



# Anhydride Production as an Additional Mechanism of Poly(3-hydroxybutyrate) Pyrolysis

著者	Ariffin Hidayah, Nishida Haruo, Shirai Yoshihito, Hassan Mohd Ali
journal or publication title	Journal of Applied Polymer Science
volume	111
number	1
page range	323-328
year	2008-10-03
URL	<a href="http://hdl.handle.net/10228/00006735">http://hdl.handle.net/10228/00006735</a>

doi: [info:doi/10.1002/app.29034](https://doi.org/10.1002/app.29034)

**Anhydride Production as an Additional Mechanism of Poly(3-hydroxybutyrate) Pyrolysis**

Journal:	<i>Journal of Applied Polymer Science</i>
Manuscript ID:	APP-2008-05-1659.R1
Wiley - Manuscript type:	Research Article
Keywords:	biopolymers, pyrolysis, recycling, thermogravimetric analysis (TGA)



Peer Review

## Anhydride Production as an Additional Mechanism of Poly(3-hydroxybutyrate) Pyrolysis

*Hidayah Ariffin,<sup>2,3</sup> Haruo Nishida,\*<sup>1</sup> Yoshihito Shirai,<sup>1,2</sup> and Mohd Ali Hassan<sup>3</sup>*

<sup>1</sup> Eco-Town Collaborative R&D Center for the Environment and Recycling, Kyushu Institute of Technology, 2-4 Hibikino, Wakamatsu-ku, Kitakyushu 808-0196, Japan

<sup>2</sup> Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, 2-4 Hibikino, Wakamatsu, Kitakyushu 808-0196, Japan

<sup>3</sup> Faculty of Biotechnology and Biomolecular Science, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Hidayah Ariffin: hid\_a@yahoo.com, Haruo Nishida: nishida@lsse.kyutech.ac.jp, Yoshihito Shirai: shirai@life.kyutech.ac.jp, and Mohd Ali Hassan: alihass@biotech.upm.edu.my

\* Corresponding author.

E-mail: nishida@lsse.kyutech.ac.jp.

Telephone: +81-93-695-6233. Fax: +81-93-695-6060.

**Abstract**

Anhydrides production is newly proposed as an additional pyrolysis mechanism of a biopolymer: poly(3-hydroxybutyrate) (PHB). In spite of many suggestions of multiple degradation mechanisms, simple random chain scission by  $\beta$ -elimination has been accepted as an exclusive mechanism of the thermal degradation of PHB. However, a wide range of activation energy value of the degradation and the deviation from the random chain scission statistics have suggested the presence of other kinds of mechanism out of the random scission. In order to confirm other mechanisms out of the random scission, minor pyrolyzates from PHB were characterized with  $^1\text{H}/^{13}\text{C}$  NMR, FTIR, and FAB-MS. As a result, crotonic anhydride and its oligomers were detected as minor products from condensation reactions between carboxyl groups. The anhydrides production must be one out of conforming to the random degradation statistics and contribute to the complexity of PHB pyrolysis. An expected thermal degradation pathway of PHB was proposed.

**Keywords:**

biopolymers, pyrolysis, mechanism, thermogravimetric analysis (TGA), recycling

## INTRODUCTION

Poly(3-hydroxybutyrate) (PHB) is a well known microbial and biodegradable polymer. Many prokaryotic microorganisms have been found to accumulate PHB up to 90% of their cellular dry weight.<sup>1</sup> PHB has been attracting much interest from researchers not only as an environmentally compatible thermoplastic, but also as a polymeric material from renewable resources with a high melting temperature of around 180 °C.<sup>2</sup> Various approaches have been used to improve PHB, i.e. by producing PHB in transgenic plants in order to increase the production and also by copolymerizing PHB to improve its physical properties.

The main setback of PHB when used as a thermoplastic is its thermal instability during melt-processing. Therefore, intense interest has been shown in the thermal degradation of PHB and other related poly(hydroxyalkanoate)s. Recently, it has been demonstrated that PHB is a chemically recyclable material with end products such as crotonic acid, linear oligomers having a crotonate end group<sup>3</sup>, and a cyclic trimer.<sup>4</sup> If materials originating from renewable resources can be efficiently recycled through precise control of their thermal degradation, an ideal recycling system for plastic products, in which the resources and production energy of the materials are minimized, could be constructed.

The thermal degradation behavior of PHB has been discussed in many reports, in which a random chain scission by  $\beta$ -elimination has been considered as the dominant mechanism based on typical structures of pyrolysis products, i.e. crotonic acid and oligomers with a crotonate end-group.<sup>3,5</sup> Recently, Kawalec et al.<sup>6</sup> newly proposed an E1cB mechanism proceeding via  $\alpha$ -deprotonation by a carboxylate anion to produce the same products. Several thermoanalytical methods have been used to investigate the thermal degradation behavior of PHB, including thermogravimetry (TG)<sup>6-14</sup> differential scanning calorimetry (DSC)<sup>7</sup>, fast atom bombardment mass spectrometry (FAB-MS)<sup>5</sup>, electrospray ionization mass spectrometry (ESI-MS)<sup>6</sup>, pyrolysis-mass spectrometry (Py-MS)<sup>7,15</sup>, pyrolysis-gas chromatography (Py-GC)<sup>16</sup>, pyrolysis-GC/mass spectrometry (Py-GC/MS)<sup>7,12,17</sup>, TG/Fourier transform infrared

spectroscopy (TG/FTIR)<sup>18</sup>, pyrolysis-GC/FTIR (Py-GC/FTIR)<sup>18,19</sup>, size exclusion chromatography (SEC)<sup>13,20-22</sup>, and NMR.<sup>4,7</sup>

