



## Biodegradation of Poly(R,S)- $\beta$ -hydroxybutyrate and its copolymer with $\delta$ -Valerolactone Synthesized by Aluminoxane Catalyst

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**Abstract.** Poly(R,S)- $\beta$ -hydroxybutyrate (PHB) and its copolymers with  $\delta$ -valerolactone were synthesized by ring-opening copolymerization of (R,S)- $\beta$ -butyrolactone ( $\beta$ -BL) and  $\delta$ -valerolactone ( $\delta$ -VL) monomers in the presence of a tetraisobutyldialuminoxane catalyst. The biodegradability of these polymers by using activated sludge was studied in an aerobic medium. The objective of this work was to determine the influence of structure and crystallinity of polymers on their degree of biodegradation and initial degradation rate. It was shown that the degradation rate for bacterial P(R)-HB with 100 % (R) isotactic structure was the highest and the final biodegradation was reached around 94 % after incubation time of 35 days. Whereas the final biodegradation about 88 % was obtained for atactic P(R,S)- $\beta$ -HB synthesized by using aluminoxane catalyst. The influence of the structure and crystallinity on the initial biodegradation rate were observed for the copolymers in various composition of comonomers. All these copolymers with the PHB ratio more than 10 % exhibit highly degree of biodegradation, about 85 % after 35 days of incubation time.

**Keywords:** Copolymers; biodegradation; aluminoxane; crystallinity; tacticity.

### 1 Introduction

Poly(R)- $\beta$ -hydroxybutyrate is produced by a wide range of bacteria and serves as an intracellular carbon and energy reserve material. The natural P(R)- $\beta$ -HB prepared by the biosynthetic route is an optically active polymer and an environmental biodegradable thermoplastic. Because of the repeating unit structure is isotactic stereoregular (100 % R isotactic), so P(R)-HB has a high crystallinity with melting point close to 180°C [1-3]. These PHB properties resemble to polypropylene properties and easy to be biodegraded in environment, so that PHB has attracted attention to be developed furthermore as an environmental friendly plastics.

A chemical synthesis of PHB by polymerization of racemic  $\beta$ -BL monomer could be used as an alternative method to synthesize polyester with various isomer and stereochemical structure. Synthetic polyesters analogous to the

bacterial PHB properties were prepared by ring-opening polymerization of racemic  $\beta$ -BL monomer in the presence of organometallic catalyst [3-5]. In contrast to biosynthesis route producing only PHB with 100 % R isotactic structure, the ring-opening polymerization of racemic  $\beta$ -BL with a certain catalyst system allows to modify structure and properties of polyesters.

Studies on enzymatic degradation of polymer involve an understanding of a number of fundamental aspect of both enzyme system and nature of the substrate. In the case of the enzyme, some factors such as the identity and location of the active sites, as well as optimal conditions for physiological activity including temperature and pH are important parameters to be considered. As the substrate, the morphology, accessibility and mobility of chain segments of the appropriate configuration for enzyme attack are important factors determining the extent of biodegradation process.

Doi *et al.* [6] and Jesudason *et al.* [7] showed that low-crystalline PHB fraction are hydrolyzed more readily than more crystalline for predominantly isotactic PHB in the presence of PHB depolymerase from *A. faecalis*. The isotactic P(S)-HB film is not degraded by PHB depolymerase, indicating that the enzyme is not capable for hydrolyzing the sequence of (S)-HB units. More recently, Hocking *et al.*[8] fractionated racemic PHB polymers into several components with a wide range of isotacticity varying from 0.35 to 0.88, and studied the stereochemical effects on the rate of enzymatic degradation with PHB depolymerase from *P. lemoignei* and *A. fumigatus*. Low-crystalline racemic PHB fractions (55-60 % isotactic diad) showed significantly higher rates of enzymatic degradation than those of more crystalline racemic PHB with either predominantly isotactic or predominantly syndiotactic crystal structure.

In previous study was shown that the biodegradability of PHB prepared by ring-opening polymerization of racemic (R,S)- $\beta$ -butyrolactone in the presence of tetraisobutyldialuminumoxane catalyst depends on the degree of crystallinity and stereoregularity of R configuration in polymers [9].

In this study we report the biodegradability of its copolymers prepared by ring-opening polymerization of racemic (R,S)- $\beta$ -butyrolactone and  $\delta$ -valerolactone monomers in the presence of tetraisobutyldialuminumoxane catalyst. The biodegradation test of polymers was carried out in the presence of activated sludge by determining the value of the biochemical oxygen demand (%BOD) for biodegradation process take place.

