The Enzymatic Degradation of Commercial Biodegradable Polymers by Some Lipases and Chemical Degradation of Them

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Summary: Biodegradable polyesters, poly(butylene succinate adipate) (PBSA), poly(butylene succinate) (PBS), poly(ethylene succinate) (PES), poly(butylene succinate)/poly(caprolactone) blend (HB02B) and poly(butylene adipate terephthalate) (PBAT), were evaluated about degradability for enzymatic degradation by lipases and chemical degradation in sodium hydroxide solution. In enzymatic degradation, PBSA was the most degradable by lipase PS, on the other hand, PBAT containing aromatic ring was little degraded by eleven kinds of lipases. In 1N NaOH solution, degradation rate of PES with ethylene unit was extremely fast, in comparison with other polyesters. Interestingly the degradation rate of PBSA in enzymatic degradation by lipase PS was faster than in chemical degradation.

Keywords: biodegradable; chemical degradation; enzymatic degradation; lipase; polyesters

Introduction

Polymers universally used such as PE, PP and PS have caused serious environmental pollution for they are little degraded in the environment. To solve this problem, biodegradable polymers degraded in soil, compost, ponds and the sea have been developed and various commercial biodegradable polymers have been produced. Also, the search for microorganisms biodegrading biodegradable polymers such as polyester, copolyester, copolyesteramide, copolyesterether and copolyestercarbonate [1, 2] and the study of high efficient compost systems for biodegradable polymers [3] have been advanced by a large number of researchers. It has been reported that poly(butylene succinate adipate) (PBSA) and poly(butylene succinate) (PBS) were degraded in activated sludge, soil and compost. [4, 5] In addition, it has been proved that PBS was degraded by

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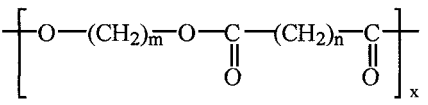
lipase AK at a relatively high temperature of 70 °C, [6] and was hydrolyzed in 1N NaOH solution. [7] In this work, PBSA, PBS, poly(ethylene succinate) (PES), poly(butylene succinate)/poly(caprolactone) blend (HB02B) and poly(butylene adipate terephthalate) (PBAT) were evaluated for enzymatic degradation by eleven kinds of lipases and chemical degradation in sodium hydroxide solution.

Experiment

Materials

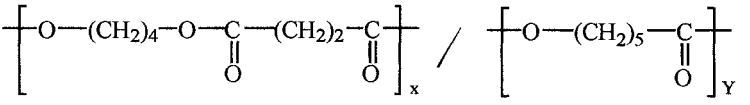
In this work, poly(butylene succinate adipate) (PBSA), poly(butylene succinate) (PBS) (SHOWA HIGHPOLYMER CO.,LTD.), poly(ethylene succinate) (PES) (NIPPON SHOKUBAI CO.,LTD.), poly(butylene succinate)/poly(caprolactone) blend (HB02B) (DAICEL CHEMICAL INDUSTRIES, LTD.) and poly(butylene adipate terephthalate) (PBAT) (BASF) were used in enzymatic and chemical degradation tests. PES films were prepared by solvent-casting method and other polyester films were prepared by thermal moulding.

Aliphatic polyesters: (PBSA, PBS, PES)



PBSA: m = 4, n = 2, 4
PBS: m = 4, n = 2
PES: m = n = 2

Polyester blend (PBS/PCL): (HB02B)



Aromatic polyester: (PBAT)

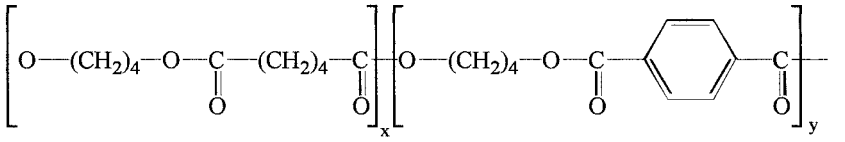


Fig. 1. Structures of Biodegradable Polyesters.

