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Accelerated biodegradation of poly(vinyl alcohol) by a glycosidation of the hydroxyl groups

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Abstract

Biodegradability of *N*-acetyl-D-glucosamine (GlcNAc)-substituted poly(vinyl alcohol) (PVA) (**1**) in a soil suspension (pH 6.5) was investigated at 25°C for 40 days. Biochemical oxygen demand (BOD) of **1** with degree of substitution of 0.2–0.3 (DP = 430–480) was higher than that of PVA under the degradation condition. Size exclusion chromatography (SEC), ^{1}H NMR, and FT-IR measurements of the recovered sample indicated that biodegradation of PVA main chain was accelerated by partial glycosidation of hydroxyl groups in PVA.

Keywords: Poly(vinyl alcohol); Glycosidation; Accelerated biodegradation

1. Introduction

Plastic waste increases at a rate of several tons per year and the time required for their biodegradation is often unknown. Poly(vinyl alcohol) (PVA) has multiple applications because it is water soluble and is an excellent barrier to scents, flavors, oils and fat [1]. Although PVA is the only carbon–carbon backbone polymer that is biodegradable under both aerobic [2–6] and anaerobic [7,8] conditions, the degradation rate under natural environmental conditions is too slow to apply as an industrial degradable polymer [8]. However approximately 800,000 tons of PVA were produced in the world in 1995 [9]. Exploring new chemical modification as well as building up miscible blends of PVA with other biodegradable polymers are the two most interesting approaches to improve the poor biodegradability of PVA. Although PVA/poly(3-hydroxybutylate) [10], PVA/chitin derivatives [11], and PVA/deoxyribonucleic acid [12] have been reported as the examples for the latter, only a few study on the former subject have been published until now [13].

Recently, we succeeded in a new chemical modification of hydroxyl groups of PVA by a glycosidation reaction and the PVA derivative with pendant sugar residue exhibited different solubilities and thermal properties from PVA [14]. Tokiwa et al. reported biodegradation of a sugar branched polymer consisting of glucose, fatty acid as a spacer arm, and PVA as the main chain, which was synthesized by radical polymerization of 6-*O*-vinyladipoyl-D-glucose [15,16]. They reported that the polymer with high molecular weight (DP > 100) scarcely degraded [16]. Acceleration of biodegradation of high molecular weight PVA is important from environmental viewpoints. In this paper, we propose a new concept of acceleration of biodegradability of PVA by introducing sugar residues directly into the hydroxyl groups in PVA, i.e., partially glycosidation.

2. Experimental

2.1. Materials

Poly(vinyl alcohol) (PVA) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). ^{1}H NMR spectrum of the PVA in dimethyl sulfoxide (DMSO-d$_6$) indicated that the saponification value was determined to be 97% and the PVA had 27% syndiotactic, 49% heterotactic, and 24% isotactic triads [17]. Number average molecular weight (M_n) and polydispersity index (M_w/M_n) of the PVA were 1.48×10^4 and 2.2_1, respectively. Mercury (II) cyanide was obtained from Wako Pure Chemicals (Osaka, Japan). 2-Propanol, benzene, and chloroform (CHCl$_3$) were purified by distillation.

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2.2. Biodegradation test

Biochemical oxygen demand (BOD) was determined by oxygen consumption with BOD tester (Model 200F, TAITEC Koshigaya-shi, Japan), basically according to the ISO standard guidelines (ISO 14851) at 25°C. The soil was obtained from the Nagoya University Farm. A soil suspension was prepared by stirring of the soil in water (150 g/l) at 25°C for 30 min. Conditioned soil suspensions were prepared in a similar way expect for addition of GlcNAc-substituted PVA **1b** or a mixture of PVA and GlcNAc (47/53, wt/wt) and successive incubation at 25°C for 12 h. The supernatants (15 ml) were added to incubation media (150 ml). The incubation medium contained the following (mg/l): K_2HPO_4, 217; KH_2PO_4, 85; Na_2HPO_4, 447; NH_4Cl, 5; $CaCl_2$, 27; $MgSO_4 \cdot 7H_2O$, 23; and $FeCl_3 \cdot 6H_2O$, 0.25. The concentration of polymers in the media was 100 mg/l. After the test, the suspension was filtered and the filtrate was evaporated and dialyzed against distilled water using a dialyzing tube (Seamless Cellulose Tubing, VT351, M. W. Cut off 3,500, Nacalai Tesque, Inc.) for 12 h. The solution was lyophilized to give polymeric material, which was purified by reprecipitation from water to methanol. The recovered sample was analyzed by SEC, IR, and ^1H NMR measurements.