These reports have shown that the activation energy value ( $E_a$ ) of the degradation to be in a wide range of 110-380 kJ·mol<sup>-1</sup>. In order to avoid the influence of some complex factors on pyrolysis, the  $E_a$  value of the  $\beta$ -elimination was calculated from molecular weight changes in a lower temperature range of 170-200 °C. Interestingly, in spite of the same expected degradation mechanism of  $\beta$ -elimination, obtained  $E_a$  values were in a medium range of 178-247 kJ·mol<sup>-1</sup>. Even though many approaches have been taken on the subject of PHB thermal degradation, a clear explanation for the wide variation in  $E_a$  value has yet to be provided. Despite this uncertainty, the  $\beta$ -elimination scission has been widely held as the exclusive degradation mechanism of PHB.

There were suggestions that some different reactions occur in the course of PHB thermal degradation. Lehrle et al.<sup>16,23</sup> reported that the analytical results of partial pyrolysis of PHB showed some deviations from the theoretical predictions of random scission statistics. The deviations were probably due to reactions induced by the end groups. Kopinke et al.<sup>7</sup> found that the isothermal degradation curve at 270 °C did not agree with the model curves of random degradation reactions. However, a clear confirmation of the disagreement has not yet been reported.

In this paper, in order to confirm that other reactions out of the random scission occur during the PHB thermal degradation, minor pyrolyzates from PHB were characterized to discuss its thermal degradation mechanism in more detail.

## EXPERIMENTAL

### Materials

PHB (3HB unit 100 %;  $M_n$  124,000 and  $M_w$  230,000 based on polystyrene standards; Ca content 8,100 ppm) was kindly supplied by the Kyoto Institute of Technology, and was purified by repeated dissolving in hot chloroform, precipitation in cold methanol, and then drying in vacuo. PHB films were prepared

by dissolving the samples in chloroform (2 % w/v) and casting on the surface of a Petri dish prior to the thermal degradation analysis. After the evaporation of the solvent, the formed films were washed by methanol and then vacuum dried.

## Measurements

The Ca and Mg contents of PHB were quantitatively measured with a Shimadzu AA-6500F atomic absorption flame emission spectrophotometer (AA). The sample was degraded by a 25% ammonia solution, dissolved in 1M-hydrochloric acid, and then measured by AA.

Molecular weights of the samples were measured by size exclusion chromatography (SEC) on a TOSOH HLC-8120 GPC system with a refractive index (RI) detector at 40 °C using TOSOH TSKgel Super HM-M column and chloroform eluent (0.6 mL·min<sup>-1</sup>). The calibration curves for GPC analysis were obtained using polystyrene standards with a low polydispersity ( $5.0 \times 10^2$ ,  $1.05 \times 10^3$ ,  $2.5 \times 10^3$ ,  $5.87 \times 10^3$ ,  $9.49 \times 10^3$ ,  $1.71 \times 10^4$ ,  $3.72 \times 10^4$ ,  $9.89 \times 10^4$ ,  $1.89 \times 10^5$ ,  $3.97 \times 10^5$ ,  $7.07 \times 10^5$ ,  $1.11 \times 10^6$ , TOSOH Corporation). The sample (12 mg) was dissolved in chloroform (2 mL) and the solution was filtered through a membrane filter with 0.45 μm pore size.

Proton (<sup>1</sup>H) and carbon (<sup>13</sup>C) NMR spectra were recorded on a 500 and 125-MHz JEOL JNM-ECP500 FT NMR system. Chloroform-*d* (CDCl<sub>3</sub>) was used as solvent. Chemical shifts were reported as δ values (ppm) relative to internal tetramethylsilane (TMS) and CDCl<sub>3</sub> unless otherwise noted. Expected <sup>1</sup>H NMR chemical shifts were predicted using a ChemNMR program in a CS ChemDraw Ultra version 6.0.

Thermogravimetric measurement (TG) was conducted on a Seiko Instruments Inc. EXSTAR 6200 TG system in aluminum pans (5 mmφ). A blank aluminum pan was used as reference. The pyrolysis data were collected at regular intervals (about 20 times·°C<sup>-1</sup>) by an EXSTAR 6000 data platform, and recorded into an analytical computer system.

Pyrolysis-gas chromatograms/mass spectra (Py-GC/MS) were recorded on a Frontier Lab double-shot

pyrolyser PY-2020D with a Frontier Lab SS-1010E selective sampler and a Shimadzu GCMS-QP5050 chromatograph/mass spectrometer. High purity helium was used as carrier gas at a static flow rate of  $100 \text{ mL}\cdot\text{min}^{-1}$ . The volatile products were introduced into MS through an Ultra Alloy<sup>+</sup>-5 capillary column ( $30 \text{ m} \times 0.25 \text{ mm i.d.}$ ; film thickness,  $0.25 \mu\text{m}$ ) and analyzed directly with the quadrupole mass spectrometer ( $70 \text{ eV}$ , electron impact ionization: EI).

Fast Atom Bombardment Mass Spectrometry (FAB-MS) spectra were recorded with a JEOL JMS-70 mass spectrometer using glycerol and *m*-nitrobenzyl alcohol as matrices, operating in FAB positive ion mode.

