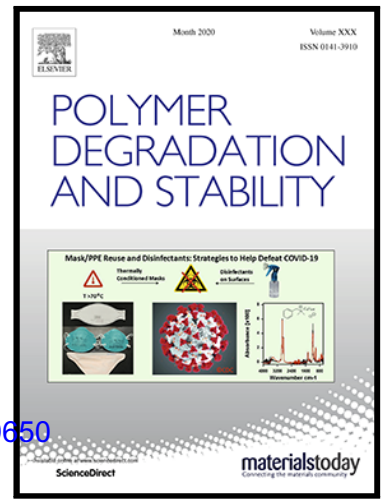

Biodegradability of poly(butylene n-alkylenedionate)s composed of long-methylene chains as alternative polymers to polyethylene

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PII: S0141-3910(21)00170-1
DOI: https://doi.org/10.1016/j.polymdegradstab.2021.109650
Reference: PDST 109650

To appear in: *Polymer Degradation and Stability*

Received date: 19 April 2021
Revised date: 1 June 2021
Accepted date: 6 June 2021

Please cite this article as: Yuya Tachibana , Toyokazu Tsutsuba , Kohei Kageyama , Ken-ichi Kasuya , Biodegradability of poly(butylene n-alkylenedionate)s composed of long-methylene chains as alternative polymers to polyethylene, *Polymer Degradation and Stability* (2021), doi: https://doi.org/10.1016/j.polymdegradstab.2021.109650

Original Article

Biodegradability of poly(butylene *n*-alkylenedionate)s composed of long-methylene chains as alternative polymers to polyethylene

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Highlights:

- PBADs composed of long-methylene chains and PE have similar thermal and mechanical properties.
- PBADs were hydrolyzed to water-soluble products by bacterial degradation.
- The hydrolysates of PBADs are easily metabolized by microorganisms.
- PBADs composed of long-methylene chains are poorly metabolized by microorganisms.

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Abstract:

Poly(butylene *n*-alkylenedionate)s (PBADs), which are polymers that are potentially biodegradable in various environments, exhibit excellent thermal and mechanical properties similar to those of polyethylene because of their long alkyl chains. However, although it has not yet been experimentally verified, it is known that differences in the alkyl chain length affect the biodegradability of PBADs. In this study, we synthesized PBADs with alkylene diacid units, with the number of methylene chains ranging from 11 to 14. We subsequently investigated the relationship between their biodegradability via clear-zone formation and BOD-biodegradation testing and their physical properties, including their structural characteristics, using instrumental analysis. Although the monomers 1,4-butanediol (BD) and α,ω-dicarboxylic acids (DCA) exhibited good BOD-biodegradability, PBADs did not, indicating that PBADs with 11–14 methylene chains do not readily degrade in natural environments.

Keywords: Biodegradable polymer, polyester, alkylenedionic acid, 1,4-butanediol, clear zone formation, hydrolysis

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1. Introduction

Synthetic polymer materials, commonly known as plastics, have been widely employed in both academic and industrial fields because of their excellent thermal and mechanical properties. However, owing to their low degradability, the disposal of plastic waste into nature has led to serious environmental pollution[1,2]. To overcome this issue, the development of biodegradable polymers that can eventually be mineralized through microorganism metabolism is highly desirable[3–5]. Some examples of well-known commercially available biodegradable polymers are aliphatic polyesters, poly(lactic acid) (PLA)[6,7], polycaprolactone (PCL)[8], poly(3-hydroxybutyrate) (P3HB)[9.10], and poly(butylene succinate) (PBS)[11–15].

Several researchers have reported that when the number of sequential methylene chains in the main chain of an aliphatic polyester is increased, the properties of the polyester, such as the melting point, approach those of polyethylene (PE). Such polymers are consequently referred to as PE-like polymers[16–20]. This observation suggests that polyesters consisting of long methylene chains could be used as effective PE alternatives, as they possess the desired properties of PE with the added advantage of biodegradability. Despite the significant potential applications of aliphatic polyesters, to the best of our knowledge, there are no detailed reports regarding the biodegradability of long-chain aliphatic polyesters. We recently reported a comparative study on the biodegradability of poly(butylene *n*-alkylene dicarboxylate) (PBAD)s with 2–10 methylene chains in dicarboxylic acid (DCA). Biochemical oxygen demand (BOD)-biodegradation testing is based on the metabolization of PBAD by microorganisms, whereas the clear-zone formation method is based on the enzymatic hydrolyzability of polymers by extracellular enzymes[21]. The BD and DCAs as hydrolysates of PBADs were easily metabolized, and some microorganisms formed a clear zone on the PBAD-emulsified media. The BOD-biodegradability of PBADs was found to depend on the number of methylene chains, with poly(*n*-butylene dodecanodioate) (PBDd) (n

= 10) demonstrating a low BOD biodegradability of 4%. However, this study did not verify whether PBADs with more than 10 methylene chains were biodegradable[21]. Although PBADs with long methylene chains have the potential to be used as biodegradable plastics that exhibit excellent properties similar to those of PE, the correlation between the primary structure of PBADs and their biodegradability has not yet been elucidated. In addition, PBADs with long methylene chains have not been synthesized sequentially, and only the synthesis of poly(butylene tetradecanoediate) (PBTed) has been reported[22].