*2.3. Preparation of Isopropyl 2-Acetamido-β-D-glucopyranoside (**2**)*

In a 100 ml of three-necked flask, 2-acetamido-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride 2.0 g (5.5 mmol) and $Hg(CN)_2$ 2.0 g (7.9 mmol) were dissolved in dry benzene (20 ml) under nitrogen atmosphere. To the solution, 0.5 ml (6.5 mmol) of 2-propanol was poured and the mixture was placed in an oil bath at 80°C for 20 h. The reaction mixture was filtered and the filtrate was evaporated. The powder obtained was dissolved in $CHCl_3$, and washed with 30% KI aqueous solution. The organic layer was condensed and purified by recrystallization in ethanol to give isopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (0.48 g, 22% yield). The acetylated GlcNAc derivative, 300 mg (0.78 mmol), was dissolved in methanol (3.0 ml) and then 12 mg (0.22 mmol) of NaOMe was added into the solution at 27°C. After the deacetylation proceeded for 3 h, the solution was made neutral with Dowex 50WX-8-400, and the suspension was filtered. The filtrate was evaporated to give isopropyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**2**, 158 mg, 80% yield). ^1H NMR (400 MHz, D_2O, δ ppm): 1.13 (d, 3H, $J = 6.0$ Hz, $CHCH_3$), 1.20 (d, 3H, $J = 6.0$ Hz, $CHCH_3$), 2.04 (s, 3H, $NHCOCH_3$), 3.44 (br, 2H, H-4 and H-5), 3.55 (t, 1H, $J = 8.8$ Hz, H-3), 3.63 (t, 1H, $J = 8.0$ Hz, H-2), 3.74 (br d, 1H, $J = 9.6$ Hz, H-6a), 3.92 (br d, 1H, $J = 12.4$ Hz, H-6b), 4.02 (m, 1H, $CHCH_3$), 4.54 (d, 1H, $J = 8.4$ Hz, H-1β), ^{13}C NMR (100 MHz, D_2O, δ ppm): 23.8 ($NHCOCH_3$), 24.6 and 24.9 ($CHCH_3$), 58.5 (C-2), 63.2 (C-6), 72.6 (C-4), 76.2 and 76.5 (C-3 and $CHCH_3$), 78.4 (C-5), 102.2 (C-1β), 177.1 ($NHCOCH_3$). IR (KBr disk, cm^{-1}): 3285 (ν_{O-H}), 2969 (ν_{C-H}), 1655 ($\nu_{C=O}$), 1553 (δ_{N-H}), 1431 (δ_{C-H}), 1130, 1081, 1029 (ν_{C-O}).

2.4. Measurements

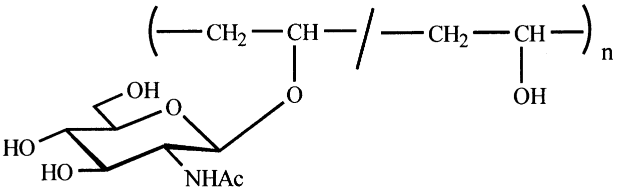
FT-IR spectra were recorded in KBr using a JASCO FT/IR-430 spectrometer and 100% KBr disk was used as blank. ^1H NMR spectra were taken on JEOL JNM-GX400 (400 MHz for ^1H) at room temperature. M_n and M_w/M_n of the polymers were determined by SEC calibrated with poly(ethylene oxide) standards using a system of JASCO Model PU-980 with both JASCO 830-RI and UV-970 detectors and Shodex B-804 + B-805 columns [eluent: 0.05 M K_2HPO_4 aq., temperature: 27°C].

3. Results and discussion

*3.1. Biodegradation of GlcNAc-substituted PVA **1** in soil suspension*

According to our preceding paper [14], GlcNAc-substituted PVA **1** was synthesized by a reaction of hydroxyl groups with sugar oxazoline derivatives, readily prepared by trimethylsilyl trifluoromethanesulfonate method [18] from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose and subsequent deacetylation by Zemplen method [19]. The structure of **1** used in this study is shown in Fig. 1. Biodegradation in soil suspension (pH 6.5, 25°C) was monitored by BOD and the constituents were calculated from BOD values and theoretical oxygen demand (TOD) value. In the BOD measurement, GlcNAc-substituted PVA **1a** with degree of substitution ($[Sugar]/[-OH]_0$) of 0.31 ($M_n = 4.60 \times 10^4$, DP = 427, $M_w/M_n = 2.5_3$) was degraded by 18% of BOD/TOD in 40 days, while PVA was hardly degraded (2%). In **1a**, the proportion of PVA main chain is 28 wt%.