Fourier transfer infrared (FTIR) spectra were recorded on a Perkin Elmer Spectrum GX2000R. Reflection spectra of as-pyrolyzed samples were measured on a Golden Gate Diamond ATR(10500) module with a germanium crystal, by the single-reflection ATR method.

### **Dynamic pyrolysis in TG and Py-GC/MS**

PHB film sample (4-6 mg) in the aluminum pan was set in TG and heated at prescribed multiple heating rates ( $\varphi$ ) of 1, 3, 5, 7 and  $9 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$  in the range of 60 to  $400 \text{ }^\circ\text{C}$  under a steady flow of nitrogen ( $100 \text{ mL}\cdot\text{min}^{-1}$ ).

PHB film sample ( $\sim 0.5 \text{ mg}$ ) was put in the pyrolyzer of Py-GC/MS and heated from  $40 \text{ }^\circ\text{C}$  to a prescribed temperature at a heating rate of  $9 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ . The volatile pyrolysis products were conducted into the GC through a selective sampler. The temperature of the column oven was first set at  $40 \text{ }^\circ\text{C}$ . After the pyrolysis process had completed, the column was heated according to the following program:  $40 \text{ }^\circ\text{C}$  for 1 min;  $40\text{--}320 \text{ }^\circ\text{C}$  at a heating rate of  $20 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ ;  $320 \text{ }^\circ\text{C}$  for 30 min. Mass spectrum measurements were recorded  $2 \text{ times}\cdot\text{s}^{-1}$  during this period.

### **Isothermal Pyrolysis in glass tube oven**

About 200 mg of PHB film sample was put into a Shibata GTO-350D glass tube oven. The oven was



heated gradually in two stages; first, the temperature was increased from room temperature to 200 °C and kept at 200 °C for 30 min *in vacuo*. During this period, the air in the oven chamber was completely removed. In the second stage, the temperature was further increased to 260 °C, at which temperature the pyrolysis occurred, and then the temperature was again kept constant for 30 min. Vaporized pyrolyzates were condensed in a chilled trap, collected by dissolving in acetone, and dried in a vacuum rotary evaporator, resulting in white solids. The obtained solid product was analyzed by  $^1\text{H}/^{13}\text{C}$  NMR and FTIR for characterization.

## RESULTS AND DISCUSSION

### PHB pyrolysis at multiple heating rates

The thermal degradation behavior of PHB was examined using the dynamic pyrolysis with a multiple heating rate method by measuring the weight loss of the film samples with TG in  $\text{N}_2$  atmosphere. The multiple heating rates, *i.e.*  $\phi=1, 3, 5, 7$  and  $9\text{ }^\circ\text{C}\cdot\text{min}^{-1}$ , were employed as a function of linear increase in temperature. In Figure 1, the TG curves, which were normalized in accordance with sample weight = 1, are illustrated. All the TG curves showed a smooth decomposition from the beginning until completion, with TG traces shifted up the temperature scale by an increase in the heating rate. After the TG analysis, no carbonaceous residue was observed, indicating that the PHB film was degraded completely by specific degradation mechanisms.

$E_a$  value of PHB degradation was calculated according to Doyle's approximation<sup>24,25</sup> and showed in Figure SM-1 (in Supplementary Materials section). The apparent  $E_a$  value of PHB was shown as entirely constant  $E_a$  values in the ranges of  $117 \pm 3\text{ kJ}\cdot\text{mol}^{-1}$ , suggesting that the weight loss over the whole period resulted mainly from one reaction. This result corresponds with  $111 \pm 7$  and  $119 \pm 2\text{ kJ}\cdot\text{mol}^{-1}$  reported by Aoyagi et al.<sup>12</sup> and Kim et al.<sup>13</sup>, respectively. However, there were slight increase and decrease for both of the plots at the beginning and end of the pyrolysis, respectively. This observation suggests the occurrence of some minor side-reactions over the whole process.

**[Figure 1]****Composition of pyrolyzates**

It has been assumed that the main PHB thermal degradation mechanism is  $\beta$ -elimination due to the presence of crotonyl and carboxyl groups at almost all chain ends of the products, as reported previously.<sup>3,5,7,19</sup> In this study, the pyrolysis products were determined by Py-GC/MS,  $^1\text{H}/^{13}\text{C}$  NMR, FTIR, and FAB-MS.

In order to investigate the dynamic decomposition process in Py-GC/MS (EI), five temperature ranges were selected based on the TG curves of PHB. In the dynamic decomposition process in Py-GC/MS, the fastest heating rate:  $\varphi=9\text{ }^\circ\text{C min}^{-1}$  was chosen to obtain some intermediates as the final product. Typical total ion current (TIC) chromatogram of the evolved gas from PHB in the temperature range of 40-270  $^\circ\text{C}$  heated at  $\varphi=9\text{ }^\circ\text{C}\cdot\text{min}^{-1}$  are illustrated in Figure 2. All of the TIC chromatograms in other temperature ranges: 40-250, 40-260, 40-280, and 40-290  $^\circ\text{C}$  showed similar profiles by having two peaks in nearly the same retention times, *i.e.* 2-5 and 17-18 min, which were attributed to crotonic acid monomer and relating dimers, respectively. The assignment of the first peak (41.2 TIC intensity-%) is based on the reference peak of crotonic acid. In EI-MS spectrum of the first peak, characteristic signals of crotonic acid, *e.g.* at  $m/z = 68$  and  $86$ , were detected. The second peak (58.8 TIC intensity-%) was assigned to be that of dimers due to the characteristic EI-MS signals recorded at  $m/z = 103, 113, 126$  and  $154$ . Trimers, which were detected in many previous studies<sup>7,12,17</sup> were not detected under the conditions employed as seen in Figure 2. These results indicate that the same components, *i.e.* crotonic acid and dimers were produced over the whole degradation period.

