment of 16S rRNA, ATCC 9999T was reclassified: Aneurinibacillus migulanus comb. nov. Based on DNA-DNA hybridization with DSM 2895T, the two other GS-producing strains from DMSZ (DSM 5668 and DSM 5759) were assigned to *A. migulanus* as well [20].

Poly (Lactic Acid) (PLA) PLA ([-O(CH3)CHCO-]n) is a biodegradable and biocompatible thermoplastic which can be produced by fermentation from renewable resources. It can also be synthesized either by condensation polymerization of lactic acid or by ring opening polymerization of lactide in the presence of a catalyst. This polymer exists in the form of three stereoisomers: poly (L-lactide) (L-PLA), poly(D-lactide) (D-PLA) and poly(DL-lactide) (DL-PLA). The manufacture of PLA from lactic acid was pioneered by Carothers [21].

Ecological studies on the abundance of PLA-degrading microorganisms in different environments have confirmed that PLA-degraders are not widely distributed, and thus it is less susceptible to microbial attack compared to other microbial and synthetic aliphatic polymers [39]. The degradation of PLA in soil is slow and that takes a long time for degradation to start. Microbial degradation of PLA using Amycolatopsis sp. was first reported by [37]. Since then, a number of research studies dealing with microbial and enzymatic degradation of PLA have been published [22]. Many strains of genus Amycolatopsis and Saccharotrix were able to degrade both PLA and silk fibroin. The main amino acid constituents of silk fibroin are L-alanine and glycine and there is a similarity between the stereochemical position of the chiral carbon of L-lactic acid unit of PLA and L-alanine unit in the silk fibroin. Silk fibroin is one of the natural analogues of poly(L-lactide), thus, the PLA degrading microorganisms may probably identify the L-lactate unit as an analogue of L-alanine unit in silk fibroin. Several proteinous materials such as silk fibroin, elastin, gelatin and some peptides and amino acids were found to stimulate the production of enzymes from PLA-degrading microorganisms [23-26].

Generally, an increase in molecular weight results in a decline of polymer degradability by microorganisms. In contrast, monomers, dimers, and oligomers of a polymer's repeating units are much easily degraded and mineralized. High molecular weights result in a sharp decrease in solubility making them unfavorable for microbial attack because bacteria require the substrate to be assimilated through the cellular membrane and then further degraded by cellular enzymes. At least two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases. During degradation, exoenzymes from microorganisms break down complex polymers yielding smaller molecules of short chains, e.g., oligomers, dimers, and monomers, that are smaller enough to pass the semi-permeable outer bacterial membranes, and then to be utilized as carbon and energy sources. The process is called depolymerization. When the end products are CO_2, H_2O, or CH_4, the degradation is called mineralization. It is important to note that biodeterioration and degradation of polymer substrate can rarely reach 100% and the reason is that a small portion of the polymer will be incorporated into microbial biomass, humus and other natural products [27]. Dominant groups of microorganisms and the degradative pathways associated with polymer degradation are often determined by the environmental conditions.

When O_2 is available, aerobic microorganisms are mostly responsible for destruction of complex materials, with microbial biomass, CO_2, and H_2O as the final products. In contrast, under anoxic conditions, anaerobic consortia of microorganisms are responsible for polymer deterioration. The primary products will be microbial biomass, CO_2, CH_4 and H_2O under methanogenic (anaerobic) conditions (e.g. landfills/ compost) [27], [28].

Bacteria are important to biodegradation process including interalia, Bacillus (capable of producing thick-walled endospores that are resistant to heat, radiation and chemical disinfection), *Pseudomonas, Klebsiella, Actinomycetes,Nocardia,Streptomyces,Thermoactinomycetes, Micromonospora, Mycobacterium, Rhodococcus, Flavobacterium, Comamonas, Escherichia, Azotobacter* and *Alcaligenes* (some of them can accumulate polymer up to 90% of their dry mass) [29]. Temperature is one of the most important factors affecting microorganism growth. Sources of carbon, nitrogen, and pH are also important. Fungi active in the biodegradation process are *Sporotrichum, Talaromyces, Phanerochaete, Ganoderma, Thermoascus, Thielavia, Paecilomyces, Thermomyces, Geotrichum, Cladosporium, Phlebia, Trametes, Candida, Penicillium, Chaetomium*, and *Aerobasidium* [30], [31].

