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Synthesis of poly (1,4-dioxan-2-one) catalyzed by immobilized lipase CA

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ABSTRACT

Polymerization of 1,4-dioxan-2-one was carried out more detailed with immobilized lipase CA as the catalyst. The effect of the enzyme amount, reaction temperature and water content on polymerization was investigated, respectively. Both the conversion of monomer and the M_v of poly(1,4-dioxan-2-one) increased with the increase of enzyme amount, and maximized at 80 °C. At the beginning of polymerization, water molecules act as initiators. As the reaction time increased, linear condensation had gradually became dominant and water was released into the reaction system. Excess water may act as a chain cleavage agent. To obtain poly(1,4-dioxan-2-one) with an ideal molecular weight, polymerization of 1,4-dioxan-2-one was conducted by adding solvent and MS to reaction system, and product with a higher molecular weight (M_v = 58,000) was gained.

1. Introduction

As an aliphatic polyester with excellent biodegradability, bioabsorbability, biocompatibility, and flexibility, poly(1,4-dioxan-2-one) (PPDO) has attracted great concern of researches in recent years. It has been approved by the Food and Drug Administration (FDA) to be used as surgical suture material [1]. In addition, PPDO can be applied to bone and tissue fixation and drug delivery system [2–8].

PPDO was synthesized extensively by chemical polymerization with organic metallic catalysts such as organic-tin [6,9], organic-aluminum [10,11], organic-zinc [12], organic rare earth compounds [13], etc. As its usage is focused mainly on pharmacological and surgical applications, the metallic catalysts should be removed before use. The purification process will increase the cost of PPDO. To suppress the harmful effects of metallic residues in PPDO for medical applications, some nontoxic catalysts were investigated, and enzymes were the expected catalysts for preparing harmless PPDO. The reported routes for lipase-catalyzed polymerization are shown in Scheme 1 [14].

There were many reports on the enzymatic synthesis of lactones [15–21]. However, only one study on the enzymatic synthesis of PPDO has been illustrated [14]. Nishida et al. reported that immobilized lipase CA for the bulk polymerization of PDO showed higher polymerization activity than other enzymes. Both the conversion and M_w of PPDO increased with increasing enzyme loading amount. The water component acts not only as a substrate of the initiation process but also as a chain cleavage agent. However, there were still some questions, such as: how does reaction temperature influence polymerization, how does water content change during the enzymatic reaction and what is the relationship of water content change, monomer conversion and M_w of PPDO? So, it is necessary that investigation on the enzymatic synthesis of PPDO was performed in more detail. In this paper, we further studied polymerization of PDO catalyzed by immobilized lipase CA. Moreover, a strategy for increasing molecular weight of PPDO was introduced.

2. Experimental

2.1. Materials

Immobilized lipases CA (Novozym 435) with specific activity 10,000 PLU/g, derived from Candida antarctica, were purchased from Novo Nordisk Bioindustrials, Inc. in China. The enzymes were dried in vacuo at 25 °C for 1 day before use. 1,4-dioxan-2-one (PDO), a gift from the Pilot Plant of Center for Degradable and Flame-Retardant Polymeric Materials (Chengdu, China), was dried over CaH_2 for 48 h, distilled under reduced pressure, stored under nitrogen, and twice distilled in vacuo immediately before use. Other solvents were purchased from Hehong Chemical Factory (Chengdu, China).

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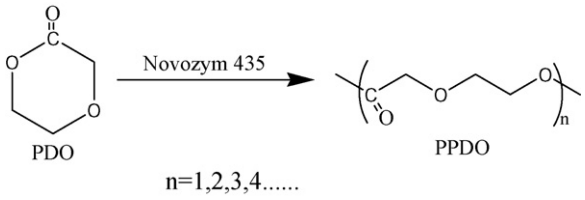

Scheme 1. Synthesis of poly(*p*-dioxanone).