SEC measurement with differential refractive index (RI) detector was carried out in order to confirm the cleavage of the PVA main chain. The SEC chromatogram of PVA after



GlcNAc-substituted PVA 1

1a: $[Sugar]/[-OH]_0 = 0.31$, $M_n = 4.60 \times 10^4$

1b: $[Sugar]/[-OH]_0 = 0.24$, $M_n = 4.53 \times 10^4$

1c: $[Sugar]/[-OH]_0 = 0.21$, $M_n = 4.18 \times 10^4$

Fig. 1. Structure of GlcNAc-substituted PVA **1** used in this study.

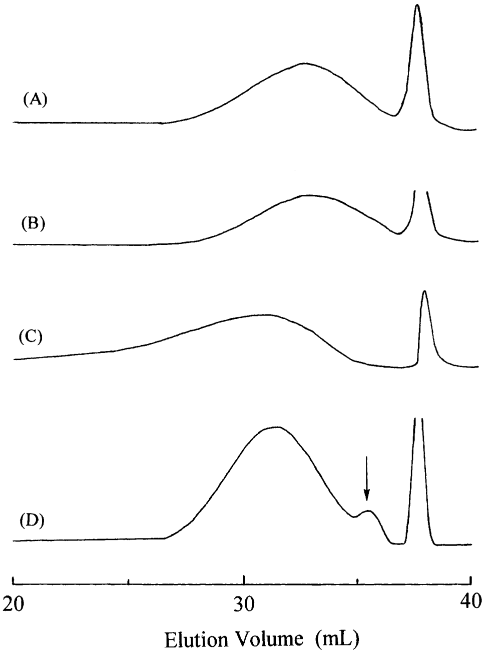


Fig. 2. SEC traces of (A) PVA, (B) PVA after biodegradation test for 40 days in soil suspension, (C) GlcNAc-substituted PVA **1a**, and (D) GlcNAc-substituted PVA **1a** after biodegradation test for 40 days in soil suspension detected by differential refractive index (RI). Flow rate, 1.0 ml/min; temp., 27°C.

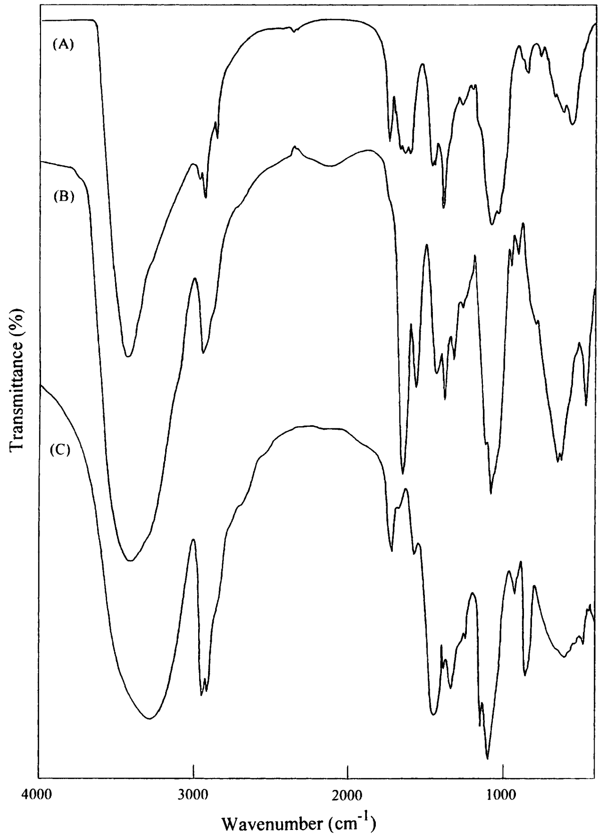


Fig. 3. FT-IR spectra of (A) isolated intermediate molecular compound (B) GlcNAc-substituted PVA **1a** (C) PVA (KBr disk).