The biodegradation process can be divided into (1) aerobic and (2) anaerobic degradation If oxygen is present, aerobic biodegradation occurs and carbon dioxide is produced. If there is no oxygen, an anaerobic degradation occurs and methane is produced instead of carbon dioxide. When conversion of biodegradable materials or biomass to gases (like carbon dioxide, methane, and nitrogen compounds), water, salts, minerals and residual biomass occurs, this process is called mineralization. Mineralization will be completed when all the biodegradable materials or biomass are consumed and all the carbon is converted to carbon dioxide. Biodegradable materials have the proven capability to decompose in the most common environment where the material is disposed, within one year, through natural biological processes into non-toxic carbonaceous soil, water or carbon dioxide. The chemical structure (responsible for functional group stability, reactivity, hydrophylicity and swelling behavior) is the most important factor affecting the biodegradability of polymeric materials. Other important factors are inter alia, physical and physico-mechanical properties, e.g., molecular weight, porosity, elasticity and morphology (crystalline, amorphous) [29].

The objective in this study was developed a rapid and simple methods that more suitable for evaluating the degradation of solid Poly Lactic Acid (PLA) films.

II. Material and Methods

A. Microbial Degradation of PLA

The composition of this medium contained 10 g Bacto tryptone; 5 g beef extract; 5 g NaCl; and 1000 mL distilled water; the pH value was adjusted to 7.0 with 1 M NaOH and 1 M HCl, followed by sterilization at 121 ℃ for 15 minute. A loop of *Aneurinibacillus migulanus* was transferred from a nutrient agar plate to nutrient broth tubes. The inoculums having a cell density of 106-107 cfu/mL was obtained by growing the culture overnight (24 h) in liquid broth medium at 30 ℃, 180 rpm [32]. After that the mixture cured enzymes were centrifuged (10,000xg, 10 min, 4 ℃) and the supernatant were stored at 4 ℃. This supernatant was further used for next step.

B. The Concentration of Total Organic Carbon (TOC) Analysis

The absorbance at OD_{600} nm was measured using a Shimadzu, Japan spectrophotometer. The water-soluble TOC concentration in the solution was measured using a TOC-5000 analyzer (Shimadzu Co., Kyoto, Japan) [33].

C. New Method Preparation

Each PLA film was dissolved in dichloromethane at 1 wt%, and then 300 mL of the solution was mixed with 10 mL of Methylene blue staining reagent (7% w/v; Nacalai Tesque, Inc. Kyoto, Japan). PLA solution was mixed with 20 mL Methylene blue staining reagent in each well (ϕ 6 mm), The weight of PLA films were calculated to be 0.0020 and 0.0030 g, respectively (based on the dichloromethane density, 1.318 g/mL) (Fig. 1). Hand-made dye-containing PLA films were used as substrates. After treatment of the film with an enzyme solution for 24 h at 30 ℃, the relative amount of dye released was measured using a colorimetric detector.

D. Evaluation of the Relative PLA-Degradation Activity Using Dye-Containing PLA Films (Dye-PLA Assay)

Proteinase K from *Engyodontium album* (formerly *Tritirachium album*), CAS RN 39450-01-6, E.C. 3.4.21.64, and Synonym: *Endoproteinase* K. Molecular mass: 28,930 Da (amino acid sequence) or 28,500 Da (SDS-PAGE). The product is from Sigma-Aldrich. Lipase B is from Cadida Antarctica, recombinant from *Aspergillus oryzae* (sigma) power. Beie, -9 U/mg, lot result: 14.4 U/mg. Then 40 mL of the appropriately diluted enzymes (lipase B, proteinase K, and A. migulanus) in 20 mM TriseHCl (pH 8.8) were separately dropped onto the BP films (Figure 2A). Then the samples were incubated at 30 ℃ for 24 hrs [13]. The reaction solutions on the PLA films were collected into a 96-well microplate (round bottom), and the OD_{600} values were measured using an Epoch Elisa reader multi spectrophotometer (BioTeck Instrument, Inc, Taiwan).