2.2. Synthesis of PPDO

The polymerization was carried out in bulk. Prescribed amounts monomer and immobilized lipases CA were mixed in a dry vial and sealed. All procedures were carried out in an atmosphere of highly purified nitrogen. The reactor was immersed into a temperature-adjusted oil bath with magnetic stirring for predetermined intervals. The reaction mixture was dissolved in chloroform and then the enzyme was separated by filtration. One fraction of the organic phase was used to measure the conversion of monomer by gas chromatography (GC). The other fraction was added cool methanol. The cloudy methanolic solution was centrifuged (3000 rpm, 30 min). The white precipitate was placed under vacuum to remove methanol and the dry polymer was prepared for ^{1}H NMR, ^{13}C NMR and IR spectroscopy.

2.3. Determination of PPDO structure

The structure of PPDO was determined by NMR and IR spectroscopy. The ^{1}H NMR spectra and ^{13}C NMR were recorded in $CDCl_3$ on a Varian Germini 400 MHz NMR spectrometer. The spectra were obtained with a pulse angle of 25°, a delay time of 10 s, and an acquisition time of 2 s. All chemical shifts were reported in parts per million with tetramethylsilane as a reference. The infrared absorption spectra were performed on a Nicolet FTIR 170SX infrared spectrometer using KBr wafer.

2.4. Determination of PPDO molecular weight

As the conventional solvents such as chloroform, tetrahydrofuran, and toluene used in GPC measurements cannot resolve the resulting polymers with higher molecular weights, only the viscosity–average molecular weights of the resulting polymers were measured in phenol/1,1,2,2-tetrachloroethane (2:3 w/w) solution using an Ubbelohde viscosimeter thermostated at 25 °C. The molecular weights of PPDO can be calculated from the intrinsic viscosity [η] according to Mark–Houwink equation $[\eta] = KM_v^{\alpha}$, where $\alpha = 0.63$ and $K = 79 \times 10^{-3}$ cm^3 g^{-1} [22].

2.5. Determination of monomer conversion

The monomer conversion was determined with a Shimadzu GC14-B GC equipped with an OE-54 capillary column (Altech, 0.25 mm × 30 m), hydrogen flame ionization detector, and CR-6A CHROMATOPAC. The injection volume was 1.0 mL. The pressures of nitrogen gas, hydrogen gas, and air were 600, 70, and 50 kPa, respectively. The temperature of the injection pool and detector were 250 °C. Upon injection, the column oven was held at 180 °C for 3 min and programmed to rise at 10 °C/min to a final temperature of 210 °C which was maintained for 2 min. The calibration curve was made with the monomer.

2.6. Measurement of water content in reaction system

The total reaction water content was determined with a 831 KF Coulometer (Metrohm Ltd. CH-9101 Herisau Switzerland), in which 0.10 g of reaction mixture was dissolved in 3.0 mL 1,1,2,2-tetrachloroethane. The water content of the supernatant was measured with a Karl Fischer titrator relative to a 3.0-mL 1,1,2,2-tetrachloroethane control.

3. Results and discussion

3.1. Structural characterization

The ^{1}H NMR ($CDCl_3$, d, ppm) spectrum of the product showed the three characteristic signals: 4.16, 3.79, and 4.35. The ^{13}C NMR ($CDCl_3$, d, ppm) spectrum of the product also showed the characteristic signals: 170.0, 69.2, 68.3, and 63.9. These data was in accord with published result [1,22,23]. The IR spectrum of PPDO had the characteristic absorption at 3445, 2960, 2926, 1745, 1434, 1386, 1203, 1130, and 1060 cm^{-1}. The broad absorption band at 3445 cm^{-1} is attributed to the vibrations of ν_{O-H} of hydroxyl group. The two bands at 2960 and 2926 cm^{-1} are the characteristic absorption peaks of ν_{C-H}. The band at 1745 cm^{-1} is ascribed to $\nu_{C=O}$ of carbonate group. The band at 1434 cm^{-1} is δ_{C-H} of methylene group. The bands at 1203, 1130 and 1060 cm^{-1} are the characteristic absorption peaks of ν_{C-O}.