In this study, we carried out the polycondensation of the diol unit 1,4-butanediol (BD) and *n*-alkylene DCAs (including tridecanoic diacid (n = 11; TrdA), tetradecanoic diacid (n = 12; TedA), pentadecanoic diacid (n = 13; PdA), and hexadecanoic diacid (n = 14; HdA)] to synthesize poly(butylene *n*-alkylenedicarboxylate) (PBAD)s [poly(butylene tridecanediate) (PBTrd), poly(butylene tetradecanediate) (PBTed), poly(butylene pentadecanediate) (PBPd), and poly(butylene hexadecanediate) (PBHd)], respectively). An estimation of the thermal properties of the PBADs revealed PE-like characteristics. In general, the biodegradation of polyesters proceeds via two steps: enzymatic hydrolysis of the ester bonds in the main chain followed by the metabolization of low-molecular-weight hydrolysates, such as monomers and oligomers, by microorganisms[3,23]. The environmental biodegradability of the synthesized PBADs was investigated using two test methods: the evaluation of enzymatic hydrolyzability by the clear zone formation test using a solid medium emulsified with PBADs and evaluation of biodegradability by mineralization of PBADs via biochemical oxygen demand (BOD) tests under aerobic conditions.

2. Experimental

2.1. Reagents

HdA was purchased from Sigma-Aldrich Japan (Tokyo, Japan). Methanol was purchased from Kishida Chemical Co., Ltd. (Osaka, Japan). BD, chloroform ($CHCl_3$), deuterated

chloroform ($CDCl_3$, 0.05 v/v %, TMS), Na_2SO_4, KCl, HCl, Na_2SO_4, $NaHCO_3$, and NaOH were purchased form Kanto Chemical Co., Inc. (Tokyo, Japan). PdA, TedA, TrdA, and titanium tetraisopropoxide were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Succinic acid, KH_2PO_4, K_2HPO_4, NaCl, $Na_2HPO_4 \cdot H_2O$, NH_4Cl, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, $FeCl_3 \cdot 6H_2O$, yeast extract, and agar powder were purchased were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). Plysurf was purchased from DKS Co. Ltd. (Kyoto, Japan). All chemicals used were of reagent grade. BD was used after distillation under reduced pressure (165 °C, 190 Pa), and other reagents were used without further purification.

2.2. General procedure for polycondensation of BD and DCA to PBAD[21, 24]

A 20 mL flask was charged with BD (11 mmol), DCA (10 mmol), and titanium tetraisopropoxide (50 μmol). The flask was equipped with a gas inlet and outlet and heated at 160 ˚C for 2 h under a dry N_2 flow to remove the water produced during the esterification reaction. Subsequently, the polycondensation was carried out at 180 Pa and 200 ˚C for 5 h. The resulting solid was dissolved in dichloromethane (15.0 mL) and the solution was poured into methanol (150 mL). The precipitate was filtered and dried under vacuum at room temperature to give a white solid.

2.3. Characterization of PBAD

^{1}H NMR spectra (400 MHz) was recorded on a JNM-ECX400 NMR spectrometer (JEOL Ltd., Tokyo, Japan). ^{13}C NMR spectra (150 MHz) were recorded on a JNM-ECX600 NMR spectrometer (JEOL Ltd., Tokyo, Japan). $CDCl_3$ was used as the solvent, calibrated using tetramethylsilane as the internal standard. The molecular weights of the polyesters were determined by gel permutation chromatography (GPC) (HLC-8220GPC; Tosoh Co., Tokyo, Japan) with a refractive index detector and a combination of a column (TSKgel multipove

HXL-M; Tosoh Co., Tokyo, Japan) with a guard column (TSKgel guardcolumn HXL-H; Tosoh Co., Tokyo, Japan) and the molecular masses were calibrated against polystyrene standards. The columns were eluted with $CHCl_3$ (1.0 mL min^{-1} at 40 °C).