E. Statistic Analysis

The results were recorded and analyzed by SAS system for windows V. 9.3 and all experiments were done in triplicate.

III. Developed Methods

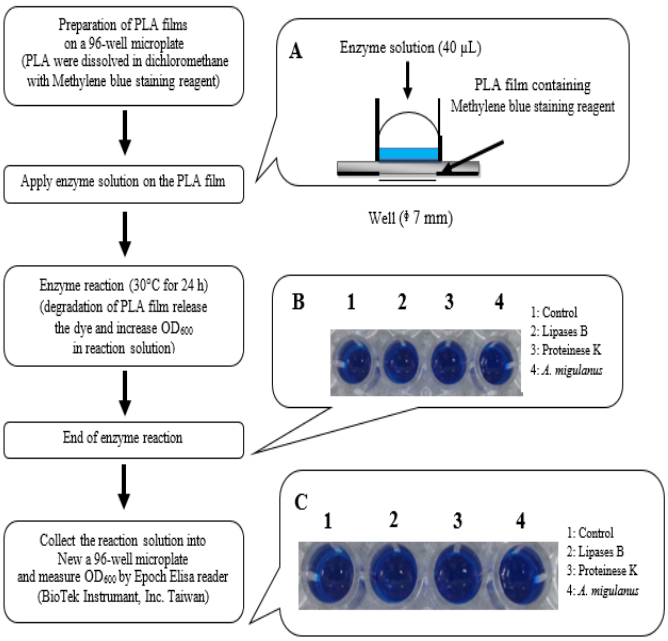

Figure 1. Evaluation of the relative PLA-degradation activity using dye-containing PLA films (dye-PLA assay). (A) Cross-sectional view of a PLA film and enzyme solution in 96-well microplate (B) Photograph of the microplate with PLA films and enzyme solutions after the reaction (30 ℃ for 24 h). (C) Reaction solutions collected after the degradation of dye-containing PLA films (Developed from [13]).

IV. Results and Discussion

A. Evaluation of the Relative BPs-Degradation Activity Using Dye-Containing PLA Films (Dye-PLA Assay)

Diluted enzymes of 40 mL (lipase B, proteinase K, and A. migulanus) in 20 mM TriseHCl (pH 8.8) were separately dropped onto the BP films (Fig. 2). Then the samples were incubated at 30 ℃ for 24 h. The OD_{600} values of the enzyme solutions were obtained after subtracting the values for the controls. After the enzymatic reactions with the dye-containing PLA films, the color changes in the reaction solutions were observed (Fig. 2, Fig. 3). The OD_{600} values of the solutions were correlated with the enzyme concentrations. Fig. 2 indicated that A. migulanus and Proteinase K, OD_{600} values were almost similarly (3.60) and lipase B was 2.87 at 30 ℃ for 24 h. As results also presented the optical density value higher than used the BPs-degrading enzyme from *Pseudozyma antarctica* JCM10317 (PaE) [13]. As the results from TOC values were same approach with optical density value with is the highest from A. migulanus (18.56±2.22 ppm) followed by Proteinase K and lipase B (8.74±2.56, 3.71±2.39 ppm) respectively (Fig. 3).

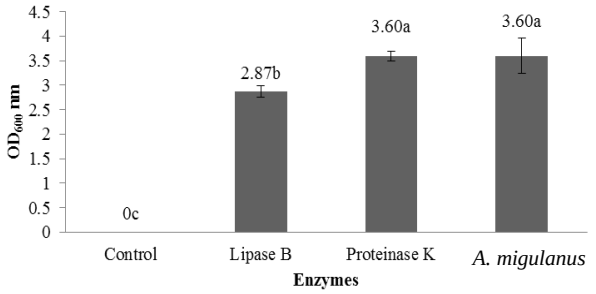

Figure 2. Comparison of the relative PLA-degrading activities of commercially available enzymes and A. migulanus. OD600 in the reaction solution after the enzymatic degradation of dye-containing PLA films (30 ℃ for 24 hrs) after subtracting the values for the control.