## 2 Experimental

### 2.1 Material

Racemic (R,S)- $\beta$ -Butyrolactone and  $\delta$ -valerolactone monomers were obtained commercially from Aldrich Chem. Co., dried over  $\text{CaH}_2$  and fractionally distilled (65°C, 15 mmHg for  $\beta$ -BL and 60°C, 0,2 mmHg for  $\delta$ -VL). Toluene was distilled and stored over  $\text{CaH}_2$ . Tetraisobutyldialuminumoxane (30 % in cyclohexane) was also obtained from Aldrich Chem. Co., stored and manipulated under a nitrogen atmosphere.

### 2.2 Preparation and Purification of Polymers

All reactants were transferred with a syringe to the reactor polymerization through natural rubber septa under nitrogen atmosphere. The reactor was connected to a vacuum line ( $10^{-6}$  mmHg). The catalyst was first added into the reactor and cyclohexane was evaporated when the solvent used was toluene. The reactor was cooled with an external bath at -78°C and then monomers were added with a syringe. The contents of the reactor were degassed during three freeze-thaw cycles and finally sealed under vacuum. Copolymerization reactions were carried out at 60°C for 7 days. After that the reactor was opened and then added ether (100 mL/g of initial monomer). The mixture was stirred with a magnetic stirrer. A solid polymer was filtered and then chloroform was added into polymer (100 mL/g of initial monomer). The polymer solution was stirred for 12 hours at room temperature and refluxed for half an hour. The clear chloroform solution obtained was concentrated to 10 mL/g of initial monomer. This solution was precipitated in ether and filtered to give the yellowish polymers. The polymer was treated with acetone or acetyl-acetone. The polymer suspension in acetone was stirred for 18 hours at room temperature, and then insoluble polymer was filtered and washed with ether [9].

### 2.3 Characterization of Polymers

The heat of fusion ( $\Delta H_m$ ), melting temperature ( $T_m$ ) and glass transition temperature ( $T_g$ ) for all polymer samples were determined by using Differential Scanning Calorimetric conducted on a Perkin Elmer DSC-7. Samples (between 5 – 10 mg) were heated at a rate of 10 °C/min from -150 °C to 200 °C, quickly cooled and then scanned a second time by using the same heating rate and temperature range as the first scan. Data used for  $\Delta H_m$  and  $T_m$  was taken from the first scan and those for  $T_g$  from the second scan.

All molecular weight data reported in the tables were obtained by GPC (Gel Permeation Chromatography). Chloroform was used as eluent at a flow rate of

1.0 ml/min. In this analysis was used sample concentration of 2 % wt/vol. and injection volume of 20-30  $\mu$ L. Polystyrene standards with low polydispersities were used to make a calibration curve.

The  $^{13}\text{C}$  NMR measurements were recorded at 100 MHz with a concentration of 1-1.5 % wt/vol. and the  $^1\text{H}$ -NMR measurements were recorded at 400 MHz at 25-30°C with the concentration of 0.1 % wt/vol.  $\text{CDCl}_3$  was used as solvent and tetramethylsilane (TMS) as internal reference.

The biodegradation test was carried out by measuring the oxygen consumption as a function of incubation time. The polymer sample was directly weighed in a bottle tester, and then solution of activated sludge as inoculums in mineral medium (10 mL of the original inoculums concentration about 3 g/L) was introduced in the bottle. The biodegradation process was followed until 35 days at constant temperature (37°C). The degree of biodegradability of the polymer samples was determined by the value of the biochemical oxygen demand (%BOD) for degradation process take place [9,10].

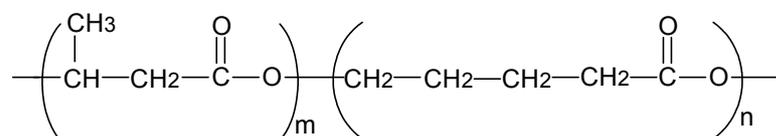
### 3 Results and Discussion

Poly(R,S)- $\beta$ -hydroxybutyrate and its copolymers with  $\delta$ -valerolactone monomer were prepared by ring-opening polymerization of racemic (R,S)- $\beta$ -BL and  $\delta$ -VL monomers in the presence of tetraisobutyldialuminumoxane catalyst. Based on analysis of  $^{13}\text{C}$  RMN and DSC, the tetraisobutyldialuminumoxane catalyst tends to produce partially stereoregular P(R,S)- $\beta$ -HB. By addition of a certain quantity of water to the catalyst and treatment of polymers by solvent extraction could increase stereoregularity and crystallinity of P(R,S)- $\beta$ -HB. This polymer is composed of two endothermic components with peak temperatures about 87°C and 163°C. The formation of several melting endothermic generally broad can be due to the presence of different crystalline structures [9].