2.4. Thermal analysis of PBAD

The thermal stability of PBAD was determined using a thermal gravimetric analyzer (STA-6000; PerkinElmer Japan Co., Ltd., Yokohama, Japan). The samples were heated to 500°C under N_2 flow at a rate of 10 °C min^{-1}. The melting temperature (T_m) and glass transition temperature (T_g) were determined using differential scanning calorimetry (DSC-4000; PerkinElmer Japan Co., Ltd., Yokohama, Japan). Each sample was heated to 120 °C and cooled to −60 °C at a rate of 10 °C min^{-1}, and then heated from –60 °C to 120 °C at a rate of 10 °C min^{-1}. The T_m and T_g values of the polyesters were determined from the second heating scan. The alpha relaxation temperatures (T_α) were determined using a dynamic mechanical analyzer (DMA-8000; PerkinElmer Japan Co., Ltd., Yokohama, Japan) with a material pocket to measure a powder sample in single cantilever bending mode at an oscillatory frequency of 1.0 Hz and an applied deformation of 0.05 mm during heating. DMA was performed at a heating rate of 2.0 °C min^{-1}. Each powder sample in the material pocket was melted at 100 °C followed by rapid cooling with liquid N_2 prior to measurement. The T_α values of the polyesters were determined from the peak top of tan δ (E''/E') plotted as a function of temperature.

2.5. Evaluation of crystallinity

Wide-angle X-ray diffraction (WAXD) measurements were conducted using an X-ray diffractometer (RINT220; Rigaku Co., Tokyo, Japan) to determine the crystallinity (Xc). The radiation source was Cu-Kα radiation with a wavelength of 1.54 nm. The voltage was set to

be 40 kV and the current was 20 mA. The powder sample were mounted on a sample holder and scanned from 3˚ to 90˚. The degree of crystallinity determined by the waveform separation. The calculated area of the amorphous halo from the peak of the derived crystal were estimated according to the following equation:

$$X_c = \frac{I_c}{I_c + I_a} \times 100$$

where I_c is the scattering intensity of the crystal, and I_a is the scattering intensity of the amorphous phase.

2.6. Preparation of PBAD-emulsified media[25]

PBAD was dissolved in dichloromethane (30 mL). The solution was emulsified with an ultrasonic disruptor (UD-200; TOMY Seiko Co. Ltd., Tokyo, Japan) in a basal medium (1.00 L) at pH 7.0 with the following components: $Na_2HPO_4 \cdot 12H_2O$, 11.6 $g \cdot L^{-1}$; KH_2PO_4, 4.6 $g \cdot L^{-1}$; $MgCl_2 \cdot 7H_2O$, 0.50 $g \cdot L^{-1}$; NH_4Cl, 1.0 $g \cdot L^{-1}$; $FeCl_3 \cdot 6H_2O$, 0.10 $g \cdot L^{-1}$; Yeast extract, 0.50 $g \cdot L^{-1}$; and 1% Plysurf, 5.0 $mL \cdot L^{-1}$. Dichloromethane was removed by heating at 40 ℃ with a magnetic stirrer. Agar (1.50 g) was added to the emulsified medium, and the mixture was autoclaved at 121 ℃ for 15 min. After cooling, the medium was solidified in a Petri dish after cooling.

2.7. Hydrolysis of PBAD by isolated bacterial strains[26]

The isolated strains (TBTK040311B, TBTK040305D, TBTK040405D, TBTK040502D, TBTK040606B, TBTK040607A, TBTK040705A, TBTK040711A, TBTK040811B, TBTK041005B, TBTK040209C, TBTK040502A, TBTK040913A, TBTK040410B, TBTK040306C, TBTK040506C, TBTK040703A, and TBTK040711C) were used in the previous study[21]. The strains streaked onto the plates using wooden picks,

following which the plates were incubated at 30 °C. The ability of each strain to hydrolyze PBAD was assessed by measuring the size of the clear zone formed after 24 h.

2.8. BOD biodegradation testing

The BOD biodegradability of PBADs and their constituents by aerobic microorganisms from soil suspension in an aqueous medium was determined by measuring oxygen consumption with a BOD instrumentation (OxiTop-C measuring head with a 300-mL BOD reactor, WTW GmbH, Weilheim, Germany) according to the ISO 14851 standard. The following stock solutions were prepared: solution A1; KH_2PO_4, 8.5 $g \cdot L^{-1}$; K_2HPO_4, 21.75 $g \cdot L^{-1}$; $Na_2HPO_4 \cdot H_2O$, 33.4 $g \cdot L^{-1}$; NH_4Cl, 0.5 $g \cdot L^{-1}$, solution B; $MgSO_4 \cdot 7H_2O$ 22.5 $g \cdot L^{-1}$, solution C; $CaCl_2$, 27.5 $g \cdot L^{-1}$, solution D; $FeCl_3 \cdot 6H_2O$, 0.25 $g \cdot L^{-1}$. To determine BOD biodegradability, 2 mL of solution A and 0.2 mL of each solution B, C, and D were added to an adequate amount of deionized water to bring the volume to 200 mL. A powder sample (~10 mg) was placed in a 300 mL BOD reactor to which 200 mL of BOD medium was added. To prepare the inoculum from soil suspension for BOD biodegradation testing, a mixture of soil (1.0 g) from Ishinomaki City, Matsushima Town, Hamamatsu City, and Wakayama City was dispersed in distilled water (10 mL) and filtered through a filter paper. The filtrate was allowed to stand for 1 day. In addition, 15 PBDA-degrading strains (TBTK040311B, TBTK040305D, TBTK040405D, TBTK040502D, TBTK040606B, TBTK040607A, TBTK040705A, TBTK040811B, TBTK040209C, TBTK040502A, TBTK040306C, TBTK040506C, TBTK040711C, TBTK041005B, TBTK040913A) were incubated in the solution medium (KH_2PO_4, 4.6 $g \cdot L^{-1}$; $Na_2HPO_4 \cdot 2H_2O$, 4.6 $g \cdot L^{-1}$; NH_4Cl, 1.0 $g \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$, 0.5 $g \cdot L^{-1}$; $FeCl_3 \cdot 6H_2O$, 0.1 $g \cdot L^{-1}$; yeast extract, 0.5 $g \cdot L^{-1}$; and succinic acid, 0.2 $g \cdot L^{-1}$) at 30 °C for 48 h. The supernatant of the soil solution (200 μL) and the solution of the 15 PBDA-degrading isolates (100 μL) were added to the BOD reactor as