**[Figure 2]**

### Characterization of pyrolyzates

Isothermal degradation of PHB in a glass tube oven was conducted at 260 °C *in vacuo* to determine the components of distilled pyrolyzates. After the weight loss of 89.9%, recovered two kinds of pyrolysis products were seen and categorized as major and minor products, respectively. Major pyrolysis product was seen as crystals, whereas the minor product was an oily liquid. The pyrolyzates were collected and analyzed by  $^1\text{H}/^{13}\text{C}$  NMR (Figures 3 and 4) and  $^1\text{H}-^1\text{H}$  COSY (Figure SM-2 in Supplementary Materials section) measurements. From the integration analysis of signals assigned to methyl groups in the  $^1\text{H}$  NMR spectrum, it was found that the major component in PHB pyrolyzates was *trans*-crotonic acid, which contributed to 67.7 % of the total PHB pyrolyzates. Other components were *cis*-crotonic acid and its derivatives (3.1 %) and oligomers (29.2 %) having crotonyl end groups. Unexpectedly, hydroxybutyric acid and its derivatives were scarcely detected in  $^1\text{H}$  NMR and  $^1\text{H}-^1\text{H}$  COSY spectra, because no signal assignable to  $\beta$ -proton neighbored on hydroxyl end-group was detected at around 4.22 ppm.<sup>26-28</sup> In order to determine the minor component of the pyrolyzates,  $^{13}\text{C}$  NMR analysis of the oily liquid product was conducted. In the  $^{13}\text{C}$  NMR spectrum, at least four kinds of carbonyl carbons were detected at 175.5, 170, 166, 161 ppm. Expected assignments of the signals are depicted in Figure 4, *i.e.* the carbonyl carbons of 3HB units (a)<sup>28,29</sup>, the carbonyl carbons of chain-end carboxyl (a'<sup>30</sup>) and crotonyl (a''<sup>30</sup> and a''') end-groups. As one component having the crotonyl end group, crotonic anhydride may be assignable to the signal at around 161 ppm. This is supported by the  $^{13}\text{C}$  NMR spectrum of standard crotonic anhydride, which shows a carbonyl carbon (a''') signal at around 161 ppm (see Supplementary Materials section).

[Figure 3]

[Figure 4]

To clarify the pyrolyzates component, the PHB pyrolyzates from isothermal degradation at 260 °C

were analyzed with FTIR and FAB-MS. FTIR spectrum of the PHB pyrolyzates is illustrated in Figure 5. Strong absorptions in ranges of 1660-1720 and 1630-1650  $\text{cm}^{-1}$  indicate the presence of  $\alpha,\beta$ -unsaturated aliphatic acids/esters ( $\nu_{\text{C}=\text{O}}$ ) and olefinic groups ( $\nu_{\text{C}=\text{C}}$ ), respectively, such as crotonic acid and related oligomeric acids. Other strong absorption at 970  $\text{cm}^{-1}$  and broad absorption in a range of 2400-3200  $\text{cm}^{-1}$  are attributable to the out of plane  $\delta_{\text{C-H}}$  of olefinic group<sup>19</sup> and  $\nu_{\text{O-H}}$  of carboxyl group.

A weak absorption band was found at 1792  $\text{cm}^{-1}$ , at which wavenumber the most detectable chemicals are acid anhydrides. Previously, Gonzalez et al.<sup>19</sup> reported that PHB pyrolyzates such as dimers showed two absorptions bands at 1783 and 1744  $\text{cm}^{-1}$ , which were assigned to the carbonyl stretching vibration bands of acid and  $\alpha,\beta$ -unsaturated ester groups. The absorption band at 1783  $\text{cm}^{-1}$  is too high wavenumber to be the band of  $\nu_{\text{C}=\text{O}}$  of such acid or  $\alpha,\beta$ -unsaturated ester groups, but it may be accountable as the band of  $\nu_{\text{C}=\text{O}}$  of anhydrides.

### [Figure 5]

To confirm the expected pyrolyzates, a positive ion FAB mass spectrum, in which  $(\text{M}+\text{H})^+$  peaks are detected, was measured. PHB was pyrolyzed in the glass tube oven at 260 °C *in vacuo* and generated pyrolyzates were condensed in a chilled trap. In Figure 6, the FAB mass spectrum of the PHB pyrolyzates is shown. The spectrum is dominated by two series of peaks at  $m/z = (87 + n \times 86)$  ( $n = 0 \sim 5$ ) and  $m/z = (155 + n \times 86)$  ( $n = 0 \sim 1$ ), corresponding to ions of compounds with one and two olefinic end-groups, respectively. Main peaks observed at  $m/z = 155$ , 173, and 259 may be assignable to crotonic anhydride ( $\text{M}=154$ ), crotonyl-3HB acid ( $\text{M}=172$ ), and crotonyl-3HB-3HB acid ( $\text{M}=258$ ), which are the related dimers and trimer. Although, in some previous reports<sup>5,7,12,15,20</sup> the same peaks ( $m/z = 154$  or 155 as  $\text{M}^+$  or  $(\text{M}+\text{H})^+$ ) have been observed in EI-MS and FAB-MS spectra, these peaks have been assigned as fragments. In Figure 6, trace peaks at  $m/z = (105 + n \times 86)$  for 3HB-acid and its oligomers were

detected, indicating that the crotonyl and carboxyl end-groups are dominantly formed by the thermal degradation of PHB.