3.2. Effect of the enzyme amount on polymerization

Experiments were conducted with immobilized lipases CA of different weight percentage based on the PDO monomer (5.0 g). The polymerizations were carried out in bulk at 60 °C for 15 h. As shown in Fig. 1, the conversion of monomer, the yield and the M_v of PPDO increased with increasing immobilized lipases CA amount. However, the addition of immobilized lipases CA more than 10 wt.% based on 5.0 g PDO led to slight increasing of these values. So, the following experiments were conducted with 10 wt.% of immobilized lipases CA. In the polymerization without the enzyme (control experiment), the monomer was found unreacted, suggesting that the polymerization proceeded through the lipase catalysis.

3.3. Effect of reaction temperature on polymerization

Reaction temperature is one of important factors for enzymatic polymerization because it not only influences reaction rate but enzyme activity. In this research, we investigated the effects of reaction temperature on polymerization. The polymerizations were conducted at temperatures from 40 to 120 °C for 15 h. The results were shown in Fig. 2. Both the conversion of PDO and M_v of PPDO maximized at 80 °C. The conversion and M_v at more than 80 °C

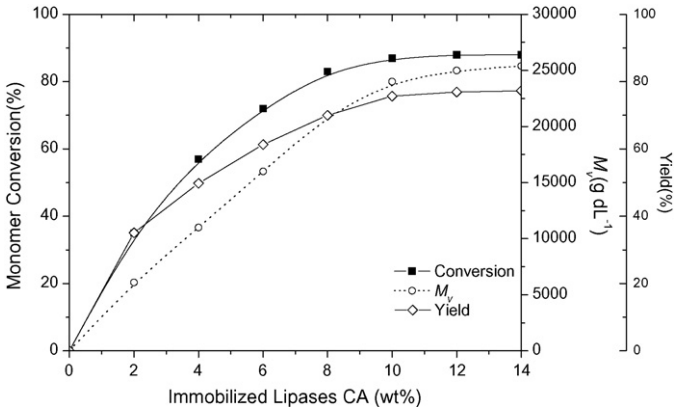

Fig. 1. Effect of the immobilized lipases CA amount on the PDO polymerization. Polymerization: 5.0 g of PDO with immobilized lipases CA at 60 °C for 15 h in bulk.

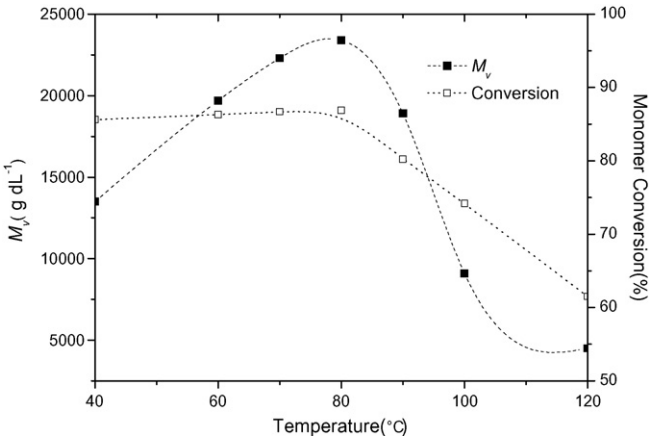

Fig. 2. Effect of the reaction temperature on the PDO polymerization. Polymerization: 5.0 g of PDO with immobilized lipases CA of 10 wt.% for 15 h in bulk.

decreased considerably, which was probably due to activity lowering of lipase CA with reaction temperature rising.