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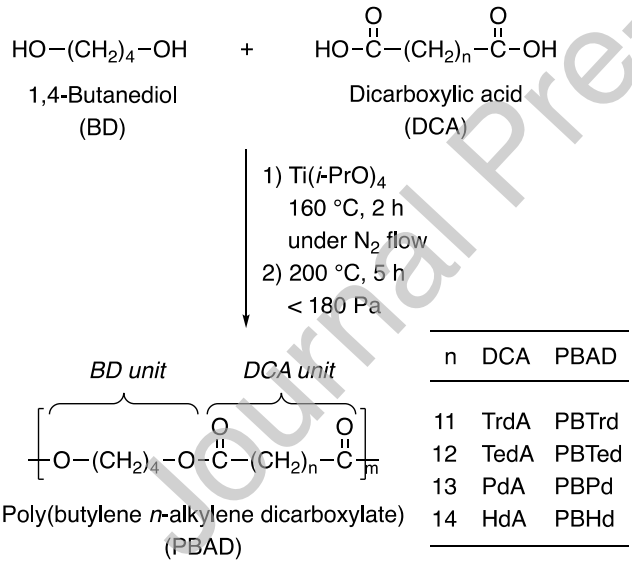
an inoculum. The OxiTop-C measuring head was attached to the head of a BOD reactor, and the reactor was incubated at 25 °C. The BOD-biodegradability of PBAD was defined as follows: BOD-biodegradability (%) = (BOD_{sample} – BOD_{blank})/ThOD × 100. Here, BOD_{sample} and BOD_{blank} are the experimentally observed values of oxygen demand of the sample and a blank medium, respectively. ThOD is a theoretically calculated value of oxygen demand of a sample, which was obtained by assuming that the film completely degraded into CO_2 and H_2O.

3. Results and Discussion

3.1. Synthesis and characterization of PBADs

PBADs containing long methylene groups (n = 11–14) in the DCA unit were synthesized according to a previous report[21,24] (Scheme 1).



n	DCA	PBAD
11	TrdA	PBTrd
12	TedA	PBTed
13	PdA	PBPd
14	HdA	PBHd

Scheme 1. Synthesis of poly(butylene *n*-alkylene dicarboxylate) (PBAD)s

BD and DCAs were polymerized with titanium tetraisopropoxide as a catalyst at 160 °C under nitrogen flow, and the transesterification polycondensation of the formed diester was carried out under reduced pressure (< 180 Pa) at 160 °C and 200 °C to afford PBTrd, PBTed, PBPd, and PBHd, respectively, as white powders. The chemical structures of

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the PBADs were characterized by ^{1}H and ^{13}C NMR (Figure 1 and S1). The peaks of methylene groups neighboring the ester moiety and the methylene protons of the aliphatic chain were clearly observed in the ^{1}H NMR spectrum of PBADs. The yields and molecular weights of the PBADs are summarized in Table 1. PBDSs were obtained in isolated yields of 66% to 93 %, and the number average molecular weights (M_n) ranged from 9. 6 × 10^3 to 19.0 × 10^3.