40 days of the degradation test did not show an important change, compared with PVA before the test (Fig. 2A and B). On the other hand, there was an important change for the RI detected chromatogram of the GlcNAc-substituted PVA **1a** (Fig. 2C and D). After the degradation test, the SEC curve shifted to low molecular weight ($M_n = 1.78 \times 10^4$, $M_w/M_n = 2.4_5$) and became bimodal accompanied with a clear peak at molecular weight of 0.3×10^4–0.4×10^4 (arrow mark in Fig. 2D). Interestingly, formation of the intermediate molecular weight polymer between those of the starting polymer and low molecular compounds corresponding to monomer unit was confirmed. The clear peak ascribed to intermediate molecular compounds ($M_n = 0.3 \times 10^4$–0.4×10^4) indicated that PVA main chain scission as well as deglycosidation occurred because the recovered sample had the [Sugar]/[–OH]$_0$ value of 0.16 even after the biodegradation test.

Isolation of the intermediate molecular compound was carried out by fraction with stepwise dialyses. The recovered sample, which was purified by dialysis with a cellulose tubing (cut off M.W. 3500, see Section 2), was dissolved in water, and dialyzed with a seamless cellulose tubing (cut off M.W. 12,000–14,000, UC36-32-100, Viskase Sales) against water. Both the outside and inside aqueous solutions of the tubing were lyophilized to give white powders, respectively. In the SEC measurement, M_n of the former was 0.33×10^4 ($M_w/M_n = 1.3_6$) and that of the latter was 2.72×10^4 ($M_w/M_n = 2.01$), indicating that the fraction was completed. IR spectrum of the former was similar to PVA [3418 cm^{-1} (ν_{O-H}), 2922 cm^{-1} (ν_{C-H}), 1075 cm^{-1} ν_{C-O})] except for absorbencies at 1715 and 1636, 1597 cm^{-1}, which were identified as those of ketone and β-diketone structures, respectively (Fig. 3A). In the ^{1}H NMR spectrum, resonances at 1.38–1.78 ppm was assigned to [–CH_2CH(OH)–]$_n$ and peaks 3.88–4.22 ppm was assigned to [–CH$_2$CH(OH)–]$_n$ were observed. The results indicated that PVA main chain of the intermediate molecular compound was partially oxidized. From these results, main chain scission of GlcNAc-substituted PVA **1** by hydrolysis [20,21] or reverse aldol reaction [22] followed by oxidation was suggested.

In the cases of GlcNAc-substituted PVA **1b** with [Sugar]/[–OH]$_0$ of 0.24 ($M_n = 4.53 \times 10^4$, DP = 484, $M_w/M_n = 2.7_6$) and **1c** with [Sugar]/[–OH]$_0$ of 0.21 ($M_n = 4.18 \times 10^4$, DP = 479, $M_w/M_n = 2.6_6$), the results similar to those of **1a** were obtained. The results are summarized in Table 1. In **1b** and **1c**, the proportions of PVA main chain are 47 and 50 wt%, respectively. Slow degradations of GlcNAc-substituted PVA **1b** and **1c** were observed within 40 days (17 and 16%, respectively). The molecular weights decreased to 2.09×10^4 and 2.04×10^4 with accompanying decrease of [Sugar]/[–OH]$_0$ value (from 0.24 to 0.12 and from 0.21 to 0.08, respectively) and a distinct shoulder ascribed to the oligomer with at molecular weight of 0.3×10^4–0.4×10^4 was observed in the SEC measurement

Table 1
Characterization of GlcNAc-subsituted PVA **1** before and after biodegradation test in soil suspension. (Polymer concentration, 100 mg/l; pH 6.5, 25°C for 40 days)

Sample	Before degradation			After degradation			
	[Sugar]/[−OH]$_0$[a]	M_n[b] × 10^{-4}	M_w/M_n[b]	[Sugar]/[−OH]$_0$[a]	M_n[b] × 10^{-4}	M_w/M_n[b]	BOD/TOD (%)
PVA	0	1.48	2.2_1	0	1.21	2.5_6	2
1a	0.31	4.60	2.5_3	0.16	1.78	2.4_5	18
1b	0.24	4.53	2.7_6	0.12	2.09	2.5_7	17
1c	0.21	4.18	2.6_6	0.08	2.04	2.3_0	16

[a] Degree of substitution, determined by ^{1}H NMR in D_2O.
[b] Determined by SEC in 0.05 M K_2HPO_4 aq. solution using poly(ethylene oxide) standards.