3.4. Effect of water content on polymerization

The activity of enzymes in non-aqueous media depends greatly on the water content of the reaction system. Gutman et al. believed water molecules surrounding the enzyme play an important role in maintaining the enzyme's conformational flexibility [24]. Bisht et al. proposed a mechanism for TMC polymerization with lipase CA in which water may act as a substrate in the initiation process [25]. Huan et al. found that water plays a different role during polymerization, in which the water is consumed at the initial stage and produced at later stage by linear condensation [26]. Matthijs et al. suggested that water dominates the initial initiation process of enzymatic polymerization [27]. Further more, Matthijs et al. found that the method of enzyme drying can have a significant effect on the results of enzymatic ring opening polymerization [28].

In order to investigate the effects of water on the enzymatic polymerization of PDO, the reactions were firstly carried out at 80 °C with water content in monomer in a range of 62.3–441.8 ppm by the addition of water during the beginning of enzymatic polymerization. Since all the immobilized lipases CA (Novozym 435) were dried in vacuo at 25 °C for 1 day before use in our experiments, we only consider the effects of water in monomer on the enzymatic polymerization. As shown in Fig. 3, the monomer conversion increased with increasing of water content in monomer during the initial stages of the reaction. However, the M_V of PPDO maximized with the water content at 152.8 ppm. Huan et al. believed that at low water content, the enzyme conformation was relatively rigid, and that in non-aqueous media, this rigidity decreased the enzyme activity and therefore the chain initiation rate as well [26]. On the other hand, although high monomer conversion was achieved at high water content (indicating an increase in chain initiation rate and thus an increase in the number of propagation chains), excess water also led to the hydrolysis of polymer, resulting in lower