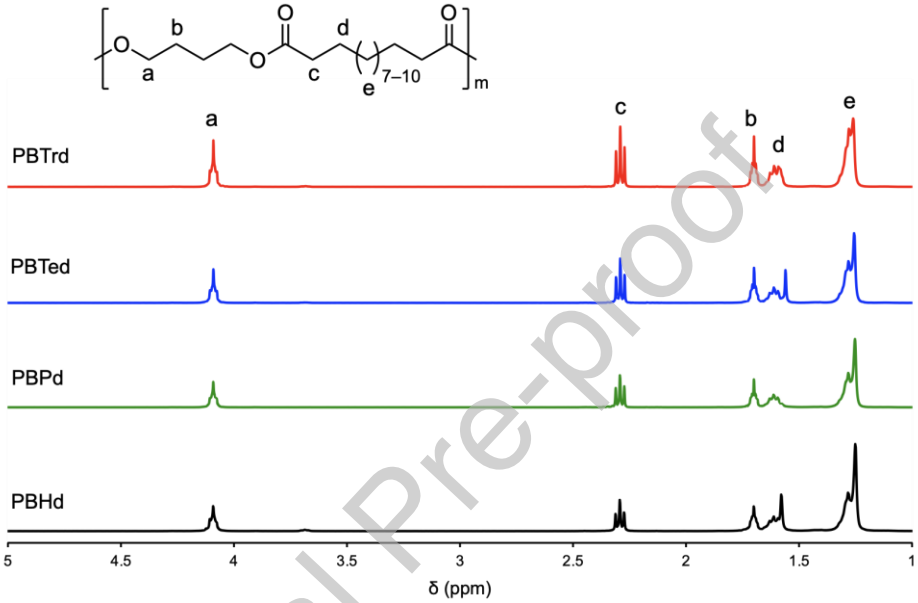

Figure 1. ^{1}H NMR spectra of PBADs (400 MHz, 298 K, $CDCl_3$)

3.2. Thermal properties and crystallinity of PBADs

The thermal properties of the PBADs were then subjected to TG-DTA, DSC, and DMA analyses (Figure S2, S3, and S4). The weight loss of 5% (T_{d5}) was obtained from TG-DTA. The alpha relaxation temperatures (T_α) was obtained from DMA. The glass transition temperature (T_g), cold crystallization temperature (T_c), the melting temperature (T_m), crystallization enthalpy (ΔH_c), and melting enthalpy (ΔH_m) were obtained from DSC. The valuses of PBTrd, PBTed, PBPd, and PBHd are summarized in Table 1. T_{d5} was observed from 365 °C to 379 °C, and no major change was observed due to the increase in the number of methylene chains in the main chain. In the DSC measurement, PBTrd, PBTed, and PBPd

did not exhibit T_g, whereas the T_g of PBHd was only observed at –26 °C. To characterize the thermal properties in detail, temperature-dependent DMA was conducted. From the results, it was observed that T_α of PBADs estimated from the maximum of the tan δ ranged from –25 °C to –23 °C. This means that the PBADs exhibit a rubbery state under ambient temperature. Meanwhile, the T_m and T_c were clearly observed at 69–80 °C and 51–67 °C, respectively. The T_m of the obtained PBADs was higher than that of PBADs with 2–10 methylene chains[21] owing to the increase in the alkyl moiety in the main chain. The relationships between T_m and the number of methylene chains in PBADs are displayed, including the PBADs with 2–10 methylene chains (Figure 3). The even-odd effect on T_m and T_c was weakened with an increase in the number of methylene chains, whereas the T_m increased subsequently. This increase in T_m occurred as the number of methylene chains approached that of PE (ca. 100–130 °C)[16].

The WAXD patterns of the powder of PBADs provided direct information on PE-like crystallinity (Figure 3). The degrees of crystallinity (X_c) of PBTrd, PBTed, PBPd, and PBHd were summarized in Table 1. In particular, PBHd, which has the longest methylene chain, showed a characteristic pattern with two main reflections at $2\theta \approx 21.5°$ (110) and 24.3° (200), suggesting that PBHd formed a PE-like crystal structure[16,28,29]. Moreover, the X_c of PBTrd, PBTed, PBPd, and PBHd were 51%, 32%, 39%, and 63%, respectively. Because the samples of PBADs was prepared by the reprecipitation after polymerization, it was difficult to compare the X_c values among PBTrd, PBTed, PBPd, and PBHd.

Table 1. Yield, molecular weights, thermal properties, and crystallinity of PBADs

PBAD	Yield[a] / %	M_n[b]	M_w/M_n[b]	$T_{d5\%}$[c] /°C	T_α[d] /°C	T_g[e] /°C	T_c[e] /°C	ΔH_c[e] /J·g^{-1}	T_m[e] /°C	ΔH_m[e] /J·g^{-1}	X_c[f] /%
PBTrd	93	16.7	2.7	366	−25	n.d.	51	97	69	96	51±7
PBTed	71	19.0	2.7	379	−24	n.d.	63	101	77	93	32±1

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PBPd	91	9.6	2.2	374	−23	n.d.	61	107	74	109	39±7
PBHd	66	12.2	6.5	365	−23	−26	67	146	80	148	63±2

n.d. : no deta. [a]Methanol insoluble part. [b]Measured by GPC (eluent: chloroform) and calibrated with polystyrene standards. [c]Measured by TGA. [d]Measured by DMA. [e]Measured by DSC during the first cooling scan with a rate of 10°C min^{-1}. [d]measured by DSC during the second heating scan with a rate of 10°C min^{-1}. [f]Measured using melt press film by X-ray diffractometer from 2θ = 5° to 60°