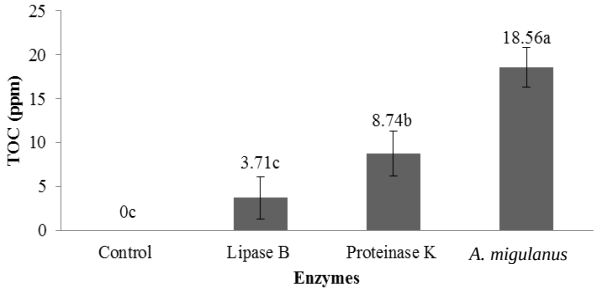

Figure 3. TOC measurements after degradation of PLA films by commercially available enzymes and A. migulanus at 30 ℃ within 24 hrs (Values are expressed as the mean (SD) at n = 3).

Similar to Williams [34] investigated the enzymatic degradation of PLA using proteinase K, bromelain and pronase. Among these enzymes, proteinase K from *Tritirachium* album was the most effective for PLA degradation. Proteinase K and other serine proteases are capable of degrading L-PLA and DL-PLA but not D-PLA. Furthermore, proteinase K preferentially hydrolyzes the amorphous part of L-PLA and the rate of degradation decreases with an increase in the crystalline part [35], [36]. The degradation of PLA oligomers was accelerated by several esterase-type enzymes, especially *Rhizopus delemar* lipase. The purified PLA depolymerase from *Amycolatopsis* sp. was also capable of degrading casein, silk fibroin, Suc-(Ala)3-pNA but not PCL, PHB and Suc-(Gly)3-pNA [37]. Their studies showed that PLA depolymerase was a kind of protease and not a lipase. It was reported that α-chymotrypsin can degrade PLA and PEA with lower activity on poly (butylenes succinate-co-adipate) (PBS/A). Moreover, several serine proteases such as trypsin, elastase, subtilisin were able to hydrolyze L-PLA [38, 39]. PLA is a biocompatible thermoplastic with a melting temperature of 175 ℃ and a glass transition temperature of 60 ℃. It is synthesized by the polymerization of L-lactic acid. PLA can be hydrolyzed by the lipase from R. delemar and the proteinase K from *Tritirachium* album and also by the polyester polyurethane depolymerase from *Comamonas acidovorans*. PLA is more resistant to microbial attack in the environment than other microbial and synthetic polyesters [29].

V. Conclusions

The results from OD_{600} and TOC can conclude that two methods are suitable for evaluation of the relative PLA-degradation activity using dye-containing PLA films (dye-PLA assay). Moreover, enzyme solution from *Aneurinibacillus migulanus* has similar ability to proteinase K from *Engyodontium* album.

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References

[1] J. H. Song, R. J. Murphy, R. Narayan, and G. B. H. Davies, "Biodegradable and compostable alternatives to conventional plastics," *Philosophical Transactions of the Royal Society B: Biological*, vol. 364, pp. 2127-2139, 2009.

[2] M. Kunioka, F. Ninomiya, and M. Funabashi, "Novel evaluation method of biodegradabilities for oil-based polycaprolactone by naturally occurring radiocarbon-14 concentration using accelerator mass spectrometry based on iso 14855-2 in controlled compost," *Polymer Degradation and Stability*, vol. 92, pp. 1279-1288, 2007.

[3] H. Pranamuda, Y. Tokiwa, and H. Tanaka, "Polylactide degradation by an *amycolatopsis* sp.," *Applied and Environmental Microbiology*, vol. 63, pp. 1637-1640, 1997.

[4] K. Nakamura, T. Tomita, N. Abe, and Y. Kamio, "Purification and characterization of an extracellular poly (l-lactic acid) depolymerase from a soil isolate, *amycolatopsis* sp. strain K104-1," *Applied and Environmental Microbiology*, vol. 67, pp. 345-353, 2001.

[5] A. Hoshino and Y. Isono, "Degradation of aliphatic polyester films by commercially available lipases with special reference to rapid and complete degradation of poly (L-lactide) film by lipase pl derived from *alcaligenes* sp.," *Biodegradation*, vol. 13, pp. 141-147, 2002.

[6] N. Hayase, H. Yano, E. Kudoh, C. Tsutsumi, K. Ushio, Y. Miyahara, *et al*., "Isolation and characterization of poly (butylenessuccinate-co-butyleneadipate)- degrading microorganism," *Journal of Bioscience and Bioengineering*, vol. 97, pp. 131-133, 2004.