### [Figure 6]

During the isothermal degradation of PHB in the glass tube oven, the pyrolysis products were trapped in two containers. The first container trapped the pyrolyzates, whilst the second one trapped only water. This indicates that dehydration occurred during the degradation of PHB. Gonzalez et al.<sup>19</sup> and Grassie et al.<sup>20</sup> reported that in the early stage of PHB degradation (temperature range: 170-200 °C) water molecules were removed as a consequence of condensation, resulting in an increase in the molecular weight of PHB. Taking into account the effect of dehydration, the production pathway of anhydrides on the PHB degradation may be reasonable. After the random  $\beta$ -elimination, a large number of oligomers having crotonyl and carboxyl end groups were generated, and subsequently, dehydration between the oligomers occurred to give oligomeric anhydrides having two crotonyl groups at both end positions.

The crotonic anhydride shows three signals in the  $^1\text{H}$  NMR spectrum (Figure 3) at nearly the same chemical shifts with those of monomer: crotonic acid, and it was observed on the Py-GC/MS profile as a peak having nearly the same retention time 15-18 min as the second peak of the dimers in Figure 2. The production of crotonic anhydride may provide an explanation to the contradiction between the pyrolyzates compositions obtained from Py-GC/MS and  $^1\text{H}$  NMR.

### Expected thermal degradation pathways of PHB

Based on the above results, an anhydride formation process, *i.e.* dehydration as a minor reaction, should be included in the thermal degradation pathways of PHB, in which the random  $\beta$ -elimination is predominant. Expected pathways of PHB are illustrated in Scheme 1, where  $l$ ,  $m$ , and  $n$  mean repetition

numbers of 3-hydroxybutyrate unit and the numbers of  $l$  and  $m$  are less than the number of  $n$ . The anhydride formation is one of mechanisms out of processes conforming to the random scission statistics. The  $\beta$ -elimination leads to oligomeric acids and finally crotonic acid with monotonous decrease in the molecular weight, while the dehydration may get in the way of the decrease. Moreover, the production of anhydrides may influence the reactions caused by carboxyl groups, such as the E1cB mechanism<sup>6</sup> and inter/intra-transesterifications, contributing to the complexity of PHB pyrolysis.

### [Scheme 1]

## CONCLUSIONS

In order to confirm minor reactions out of the random chain scission occurred during the PHB thermal degradation, minor pyrolyzates from PHB were characterized with  $^1\text{H}/^{13}\text{C}$  NMR, FTIR, and FAB-MS. As results, crotonic anhydride and its oligomers were detected as minor products from condensation reactions between carboxyl groups. The anhydrides production must be one out of conforming to the random degradation statistics and contribute to the complexity of the PHB pyrolysis. An expected thermal degradation pathway of PHB was proposed.

## Acknowledgement

The authors are extremely grateful to Mr. Masashi Ohira of Kinki University, Dr. Takayuki Tsukegi of Kyushu Institute of Technology, and Dr. Taisuke Matsumoto and Dr. Yasuko Tanaka of the Institute for Materials Chemistry and Engineering, Kyushu University for their generous support during the experiments and the AA and FAB-MS measurements.

## References and notes

1. Steinbüchel, A.; Valentin, H. E. FEMS Microbiol Lett 1995, 128, 219.

2. Marchessault, R. H.; Coulombe, S.; Morikawa, H.; Okamura, K.; Revol, J. F. *Can J Chem* 1981, 59, 38.
3. Morikawa, H.; Marchessault, R. H. *Can J Chem* 1981, 59, 2306.
4. Melchior, M.; Keul, H.; Höcker, H. *Macromolecules* 1996, 29, 6442.
5. Ballistreri, A.; Garozzo, D.; Giuffrida, M.; Impallomeni, G.; Montaudo, G. *J Anal Appl Pyrolysis* 1989, 16, 239.
6. Kawalec, M.; Adamus, G.; Kurcok, P.; Kowalczyk, M.; Foltran, I.; Focarete, M. L.; Scandola, M. *Biomacromolecules* 2007, 8, 1053.
7. Kopinke, F. D.; Remmler, M.; Mackenzie, K. *Polym Degrad Stab* 1996, 52, 25.
8. Galego, N.; Rozsa, C. *Polym Int* 1999, 48, 1202.
9. Li, S. D.; Yu, P. H.; Cheung, M. K. *J Appl Polym Sci* 2001, 80, 2237.
10. He, J. D.; Cheung, M. K.; Yu, P. H.; Chen, G. Q. *J Appl Polym Sci* 2001, 82, 90.
11. Lee, M. Y.; Lee, T. S.; Park, W. H. *Macromol Chem Phys* 2001, 202, 1257.
12. Aoyagi, Y.; Yamashita, K.; Doi, Y. *Polym Degrad Stab* 2002, 76, 53.
13. Kim, K. J.; Doi, Y.; Abe, H. *Polym Degrad Stab* 2006, 91, 769.
14. Carrasco, F.; Dionisi, D.; Martinelli, A.; Majone, M. *J Appl Polym Sci* 2006, 100, 2111.
15. Abate, R.; Ballistreri, A.; Montaudo, G.; Impallomeni, G. *Macromolecules* 1994, 27, 332.
16. Lehrle, R. S.; Williams, R. J. *Macromolecules* 1994, 27, 3782.
17. Kopinke, F. D.; Mackenzie, K. *J Anal Appl Pyrolysis* 1997, 40, 43.
18. Li, S. D.; He, J. D.; Yu, P. H.; Cheung, M. K. *J Appl Polym Sci* 2003, 89, 1530.
19. Gonzalez, A.; Irusta, L.; Fernandez-Berridi, M. J.; Iriarte, M.; Irui, J. J. *Polym Degrad Stab* 2005, 87, 347.
20. Grassie, N.; Murray, E. J.; Holmes, P. A. *Polym Degrad Stab* 1984, 6, 95.
21. Kunioka, M.; Doi, Y. *Macromolecules* 1990, 23, 1933.
22. Nguyen, S.; Yu, G.; Marchessault, R. H. *Biomacromolecules* 2002, 3, 219.