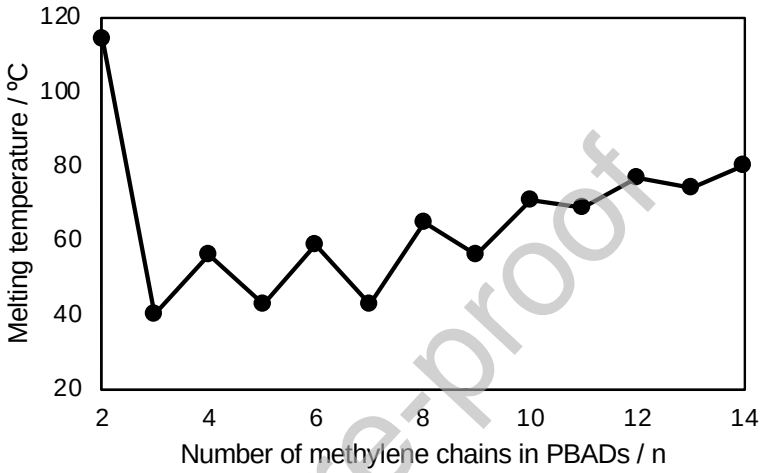

Figure 2. Relationships of melting temperature of PBAD and number of methylene chains in PBADs (n). The melting temperature (T_m)s of PBDAs of the numbers 2-10 of methylene chains in alkylenedionate moeity, were taken from our previous study[21].

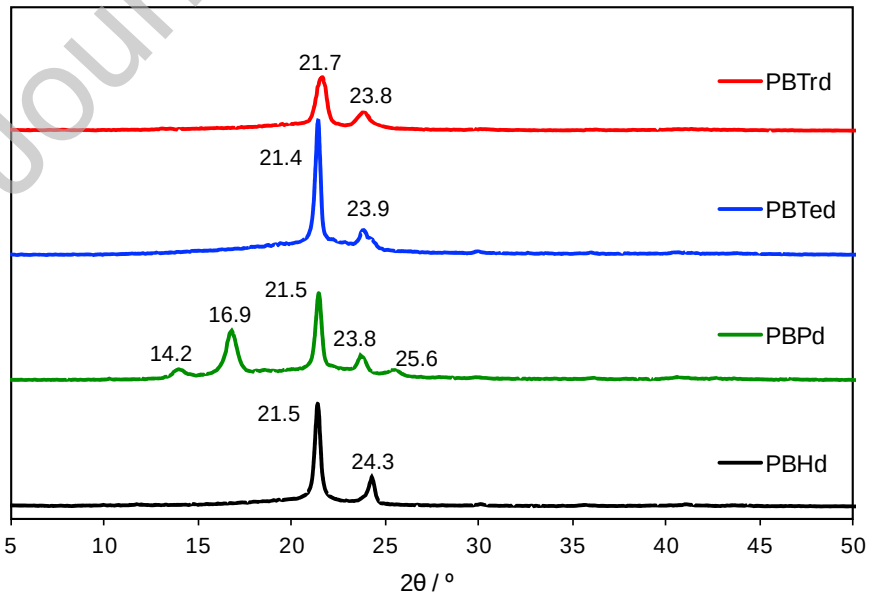

Figure 3. WAXD patterns of PBAD films.

3.3. Clear zone formation testing of PBADs

Eighteen strains that formed a clear zone on the emulsified media of PBADs composed of shorter methylene chain (n = 2–10) would produce PBAD-degrading enzymes[21]. To evaluate the enzymatic hydrolyzability, these strains were used for clear-zone formation testing of PBADs (n = 11–14). The results are illustrated in Table 2. A clear zone was formed on the emulsified media of PBTrd, PBTed, PBPd, and PBHd by 14, 10, 8, and 9 of 18 strains, respectively. Compared to PBADs (n = 3–9), the number of strains forming clear zone and the size of clear zone on PBTrd, PBTed, PBPd, and PBHd were few and small. Especially, TBTK040506C, TBTK040703A, TBTK040711C formed no clear zone or a small clear zone on PBTrd, PBTed, PBPd, and PBHd while they formed a large clear zone on PBADs (n = 3–9). These suggest that these strains relatively less hydrolyzed PBADs with m = 2 and m > 11 methylene chain numbers. Although the enzymatic hydrolyzabilities of PBTrd, PBTed, PBPd, and PBHd were lower than those of PBADs (n = 3–9) by the strains, the clear zone formation testing indicated that PBADs are hydrolyzed by the enzyme to produce water-soluble hydrolysates.