[7] K. Masaki, N. R. Kamini, H. Ikeda, and H. Iefuji, "Cutinase like enzyme from The yeast cryptococcus sp. strain S-2 hydrolyzes polylactic acid and other biodegradable plastics," *Applied and Environmental Microbiology*, vol. 71, pp. 7548-7550, 2005.

[8] H. Maeda, Y. Yamagata, K. Abe, F. Hasegawa, M. Machida, R. Ishioka, *et al*., "Purification and characterization of a biodegradable plastic-degrading enzyme from *Aspergillus oryzae*," *Applied Microbiology and Biotechnology*, vol. 67, no. 6, pp. 778-788, 2005.

[9] H. S. Seo, H. J. Um, J. Min, S. K. Rhee, T. J. Cho, Y. H. Kim, *et al*.,"*Pseudozymajejuensis* sp. nov., a novel cutinolytic stilaginomycetous yeast species that is able to degrade plastic waste," *FEMS Yeast Research*, 2007, vol. 7, pp. 1035-1045.

[10] F. Li, S. Wang, W. F. Liu, and G. J. Chen, "Purification and characterization of poly (L-lactic acid)-degrading enzymes from *Amycolatopsis orientalis* ssp. orientalis," *FEMS Microbiology Letters*, vol. 282, pp. 52-58, 2008.

[11] T. Nakajima-Kambe, K. Toyoshima, C. Saito, H. Takaguchi, Y. Akutsu-Shigeno, M. Sato, *et al*., "Rapid monomerization of poly (butylene succinate)-co-(butylene adipate) by leptothrix sp.," *Journal of Bioscience and Bioengineering*, vol. 108, pp. 513-516, 2009.