23. Lehrle, R.; Williams, R.; French, C.; Hammond, T. *Macromolecules* 1995, 28, 4408.
24. Doyle, C. D. *J Appl Polym Sci* 1961, 5, 285.
25. Doyle, C. D. *J Appl Polym Sci* 1962, 6, 639.
26. Tanio, T.; Fukui, T.; Shiakura, Y.; Saito, T.; Tomita, K.; Kaiho, T. *Eur J Biochem* 1982, 124, 71.
27. Adamus, G.; Kowalczyk, M.; Jedliński, Z.; Ballistreri, A.; Scherer, T.; Lenz, R. W. *Polym Degrad Stab* 1995, 50, 269.
28. Abe, H.; Doi, Y.; Hori, Y.; Hagiwara, T. *Polymer* 1997, 39, 59.
29. Barnard, G. N.; Sandars, J. K. M. *J Biol Chem* 1989, 264, 3286.
30. The chemical shifts were predicted using a ChemNMR program in a CS ChemDraw Ultra version 6.0.



### Figure and Scheme Captions

**Figure 1.** TG curves of PHB degradation at multiple heating rates ( $\phi=1, 3, 5, 7$  and  $9 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ ) in a constant  $\text{N}_2$  flow ( $100 \text{ mL}\cdot\text{min}^{-1}$ ).

**Figure 2.** TIC spectrum of PHB pyrolyzates at temperature range of  $40\text{-}270 \text{ }^\circ\text{C}$  at heating rate of  $9^\circ\text{C}\cdot\text{min}^{-1}$ .

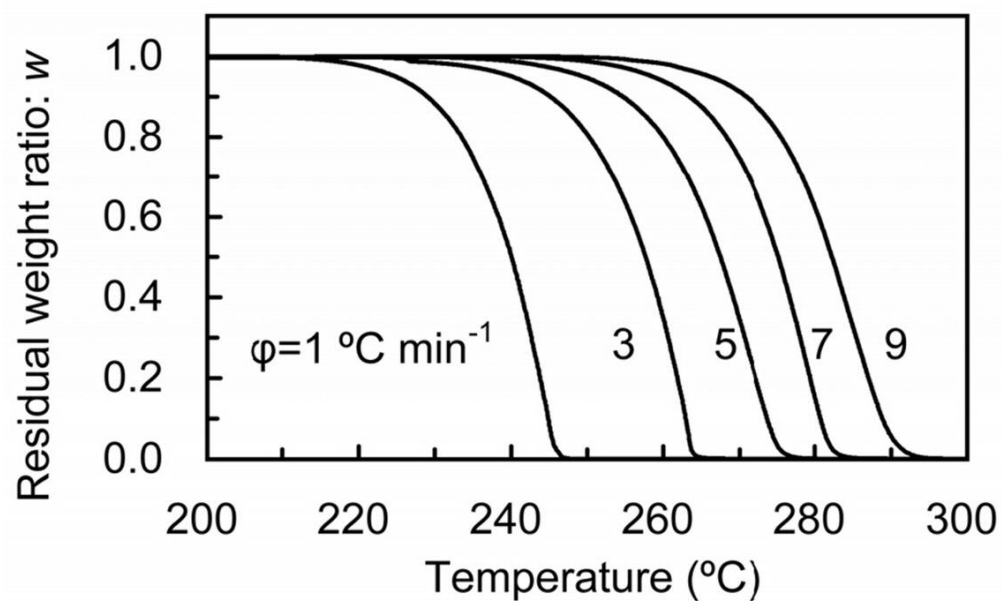
**Figure 3.**  $500 \text{ MHz } ^1\text{H}$  NMR spectrum of total PHB pyrolyzates obtained by thermal degradation *in vacuo* at  $260^\circ\text{C}$ .

**Figure 4.**  $125 \text{ MHz } ^{13}\text{C}$  NMR spectrum of minor oily PHB pyrolyzates obtained by thermal degradation *in vacuo* at  $260^\circ\text{C}$ .