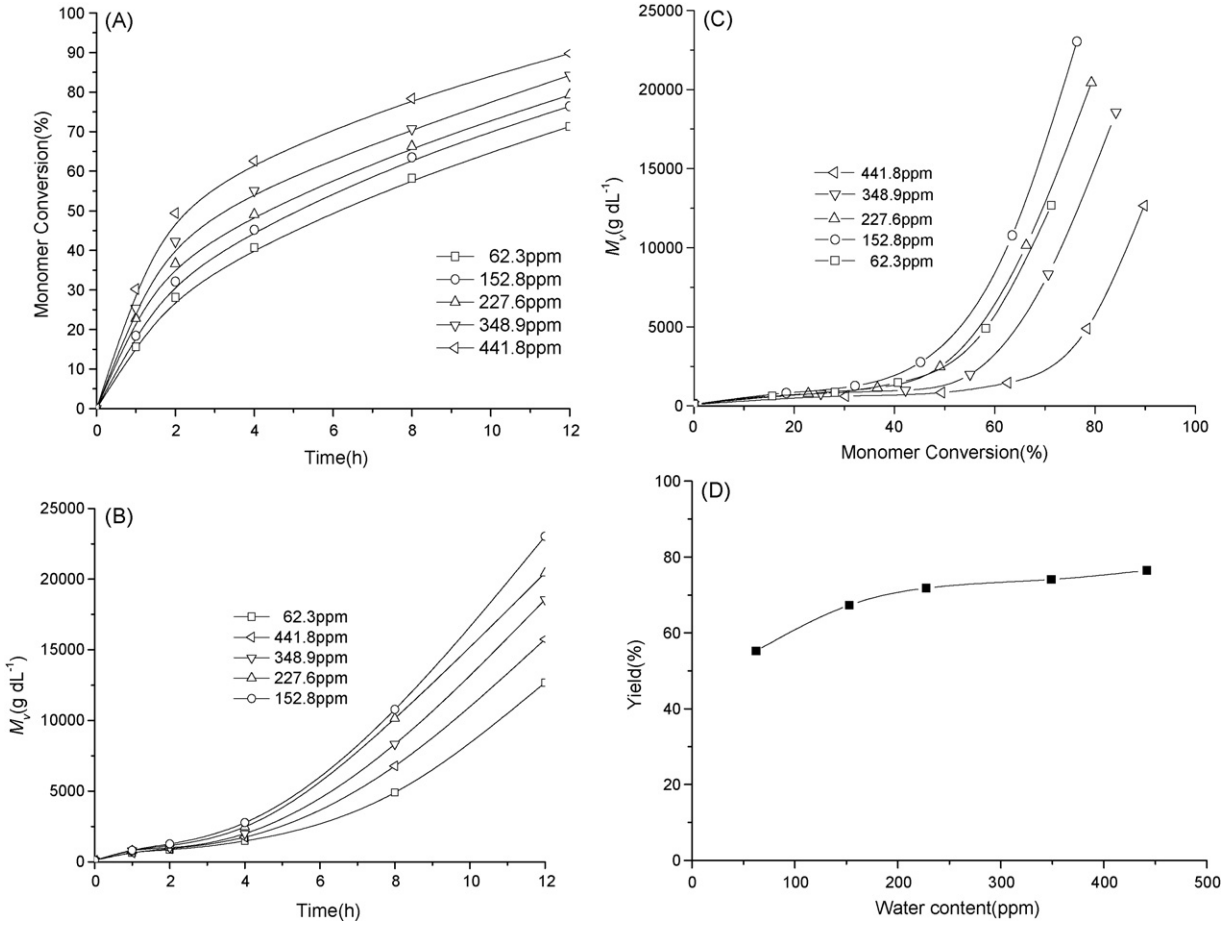

Fig. 3. Effect of water content on the PDO polymerization during the beginning of enzymatic polymerization. Polymerization: 5.0 g of PDO with immobilized lipases CA of 10 wt.% at 80 °C for 12 h in bulk.

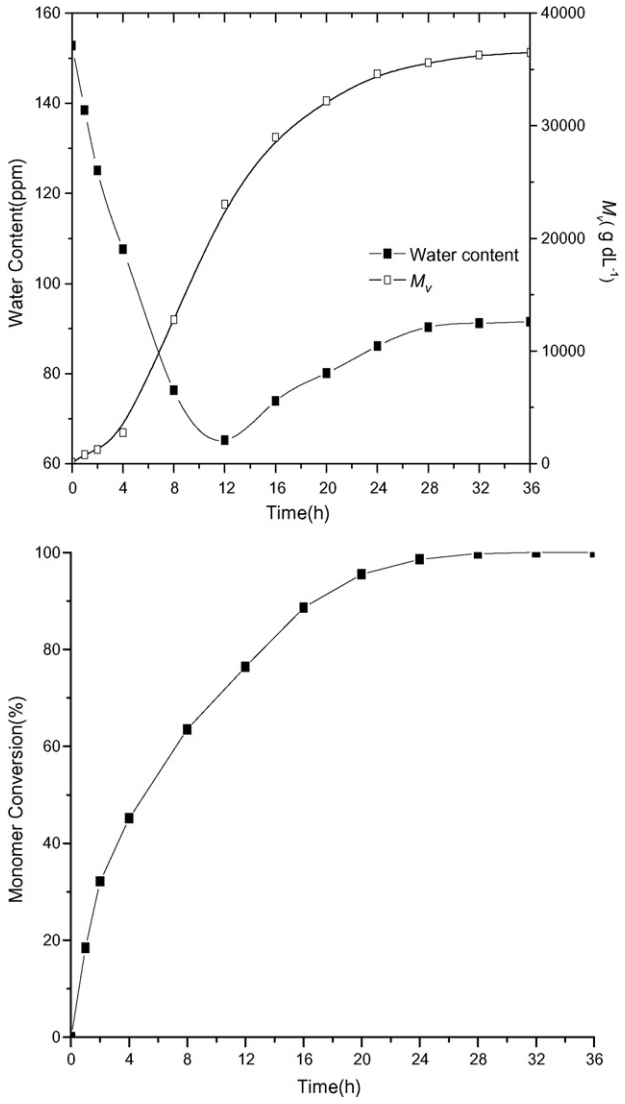

Fig. 4. Variation of water content, molecular weight, and monomer conversion during enzymatic polymerization. Polymerization: 5.0 g of PDO with immobilized lipases CA of 10 wt.% at 80 °C for 12 h in bulk.

molecular weight of polymer. So, the 152.8 ppm system produced higher molecular weight of PPDO than others.