[12] H. K. Kitamoto, Y. Shinozaki, X. H. Cao, T. Morita, M. Konishi, K. Tago, *et al.*, "Phyllosphere yeasts rapidly break down biodegradable plastics," *AMB Express*, vol. 1, pp. 44, 2011.
[13] Y. Shinozaki, T. Watanabe, T. Nakajima-Kambe, and H. K. Kitamoto, "Rapid and simple colorimetric assay for detecting the enzymatic degradation of biodegradable plastic films," *Journal of Bioscience and Bioengineering*, vol. 115, no. 1, pp. 111-114, 2013.
[14] A. Torres, S. Li, S. Roussos, and M. Vert, "Screening of microorganisms for biodegradation of poly (lactic acid) and lactic acid-containing polymers," *Applied and Environmental Microbiology*, vol. 62, pp. 2393-2397, 1996.
[15] H. Pranamuda and Y. Tokiwa, "Degradation of poly (l-lactide) by strains belonging to genus Amycolatopsis," *Biotechnology Letters*, vol. 21, pp. 901-905, 1999.
[16] K. Tomita, Y. Kuraki, and K. Nagai, "Isolation of thermophiles degrading poly (l-lactic acid)," *Journal of Bioscience and Bioengineering*, vol. 87, pp. 752-755, 1999.
[17] H. Fukuzaki, M. Yoshida, M. Asano, and M. Kumakura. "Synthesis of co poly (d, l-lactic acid) with relative low molecular weight and *in vitro* degradation," *European Polymer Journal*, vol. 25, pp. 1019-1026, 1989.
[18] S. P. McCarthy, "Advances in properties and biodegradability of continuous, immiscible, biodegradable, polymer blends," *Macromolecular Symposia*, vol. 144, pp. 63-72, 1999.
[19] A. A. Shah, F. Hasan, A. Hameed, and S. Ahmed, "Biological degradation of plastics: A comprehensive review", *Biotechnology Advances*, vol. 26, pp. 246-265, 2008.
[20] M. Berditsch, S. Afonin, and A. S. Ulrich, "The ability of *Aneurinibacillus migulanus* (*Bacillus brevis*) to produce the *antibiotic gramicidin* S is correlated with phenotype variation," *Applied and Environmental Microbiology*, vol. 73, no. 20, pp. 6620-6628, 2007.
[21] W. H. Carothers and J. W. Hill. "Studies of polymerization and ring formation and artificial fibers from synthetic linear condensation superpolymers," *Journal of the American Chemical Society (ACS Publications)*, 1932, vol. 54, pp. 1579-1596.
[22] Y. Tokiwa and B. P. Calabia, "Biodegradability and biodegradation of poly (lactide)," *Journal of Applied Microbiology*, 2006, vol. 72, pp. 244-251.
[23] A. Jarerat and Y. Tokiwa, "Degradation of poly (l-lactide) by fungus," *Macromolecular Bioscience*, vol. 1, pp. 136-140, 2001.
[24] A. Jarerat and Y. Tokiwa, "Poly (l-lactide) degradation by Saccharotrix waywayandensis," *Biotechnology Letters*, vol. 25, pp. 401-404, 2003.
[25] A. Jarerat and Y. Tokiwa, "Poly (l-lactide) degradation by *Kibdelosporangium aridum*," *Biotechnology Letters*, vol. 25, pp. 2035-2038, 2003.
[26] A. Jarerat, Y. Tokiwa, and H. Tanaka, "Microbial poly (l-lactide) degrading enzyme induced by amino acids, peptides and poly (l-amino acids)," *Journal of Polymers and the Environment*, vol. 12, pp. 139-146, 2004.
[27] A. A. Shah, F. Hasan, A. Hameed, and S. Ahmed, "Biological degradation of plastics: A comprehensive review," *Biotechnology Advances*, vol. 26, pp. 246-265, 2008.
[28] R. J. Mueller, "Biodegradability of polymers: Regulations and methods for testing," in *Biopolymers*, A. Steinbüchel, ed. Weinheim: Wiley-VCH, 2003.
[29] K. Leja and G. Lewandowicz, "Polymer biodegradation and biodegradable polymers-a review," *Polish Journal of Environmental Studies*, vol. 19, no. 2, pp. 255-266, 2010.
[30] M. Tuomela, M. Vikman, A. Hatakka, and M. Itavaara, "Biodegradation of lignin in a compost environment: A review," *Bioresource Technology*, vol. 72, pp. 169, 2000.
[31] R. Gautam, A. S. Bassi, E. K. Yanful, and E. Cullen, "Biodegradation of Automotive Waste Polyester Polyurethane Foam Using *Pseudomonas chlororaphis* ATCC55729," *International Biodeterioration & Biodegradation*, vol. 60, pp. 245-249, 2008.
[32] K. Chaisu, A. L. Charles, Y. K. Guu, and C. H. Chiu, "Optimization of Poly Lactic Acid (PLA) plastic degradation by *Aneurinibacillus Migulanus* using response surface methodology," *IPCBEE*, vol. 40, pp. 22-27, 2012.
[33] K. Tomita, T. Nakajima, Y. Kikuchi, and N. Miwa, "Degradation of poly (L-lactic acid) by a newly isolated thermophile," *Polymer Degradation and Stability*, vol. 84, pp. 433-438, 2004.
[34] D. F. E. Williams, "Enzymatic hydrolysis of polylactic acid," *The New England Journal of Medicine*, vol. 10, pp. 5-7, 1981.
[35] M. S. Reeve, S. P. McCarthy, M. J. Downey, and R. A. Gross, "Polylactide stereochemistry: Effect on Enzymatic Degradability," *Macromolecules*, vol. 27, pp. 825-831, 1994.
[36] R. T. McDonald, S. McCarthy, and R. A. Gross. "Enzymatic degradability of poly(lactide): Effects of chain stereochemistry and material crystallinity," *Macromolecules,* 1996, vol. 29, no. 23, pp. 7356-7361.
[37] H. Pranamuda, A. Tsuchii, and Y. Tokiwa, "Poly (L-lactide)-degrading enzyme produced by *Amycolatopsis* sp.," *Macromolecular Bioscience*, vol. 1, pp. 25-29, 2001.
[38] H. A. Lim, T. Raku, and Y. Tokiwa, "Hydrolysis of polyesters by serine proteases," *Biotecchnology*, vol. 27, pp. 459-464, 2005.
[39] Y. Tokiwa, B. P. Calabia, C. U. Ugwu, and S. Aiba, "Biodegradability of plastics review," *International Journal of Molecular Sciences*, vol. 10, pp. 3722-3742, 2009.

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