Lipase A from *Aspergillus niger*, Lipase AK from *Pseudomonas* sp., Lipase AY from *Candida rugosa*, Lipase D from *Rhizopus delemar*, Lipase F from *Rhizopus* sp., Lipase M from *Mucor javanicus*, Lipase N from *Rhizopus niveus* and Lipase PS from *Pseudomonas* sp. were gifted from AMANO PHARMACEUTICAL CO., LTD. and Lipase from *C. cylindracea* (LPC) and Lipase from *Rhizopus arrizus* (LPRa) were purchased from SIGMA. Lipase from *Rhizopus* sp. (LPR) was purchased from KATAYAMA CHEMICAL, INC.

Measurement

The components of PBSA, PBS, PES, HB02B and PBAT were determined by NMR spectroscopy (JEOL Model JNM-ECP 400). ^{1}H-NMR chemical shifts were calibrated by chloroform (δ 7.27 ppm) in chloroform-*d*. The number average molecular weights and the weight average molecular weights were determined by a size exclsion chromatography (SEC) with a HITACHI chromatograph system (L-6200 Intelligent Pump, L-3350 RI Monitor, L-5025 Column Oven and D-2520 GPC Integrator) equipped with Shodex K-804 and K-802.5 columns using chloroform as eluent at 40 °C. The flow rate was 1.0 mL min^{-1}. The molecular weights were determined by the universal curve plotted with standard polystyrene. The glass transition temperature (T_g) and the melting temperature (T_m) were measured by a differential scanning calorimeter (DSC) (RIGAKU Model DSC-8230). The polymer samples were scanned from –100 to 200 °C at a heating rate of 10 °C min^{-1} under a nitrogen. T_m and the heat of fusion (ΔH_m) were determined in the first heating, whereas T_g was determined in the second heating. Topological changes in the polymer surface were measured by a scanning electron microscope (SEM) (JEOL Model JSM-5200) after the films were coated with Au coating by an ion coater (SHIMADZU Model IC-50).

Enzymatic Degradation

The enzymatic degradation tests of polyesters by eleven kinds of lipases was carried out in phosphate buffer (pH 7) or citrate buffer (pH 4) at 37 °C. The enzyme concentration in this degradation test was 5 U/mg polymer. The degradation tests were also conducted by the exposure

of the polymer samples to lipases in buffer solution. The degradability of polyester was determined by the weight loss after the recovery of the samples at intervals. The polymer films were prepared by the solvent-casting method. The thickness of the films was 0.1 mm and the weight was ca. 30 mg. The films for enzymatic degradation were packaged in polyethylene net (mesh size ca. 1 mm•ca. 1 mm). The buffer solution containing lipase was replaced every 40 h so that the enzyme activities were maintained at a desired level throughout the experiment. A 50 ml bottle containing a polymer sample, a lipase and buffer solution was warmed to 37 °C by shaking. The samples were pulled out of the bottle every 20 h, washed with deionized water, and then dried to a constant weight *in vacuo* before they were weighed. The molecular weight, composition, crystallinity and surface morphology of the polyesters before and after degradation by lipases were determined by SEC, NMR, DSC and SEM observation, respectively.

Chemical Degradation

The chemical degradation tests were conducted in various concentrations of sodium hydroxide at 37 °C. The chemical degradation were carried out like the above enzymatic degradation. The sodium hydroxide solution was also replaced every 40 h. The polymer samples were put into sodium hydroxide solution preheated to 37 °C and then were shaken. The samples were pulled out of the bottles every 20 h, washed with deionized water, and then dried to a constant weight *in vacuo* before they were weighed. The molecular weight of the polyesters and their composition before and after chemical degradation were determined by SEC and NMR.