Based on the analysis of thermal properties indicate that copolymers have a single glass transition temperature located between glass transition temperature of PVL and PHB. This result is supported by the analysis of monomer reactivity ratio and diad sequence distribution of copolymers that copolymers obtained are a mixture of compatible polymers [11].

In previous research [6-10] showed that crystallinity and stereoregularity of (R) configuration are important parameters in biodegradation behavior. The biodegradability of synthetic P(R,S)- $\beta$ -HB increase with the decreasing of isotactic diad fraction (i) from 0.79 to 0.50. The highest biodegradability for synthetic P(R,S)- $\beta$ -HB was observed for atactic polymer with isotactic diad

fraction ( $i$ ) = 0.50, whereas a very low the biodegradability was observed for the predominantly syndiotactic synthetic P(R,S)- $\beta$ -HB ( $i$  = 0.41) as result of a high crystallinity and a low isotacticity structure [9].



**Figure 1** Structure of P(HB-co-VL).

**Table 1** Characteristic of copolymers P(HB-co-VL) prepared by using tetraisobutyldialuminoxane catalyst.

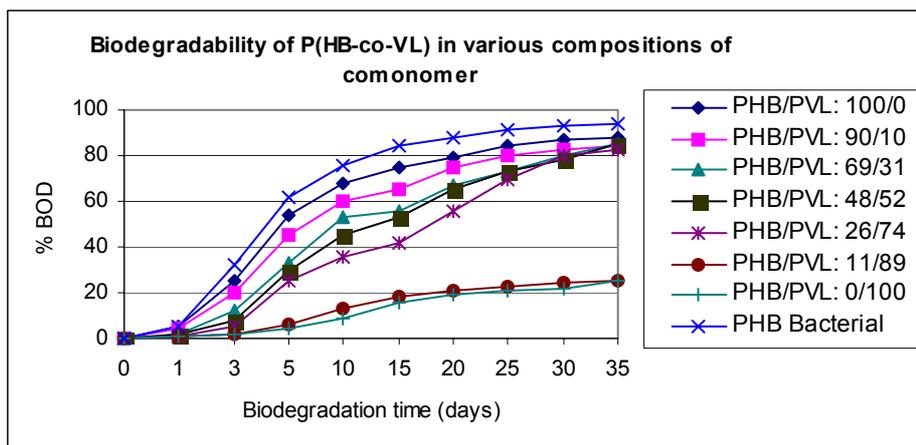
No.	Ratio PHB/PVL	$\overline{M}_w$	Tg (°C)	Tm (°C)	$\Delta H$ (J/g)	Biodegradation (% BOD) <sup>a</sup>
1	100/0	18900	2.0	-	-	88
2	90/10	7750	-4.5	-	-	84
3	69/31	8300	-16.7	-	-	85
4	48/52	11400	-30.2	-	-	85
5	26/74	13800	-43.2	-	-	83
6	11/89	17200	-54.0	35.8	34.4	25
7	0/100	13100	-62.0	52.0	64.8	25
8	Bact PHB	800000	-	179	97.0	94

<sup>a</sup>This result is the average value from two experiments undertaken in parallel. Incubation time = 35 days.

The biodegradation results of copolymer with various compositions of PHB/PVL and curve indicating % BOD as a function of incubation time can be shown at Table 1 and Figure 2, respectively. The final biodegradation of PVL was observed about 25 % after incubation time of 35 days, whereas biodegradation process was still continued slowly. It is known that PVL is continuously biodegraded until totally biodegradation, but its degradation rate is lower than that of naturally PHB produced biosynthesis route[10].

The maximal biodegradation for all copolymers P(HB-co-VL) were observed in around 85 % with exception of those with PHB/PVL composition of 11/89, its biodegradation was only 25 % after incubation time of 35 days. Based on the analysis of biodegradation results (Fig.2) could be shown that the biodegradability and initial biodegradation rate measured by curve slope of the biodegradation versus incubation time from initial time until incubation time of 25 days tend to decrease with the increase of non-substituted comonomer unit (VL) in copolymers. The initial biodegradation rate of copolymers with various composition of HB comonomer is higher than that of synthetic PVL. It was

known previous that PHB prepared by using dialuminumoxane catalyst have isotacticity of 50 %, so that this polymer is composed only amorphous atactic polymers, and finally the biodegradability of PHB is rather high, namely almost 90 % after incubation time of 35 days. On the contrary based on physical and thermal properties, the homopolymer PVL have rather high crystallinity, so that biodegradation of PVL was observed more difficult than that of copolymers, only 25 % after incubation time of 35 days. The increase of BL comonomer in copolymers influences the crystallinity and structure of copolymers, and this allows the presence of active site accessible by enzyme of microorganism. Because of P(R,S)- $\beta$ -HB prepared by using dialuminumoxane catalyst produce amorphous atactic polymer with the high biodegradability, so the rate and final biodegradation on film of copolymers increase with the increase of BL comonomer unit in copolymers. These results confirm that the crystallinity and structure of copolymers are significant factors in biodegradation behavior of copolymers. The study of these biodegradation results and the structure analysis by  $^{13}\text{C}$  NMR spectroscopy could clarify that copolymers P(HB-co-VL) are composed of homopolymers mixture (amorphous PHB and crystalline PVL) and block copolymer with variable block size [11].