To investigate the effects of water content on the M_v and monomer conversion for the entire enzymatic polymerization process, bulk polymerization of PPDO was performed at 80 °C with water content at 152.8 ppm for the initial reaction. As shown in Fig. 4, M_v increased slowly to 2800 (g L^{-1}) during the first 4 h, and then increased steadily during 4–20 h. The PDO was completely consumed by 20 h. After 20 h, M_v continued to increase, which indicated that linear condensation reactions had become important in chain propagation at this stage. The water content decreased from 152.8 to 62.8 ppm by 12 h, and then increased to 90.3 ppm by 28 h. These results suggested that: at the beginning of polymerization, water molecules participate in the chain initiation. As the reaction time increased, linear condensation had gradually became dominant and water is released into the reaction system.

3.5. Methods for increasing the molecular weight

The molecular weight is one of most important properties of polymers. The higher the molecular weight, the more stability the PPDO polymer exhibits during its thermal processing and storage. Furthermore, the most important result of increased PPDO molecular weight will be improved material mechanical properties. Therefore, obtaining high-molecular weight production is very important for degradable PPDO. In order to increase the molecular weight, some methods must be applied to regulate the water content in the reaction system and overcome the diffusion limitation stemming from the increase of viscosity during polymerization process [11]. In the present study, adding solvent and MS to reaction system was introduced. Both PDO and PPDO are soluble in 1,1,2,2-tetrachloroethane, moreover, the boiling point of 1,1,2,2-tetrachloroethane (146.2 °C) is higher than the polymerization temperature (80 °C). Hence, 1,1,2,2-tetrachloroethane was choose as solvent. According to the preceding experiments, the M_v values of the polymer stop changing after 28 h of bulk polymerization. To increase the molecular weight, 4.0 g of the above reaction mixture and 10.0 mL of dry solvent were mixed, with and without 4 Å MS. Both reaction mixtures were incubated with shaking at 80 °C until M_w of polymer did not increase. As shown in Table 1, adding solvent and MS increased obviously the molecular weight of PPDO as we expected. However, adding the solvent resulted in an even greater increase than adding MS, which indicated that the diffusion limitation may be the major factor determining the increase of molecular weight in bulk polymerization.

Table 1
Method for increasing the molecular weight.

	Bulk[a]	1,1,2,2-Tetrachloroethane	1,1,2,2-Tetrachloroethane + MS
M_v (g dL^{-1})	36,000	55,000	58,000
Yield (%)	91.3	93.5	94.3

[a] Polymerization conducted in bulk until M_v of polymer did not increase.

4. Conclusion

In this article, the enzymatic synthesis of PPDO was performed in more detail. Effects of the loading amount of enzyme, reaction temperature and water content on polymerization were investigated. Both the conversion of monomer and the M_v of PPDO increased with increasing enzyme loading amount, and maximized at 80 °C. At the beginning of polymerization, water molecules act as initiators. As the reaction time increased, linear condensation had gradually became dominant and water is released into the reaction system. Excess water may act as a chain cleavage agent. Furthermore, adding solvent and MS to reaction system was conducted to increase the molecular weight of PPDO, and PPDO with a higher molecular weight (M_v = 58,000) was obtained.