by differential RI (Fig. 4B and C, left side). Ultraviolet absorption ($\lambda = 220$ nm) indicated the existence of the intermediate molecular weight compound more clearly (Fig. 4, right side). In the SEC curve, intense peaks were observed at the same elution volumes as those of the shoulders in the differential RI chromatograms. As shown in Fig. 4, the relative peak intensities of the oligomer to the polymers (**1b** and **1c**) were lower than that of **1a** after the test. In the blank test of **1c** ([Sugar]/[−OH]$_0$ = 0.21, $M_n = 4.18 \times 10^4$) in phosphate buffer (pH 6.5) at 25°C for 40 days, neither apparent decreases of [Sugar]/[−OH]$_0$ nor M_n values were confirmed ([Sugar]/[−OH]$_0$ = 0.18, $M_n = 4.00 \times 10^4$). From the results, it seems that biodegradation of PVA main chain is accelerated by the glycosidation of the hydroxyl groups, in which the degradation rate depends on the degree of substitution ([Sugar]/[−OH]$_0$).

*3.2. Biodegradability of PVA in a conditioned soil suspension incubated with GlcNAc-substituted PVA **1***

In order to demonstrate PVA main chain scission more clearly, biodegradation test of non-modified PVA (97% saponification, $M_n = 1.48 \times 10^4$, $M_w/M_n = 2.2_1$) was carried out using a conditioned soil suspension prepared with incubation with GlcNAc-substituted **1b** previously (pH 6.5, 25°C). After 40 days, the BOD/TOD value was 10%, while the value was 2% in the original (non conditioned) soil suspension. In the SEC measurement, M_n after the test for 40 days ($M_n = 0.44 \times 10^4$, $M_w/M_n = 1.5_3$) was much lower than that of original PVA indicating that PVA main chain scission occurred in the conditioned soil, although no remarkable decrease of M_n was observed in the test using non conditioned soil ($M_n = 1.11 \times 10^4$, $M_w/M_n = 2.3_3$). From these data, biodegradation of PVA was accelerated in the conditioned soil suspension, showing that the main carbon chain of PVA was assimilated by microbes enriched in the conditioned soil. Although non-modified PVA was also degraded in a conditioned soil activated with a mixture of PVA and GlcNAc (47/53, wt/wt), the M_n of PVA after the test was higher (0.75×10^4, $M_w/M_n = 2.3_1$) than that of PVA in the conditioned soil incubated with GlcNAc-substituted PVA **1b**. The

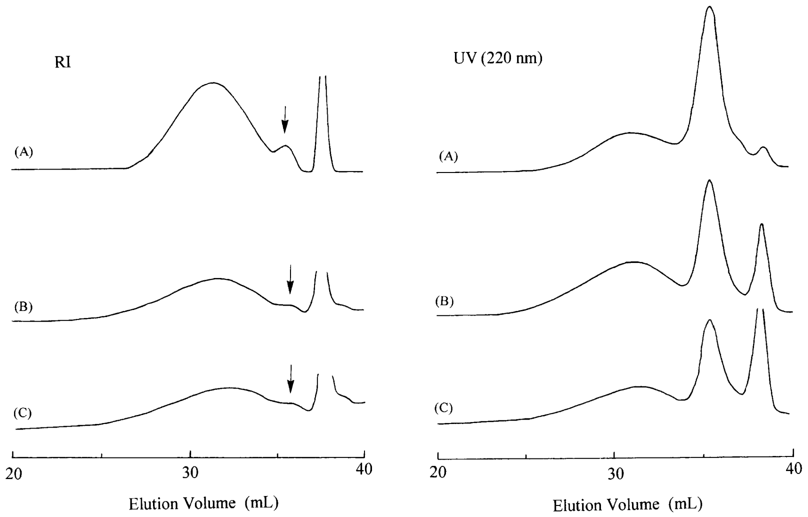
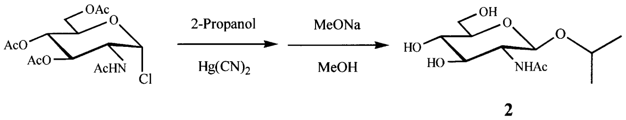


Fig. 4. SEC traces of GlcNAc-substituted PVA **1** after biodegradation test for 40 days in soil suspension detected by differential RI (left side) and detected by UV absorption at 220 nm (right side). Flow rate, 1.0 ml/min; temp., 27°C. (A) **1a** ([Sugar]/[−OH]$_0$ = 0.31), (B) **1b** ([Sugar]/[−OH]$_0$ = 0.24), (C) **1c** ([Sugar]/[−OH]$_0$ = 0.21).