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Table 2. Ability of clear zone formation on the plates containing PBAD emulsified solid medium by PBAD-degrading strains.

Strain	Accession number[a]	Phylum[b]		PBTrd	PBTed	PBPd	PBHd
			m[c]	11	12	13	14
TBTK040311B	LC034566	F		++	++	++	+
TBTK040305D	LC057687	F		+	−	+	+
TBTK040405D	LC057701	F		+	+	−	+
TBTK040502D	LC057707	F		+	−	−	+
TBTK040606B	LC057691	F		++	+	−	−
TBTK040607A	LC057709	F		+	+	+	−
TBTK040705A	LC057697	F		+	++	++	++
TBTK040711A	LC057695	F		−	−	−	−
TBTK040811B	LC057705	F		+	−	−	−
TBTK041005B	LC057710	F		++	++	++	++
TBTK040209C	LC057618	A		+	+	+	+
TBTK040502A	LC057706	A		−	++	+	−
TBTK040913A	LC057704	A		++	+	−	+
TBTK040410B	LC057699	*β*-P		−	−	−	+
TBTK040306C	LC057708	*γ*-P		+	+	−	−
TBTK040506C	LC057689	*γ*-P		+	−	−	−
TBTK040703A	LC057698	*γ*-P		−	−	−	−
TBTK040711C	LC057696	*γ*-P		+	−	+	−

a The accession numbers are for the 16S rDNA gene corresponding to each strain, deposited at the DNA Data Bank of Japan (DDBJ). b F: Firmicutes, A: Actinobacteria, *β*-P: *β*-Proteobacteria, *γ*-P: *γ*-proteobacteria. c m indicates the numbers of methylene chains in the alkylenedionates as polymer constituents.

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3.4. BOD-biodegradabilities of PBADs

The metabolization of PBADs and BD, DCAs as their hydrolysates was evaluated by BOD-biodegradation testing. For BOD-biodegradation testing, the powder samples of PBADs were prepared by the reprecipitation which was same as WAXD analysis. The DSC charts and WAXD patterns of PBADs indicate all samples are crystalline. PBTrd did not show BOD biodegradability with only a mixture of soil in spite of the fact that TrdA did BOD biodegradability (Figure S5). Therefore, 15 PBAD-degrading strains as inocula were added to the mixture of soil and used as inocula for BOD-biodegradation testing. The BOD-biodegradation curves of PBADs, BD, and DCAs are shown in Figure 4. BD, TrdA, TedA, PdA, and DdA had a short lag phase within 1 day. The slopes of the degradation phase in the BOD biodegradabilities of BD, TrdA, TedA, PdA, and DdA were 29%, 33%, 47%, 31%, and 5.8 %/day, respectively, and their BOD biodegradability reached a plateau phase after incubation for 7 days. BOD-biodegradability of BD, TrdA, TedA, PdA, and DdA reached the values of 56%, 75%, 73%, 61%, and 59 %, respectively, after incubation for 45 days. Such dicarboxylic acids and alcohols are readily catabolized via the β-oxidation pathway used to metabolize fatty acids to produce carbon dioxide, indicating that BD and DCA were immediately metabolized by microorganisms[28]. In contrast, the BOD biodegradabilities of PBADs PBTrd, PBTed, PBPd, and PBHd were 5%, 2%, 0%, and 2%, respectively, after 45 days. Although we added PBAD-degrading isolates to accelerate the hydrolysis of ester bonds, PBTrd, PBTed, PBPd, and PBHd showed low BOD biodegradability. The PBAD-degrading bacteria did not produce PBAD hydrolytic enzymes in the BOD medium used in this study, and consequently, the hydrolysis of the ester bond would not have occurred.