Acknowledgements

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References

[1] Ke-Ke Yang, Xiu-Li Wang, Wang. Yu-Zhong, J. Macromol. Sci. C43 (2002) 373–398.
[2] A. Gertzman, D.R. Thompson. U.S. Patent 4,591,630 (1986).
[3] V.V. Cachia, M.S. Juan, Bone Fixation Device. U.S. Patent 5,893,850 (1999).
[4] F.V. Mattei, N. Doddi, Synthetic Absorbable Hemostatic Composition. U.S. Patent 4,440,789 (1984).
[5] S.R. Bhattaria, N. Bhattaria, H.K. Yic, P.H. Hwangc, D.I. Chad, H.Y. Kim, Biomaterials 25 (2004) 2595–2602.
[6] S.J. Peter, M.J. Miller, A.W. Yasko, M.J. Yaszemski, A.G. Mikos, J. Biomed. Mater. Res. 43 (1998) 422–447.
[7] J.A. Hubbel, Biotechnology 13 (1995) 565–576.

[8] A. Atala, D.J. Mooney, J.P. Vacanti, R. Langer (Eds.), Synthetic Biodegradable Polymer Scaffolds, Birkh.auser, Boston, 1997.
[9] T.C. Forschner, US Patent 5,717,059 (1998).
[10] J.M. Raquez, P. Degee, R. Narayan, P. Dubois, Macromol. Rapid Commun. 21 (2000) 1063–1071.
[11] Y.H. Guo, Y.Z. Wang, K.K. Yang, X.L. Wang, Q. Zhou, Chem. J. Chin. Univ. 25 (2004) 1565–1767.
[12] H.R. Kricheldorf, D.O. Damrau, Macromol. Chem. Phys. 199 (1998) 1089–1097.
[13] K.K. Yang, H.X. Huang, X.L. Wang, Y.Z. Wang, Chem. Res. Appl. 3 (2004) 409–410.
[14] H. Nishida, M. Yamashita, M. Nagashima, T. Endo, Y. Tokiwa, J. Polym. Sci. A 38 (2000) 1560–1567.
[15] S. Namekawa, S. Suda, H. Uyama, S. Kobayashi, Int. J. Bio. Macromol. 25 (1999) 145–151.
[16] R. Marcilla, M. de Geus, D. Mecerreyes, C.J. Duxbury, C.E. Koning, A. Heise, Eur. Poly. J. 42 (2006) 1215–1221.
[17] F. Deng, R.A. Gross, Int. Bio. Macromol. 25 (1999) 153–159.
[18] S. Habauea, M. Asai, M. Morita, Y. Okamoto, H. Uyama, S. Kobayashi, Polymer 44 (2003) 5195–5200.
[19] S. Kobayashi, H. Uyama, S. Kimura, Adv. Polym. Sci. 194 (2006) 95.
[20] S. Matsumara, Chem. Rev. 101 (2001) 3793.
[21] H. Uyama, S. Kobayashi, J. Mol. Catal. B: Enzymol. 19–20 (2002) 117–127.
[22] M.A. Sabino, J.L. Feijoo, A. Müller, J. Macromol. Chem. Phys. J. 201 (2000) 2687.
[23] K.K. Yang, H.X. Huang, X.L. Wang, WangF Y.Z., Chem. Res. Appl. 3 (2004) 409–410.
[24] A.L. Gutman, K. Zuobi, T. Bravdo, J. Org. Chem. 55 (1990) 3546–3552.
[25] K.S. Bisht, L.A. Henderson, R.A. Gross, D.L. Kaplan, G. Swift, Macromolecules 30 (1997) 2705–2711.
[26] H. Dong, S.-G. Cao, Z.-Q. Li, S.-P. Han, D.-L. You, J.-C. Shen, J. Polym. Sci. A 37 (1999) 1265–1275.
[27] M. de Geus, R. Peters, C.E. Koning, A. Heise, Biomacromolecules 9 (2008) 752.
[28] M. de Geus, J. Peeters, M. Wolffs, T. Hermans, A.R.A. Palmans, C.E. Koning, A. Heise, Macromolecules 38 (2005) 4220.