**Figure 2** Biodegradability of P(HB-co-VL) in various composition of PHB/PVL.

#### 4 Conclusion

The biodegradability of these polymers in the presence of activated sludge in the aerobic medium shown that the degradation rate for bacterial P(R)-HB with 100 % (R) isotactic structure was the highest and the final biodegradation was reached around 94 % after incubation time of 35 days. Whereas the final

biodegradation about 88 % was obtained for atactic P(R,S)- $\beta$ -HB synthesized by using aluminoxane catalyst. The influence of the structure and crystallinity on the initial biodegradation rate were observed for the copolymers in various composition of comonomers. All these copolymers with the PHB ratio more than 10 % exhibit highly degree of biodegradation, about 85 % after 35 days of incubation time. These biodegradation results could clarify that copolymers P(HB-co-VL) are composed of homopolymers mixture (amorphous PHB and crystalline PVL) and block copolymer with variable block size

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### References

1. Pere, R. & Lenz, R. W., *Synthesis and polymerization of (R,S)- $\beta$ -pentyl- $\beta$ -propiolactones*, *Macromolecules*, 26 : 6697 (1993).
2. Holmes, P. A., *Applications of PHB. A microbially produced biodegradable thermoplastic*, *Phys. Technol.*, 16: 32 (1985).
3. Benvenuti, M. & Lenz, R. W., *Polymerization and copolymerization of  $\beta$ -butyrolactone and benzyl - $\beta$ -malolactone by aluminoxane catalyst*, *J. Polym. Sci., Part. A J. Polym. Sci., Part. A : Polym. Chem.*, 29: 793 (1991).
4. Zhang, Y., Gross, R. A. & Lenz, R. W., *Stereochemistry of the ring-opening polymerization of (S)- $\beta$ -butyrolactone*, *Macromolecules.*, 23 : 3206 (1990).
5. Kemnitzer, J. E., McCarthy, S. P. & Gross, R. S., *Poly-( $\beta$ -hydroxybutyrate) stereoisomers: A model study of the effects of stereochemical and morphological variables on polymer biological degradability*, *Macromolecules*, 25: 5927 (1992).
6. Doi, Y., Kumagai, Y., Tanahashi, N. & Mukai, K., *Structural Effect on Biodegradation of Microbial and Synthetic Poly(hydroxyalkanoates)*, *Biodegradable Polymer and Plastics*, Vert, M., at all. Eds., Royal Society of Chemistry, London, p. 139 (1992).
7. Jesudason, J. J., Marchessault, R. H. & Saito, T., *Enzymatic Degradation of Poly(R,S)- $\beta$ -hydroxybutyrate*, *J. Environ. Polym. Degradation.*, 1: 89 (1993).
8. Hocking, P. J., Timmins, M. R., Scherer, T. M., Fuller, R. C., Lenz, R. W. & Marchessault, R. H., *Enzymatic Degradability of isotactic versus*

- syndiotactic poly( $\beta$ -hydroxybutirate)*, *Macromol. Rapid Commun.*, 15: 447 (1994).
9. Arcana, I. M. & Surdia, N. M., *Chemical synthesis of poly(R,S)- $\beta$ -hydroxybutyrate and its characterization*, Proceeding: The Second International Workshop on Green Polymers, Science and Technology (Japan)-Dutch Polymer Institute (Netherlands), 15-20 October 2000, Bandung, Indonesia (2000).
  10. Arcana, M., Giani-Beaune, O., Schue, R., Schue, F., Amass, W. & Amass, A., *Ring-opening copolymerization of racemic  $\beta$ -butyrolactone with  $\epsilon$ -caprolactone and  $\delta$ -valerolactone by distannoxane derivative catalysts: study of the enzymatic degradation in aerobic media of obtained copolymers*, *Polymer International*, 51 (10), 859-866, 2002.
  11. Arcana, M., Tanajaya, B., Anwar, B., Radiman, C. L., & Sulfikar, M. A., *Structure analysis of copolymers prepared by copolymerization of  $\beta$ -BL and  $\delta$ -VL in the presence of aluminoxane catalyst*, Proceeding: The sixth ITB-UKM Joint Seminar on Chemistry, ITB-UKM, Bali, Indonesia, (2005).