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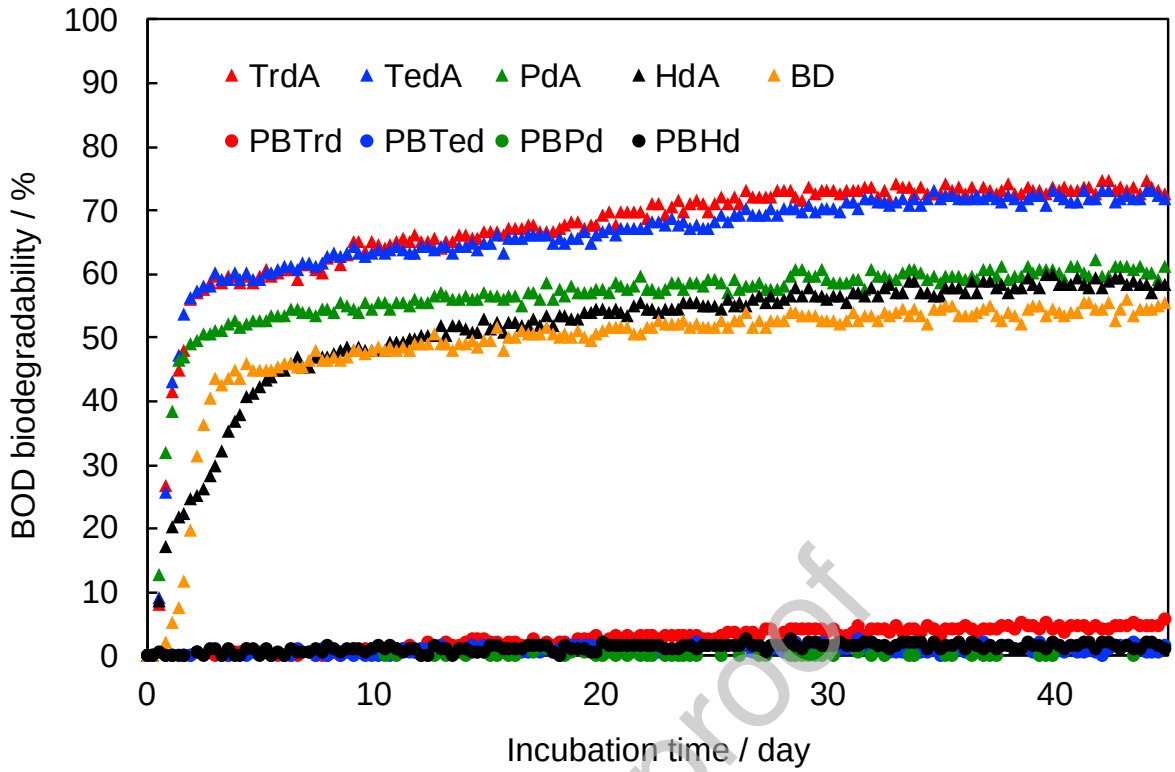

Figure 4. BOD biodegradation curves of BD, DCAs, and PBADs at 25°C for 45 days.

4. Conclusion

We synthesized PBADs (n = 11–14) composed of relatively long methylene chains with properties similar to those of PE and evaluated their biodegradability by clear-zone formation testing and BOD-biodegradation testing. The clear zone formation testing with PBADs revealed that PBADs were hydrolyzed to water-soluble products by the degrading bacteria used in this study. BOD-biodegradation testing of the components of PBADs (BD and DCAs) revealed that they were metabolized by environmental microorganisms. However, PBAD did not show BOD biodegradability. These results show that PBADs are relatively difficult to biodegrade in the actual natural environment, although they are potentially hydrolyzable and metabolizable. If such PE-like polyesters with a high number of methylene chains can be efficiently lowered in molecular weight by abiotic means using external stimuli, it may lead to the creation of PE substitutes with biodegradability. The fact that the constituents of PBADs used in this study are biodegradable, while PBADs

themselves do not biodegrade, may be considered desirable from the aspect of the stability of biodegradable plastics in the use environment. This study provides beneficial information in the field of biodegradation of polymer materials, leading to the further development of novel biodegradable polymers.

ASSOCIATED CONTENT

Author contributions

Yuya Tachibana and Ken-ichi Kasuya designed the study. Yuya Tachibana, Toyokazu, Tsutsuba, and Ken-ichi Kasuya wrote the paper. Kohei Kageyama and Toyokazu Tsutsuba carried out the synthetic experiments and evaluated the properties. All the authors participated in the analysis and discussion of the results.

Notes

The authors declare no competing financial interests.

Acknowledgments

Yuya Tachibana is grateful for financial support from JSPS KAKENHI Grant-in-Aid for Scientific Research (C) Number 16K00607. Ken-ichi Kasuya is grateful for financial support from JSPS KAKENHI Grant-in-Aid for Scientific Research (B) Number 19H04311 and the New Energy and Industrial Technology Development Organization (NEDO), project code JPNP14004. NMR measurements were performed on a JNM-ECS400 NMR spectrometer and JNM-ECA600 NMR spectrometer (JEOL Ltd., Tokyo, Japan), and WAXD measurements were performed using X-ray diffractometer (RINT2200VF; Rigaku Co., Tokyo, Japan) at the Center for Instrumental Analysis of Gunma University.

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Credit author statement

Yuya Tachibana and Ken-ichi Kasuya designed the study. Yuya Tachibana, Toyokazu, Tsutsuba, and Ken-ichi Kasuya wrote the paper. Kohei Kageyama and Toyokazu Tsutsuba carried out the synthetic experiments and evaluated the properties. All the authors participated in the analysis and discussion of the results.

Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials.

The chemical shift data of ^{1}H NMR and ^{13}C NMR; ^{13}C NMR spectra, TGA charts, DSC charts, DMA charts of PBTrd, PBTed, PBPd, and PBHd. BOD-biodegradation curves of PBTrd and TRdA with a mixture of soil inocula. Supplementary data to this article can be found online at https://doi.org/*****

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