Scheme 1.

results indicated that some GlcNAc-substituted PVA **1**-degrading microbes have a catalytic degrading activity for main chain of PVA apparently and suggested that the accelerated main chain scission of **1** was ascribed to a recognition of pendant sugar residue in the assimilating microbes. The enzyme obtained from cultivation of the soil suspension using GlcNAc-substituted PVA **1** as the screening substrate will be analyzed and the degradation mechanism will be discussed in forthcoming full paper.

3.3. Biodegradability of model compound **2**

Isopropyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**2**) was prepared as a model compound for the GlcNAc-substituted PVA **1**. As shown in Scheme 1, **2** was synthesized by the Koenigs–Knorr reaction [23] of 2-propanol and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride and successive deacetylation (see Section 2). In the biodegradation test using the soil suspension, model compound **2** (BOD/TOD = 41%) as well as GlcNAc (BOD/TOD = 46%) was easily degraded in 20 days, compared with GlcNAc-substituted PVA **1**. The result indicated that low molecular weight model compound **2** can be catabolized by microorganisms in the soil suspension irrespective of the derivatization at the anomeric carbon (hemiacetal to acetal).

4. Conclusions

On the basis of these data, we demonstrate an acceleration of biodegradation of poly(vinyl alcohol) by a partially glycosidation of the hydroxyl groups and the glycosidation was effective for controlling the biodegradation of PVA main chain. Recognition of pendant sugar residues bonded to PVA main chain was supposed to plays an important role in the degradation mechanism.

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References

[1] Fritz HG. A study on production of thermoplastics and fibers based mainly on biological materials. Stuttgart: Institut fur Kunst, 1994. p. 184.
[2] Suzuki T, Ichihara Y, Yamada M, Tonomura K. Agric Biol Chem 1973;37:747.
[3] Watanabe Y, Morita M, Hamada N, Tsujisaka Y. Agric Biol Chem 1975;39:2447.
[4] Sakazawa C, Shimao M, Taniguchi Y, Kato N. Appl Environ Microbiol A-1 1981;41:261.
[5] Matsumura S, Maeda S, Takahashi J, Yoshikawa S. Kobunshi Ronbunshu 1988;45:317.
[6] Mori T, Sakimoto M, Kagi T, Sakai T. Biosci Biotechnol Biochem 1996;60:330.
[7] Matsumura S, Kurita H, Shimokobe H. Biotechnol Lett 1993;15:749.
[8] Gartiser S, Wallrabestein M, Stiene G. J Environ Polym Degrad 1999;6:159.
[9] Moritani T, Kajitani K. Polymer 1997;38:2933.
[10] Ikejima T, Cao A, Yoshie N, Inoue Y. Polym Degrad Stab 1998; 62:463.
[11] Takasu A, Aoi K, Tsuchiya M, Okada M. J Appl Polym Sci 1999; 73:1171.
[12] Takasu A, Aoi K, Okada M. Polymer 2000;41:2847.
[13] Huang SJ, Wang IF, Quingam E. Polym Sci Technol 1983;21:75.
[14] Takasu A, Niwa T, Itou H, Inai Y, Hirabayashi T. Macromol Rapid Commun 2000;21:764.
[15] Kitagawa M, Takegami S, Tokiwa Y. Macromol Rapid Commun 1998;19:155.
[16] Tokiwa Y, Hong F, Sumida Y, Hiraguri Y, Kurane R, Kitagawa M, Shibatani S, Maekawa Y. Macromolecules 2000;33:1636.
[17] Moritani T, Kimura I, Shibatani K, Fujiwara Y. Macromolecules 1972;5:577.
[18] Nakabayashi S, Warren CD, Jeanloz RW. Carbohydr Res 1986; 150:C7.
[19] Lee RT, Lee YC. Carbohydr Res 1974;37:193.
[20] Sakai K, Hamada N, Watanabe Y. Agric Bio Chem 1985;49:827.
[21] Shimao M, Ninomiya K, Kuno O, Kato N, Sakazawa C. Appl Environ Microbiol 1986;51:268.
[22] Matsumura S, Tomizawa N, Toki A, Nishikawa K, Toshima K. Macromolecules 1999;32:7753.
[23] Koenigs W, Knorr E. Ber 1901;34:957.