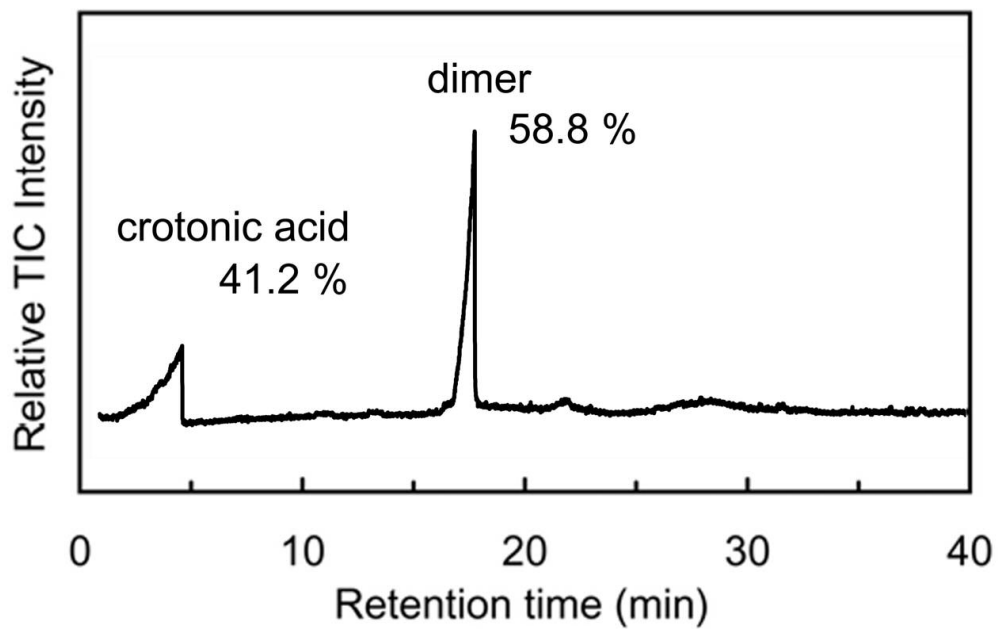
**Figure 5.** FTIR spectrum of PHB pyrolyzates at  $260^\circ\text{C}$ .

**Figure 6.** Positive ion FAB mass spectrum (glycerol matrix) of PHB pyrolyzates.

**Scheme 1.** Expected thermal degradation pathways of PHB.

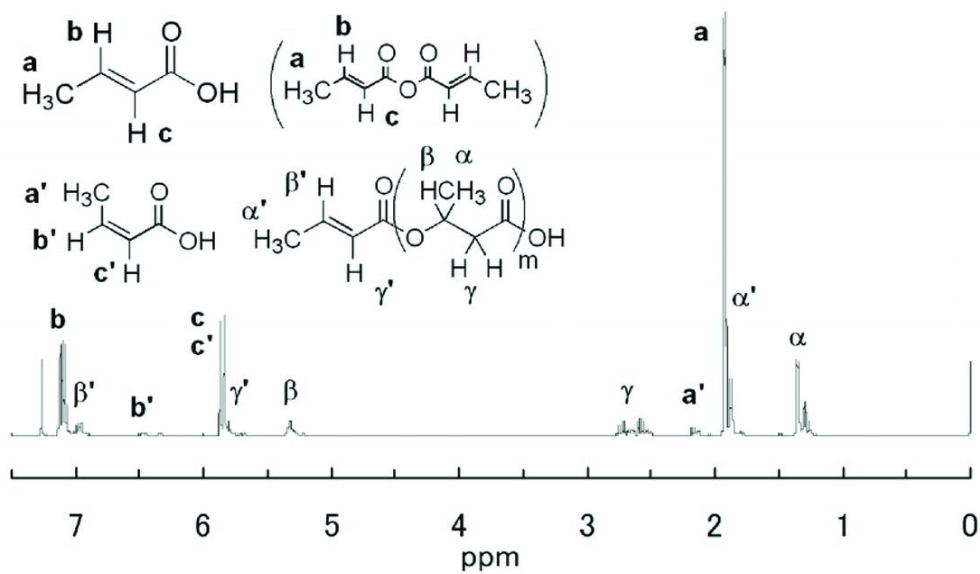


**Figure 1.** TG curves of PHB degradation at multiple heating rates ( $\phi = 1, 3, 5, 7$  and  $9$  °C•min<sup>-1</sup>) in a constant N<sub>2</sub> flow (100 mL•min<sup>-1</sup>).  
48x29mm (600 x 600 DPI)



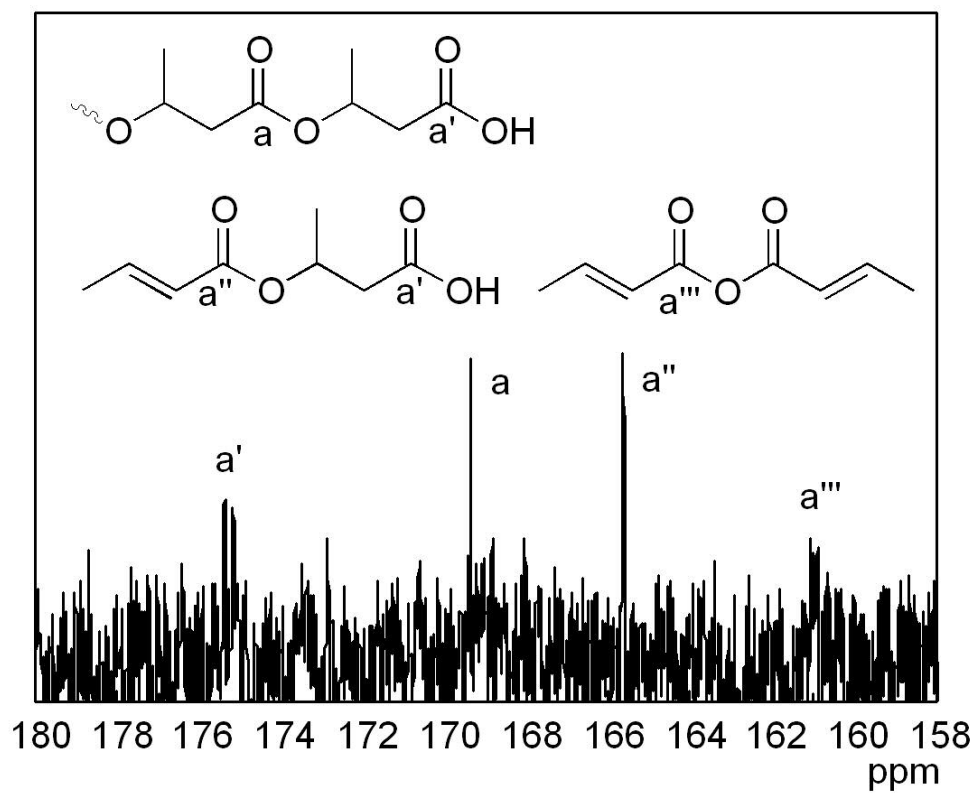
**Figure 2. TIC spectrum of PHB pyrolyzates at temperature range of 40-270 °C at heating rate of 9°C•min<sup>-1</sup>.**

53x33mm (600 x 600 DPI)



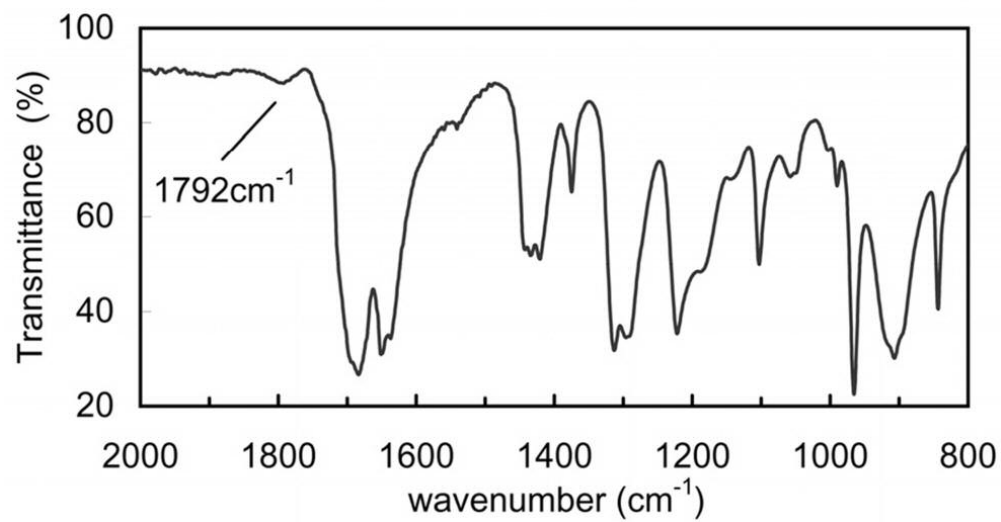
**Figure 3. 500 MHz  $^1\text{H}$  NMR spectrum of PHB pyrolyzates obtained by thermal degradation in vacuo at 260°C.**

46x27mm (600 x 600 DPI)

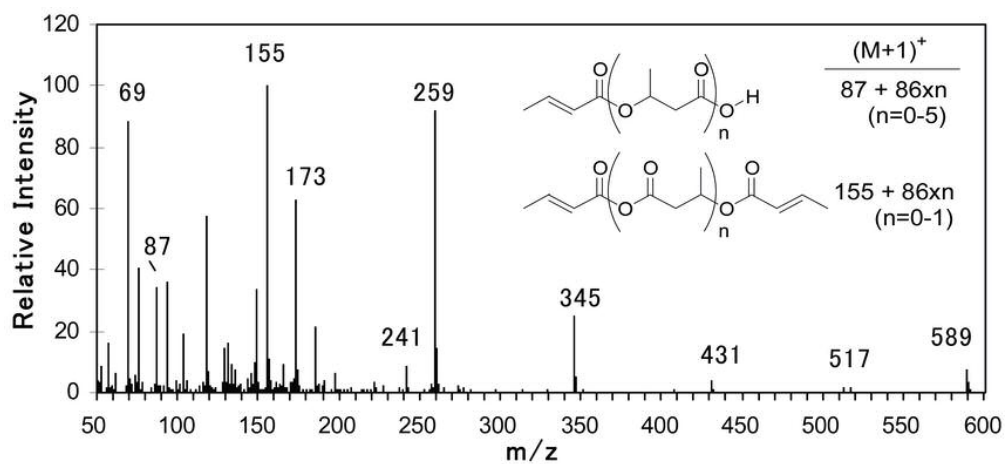


**Figure 4. 125 MHz  $^{13}\text{C}$  NMR spectrum of PHB pyrolyzates obtained by thermal degradation in vacuo at 260°C.**

93x79mm (300 x 300 DPI)



**Figure 5. FTIR spectrum of PHB pyrolyzates at 260°C.**  
41x22mm (600 x 600 DPI)



**Figure 6. Positive ion FAB mass spectrum (glycerol matrix) of PHB pyrolyzates.**  
36x16mm (600 x 600 DPI)