Results and Discussion

Properties of Polyesters

The properties of the biodegradable polyesters used in enzymatic degradation by lipases and chemical degradation are shown in Table 1. The composition of PBSA was at the molar ratio of 5: 4: 1 for butylene, succinate and adipate. The molecular weight of PBSA was $6.4 \cdot 10^4$ and its molecular weight distribution was 1.72. T_m and ΔH_m determined by first heating of DSC

measurement were 91.0 °C and 70.3 J/g, and the existence of ΔH_m showed that the polymer was crystalline. T_g was – 42.2 °C from second heating of DSC measurement.

^{1}H-NMR spectrum of PBSA showed that it is an alternating copolymer of butylene and succinate. The molecular weight of PBS was $13.2 \cdot 10^4$ (higher than that of PBSA). Thermal properties of PBS indicated that T_m was 111.7 °C, ΔH_m was 79.0 J/g and T_g was – 34.0 °C.

Table 1. Some properties of commercial biodegradable polyester.

Polyester	Constituent	Component [a] mol %	M_n [b] $\cdot 10^{-4}$	M_w/M_n [b]	T_m [c] °C	ΔH_m [c] J/g	T_g [c] °C
PBSA	But/Suc/Adi	50/40/10	6.4	1.72	91.0	70.3	- 42.2
PBS	But/Suc	50/50	13.2	1.68	111.7	79.0	- 30.4
PES	Eth/Suc	50/50	9.4	3.40	94.6	69.9	- 6.8
HB02B	PCL/PBS blend	38/62 (blend)	8.6	1.95	63.3, 112.3	32.4, 50.2	- 27.8
PBAT	But/Adi/Ter	50/26/24	4.4	2.33	53.3, 117.7	33.8	- 31.8

[a] Determined by ^{1}H-NMR. [b] Determined by SEC. [c] Determined by DSC.

It was also proved that PES is an alternating copolymer of ethylene and succinate like PBS. The molecular weight of PES was $9.4 \cdot 10^4$. Its molecular weight distribution (3.40) was wider than that of each biodegradable polyester. Although T_m (94.6 °C) and ΔH_m (69.9 J/g) were almost identical with those of PBSA, T_g (-6.8 °C) was the highest temperature among five kinds of polyesters because it contains ethylene unit.

The molecular weight of HB02B, blend polyester of polycaprolactone (PCL) and PBS, was $8.6 \cdot 10^4$ and its molecular weight distribution was 1.95. T_m showed two values (63.3 and 112.3 °C) and ΔH_m showed that two values (32.4 and 50.2 J/g), which indicates HB02B is a polymer blend. In the second heating, only one T_g (-27.8 °C) was observed, indicating that PCL and PBS are compatible with each other. PBAT consisted of butylene diol (50 mol %), adipate – (26 mol %) and terephthalate (24 mol %); this molar ratio, indicates that the polyester is an alternating copolymer of butanediol and dicarboxylic acid. The molecular weight of PBAT showed the lowest value ($4.4 \cdot 10^4$) among biodegradable polyesters. T_m of PBAT showed a higher value than that of each polyester, it is provably because PBAT contains aromatic ring. ΔH_m (33.8 J/g), which indicates the crystallinity of polymer, was the lowest among these polyesters.

Enzymatic Degradation

Enzymatic degradability of five kinds of polyesters was evaluated by eleven lipases. Enzymatic degradation was carried out in phosphate buffer (pH 7) containing lipase with concentration of 5 U/mg polymer at 37°C. This result is shown in Fig. 2. PBSA was more readily degraded by various lipases than other polyesters. Its weight loss by lipase D was 23.3 % after 240 h and the loss by lipase PS was 96.1% after 240 h. It was found that PBSA was remarkably degraded by lipase PS (Fig. 2, 7). HB02B, which is a blend of PCL and PBS, was also degraded by lipase D and lipase PS, its weight loss by lipase D was 12.9 % after 240 h and the loss by lipase PS was 55.6 % after 240 h. PBS was difficult to degrade by some lipases but lipase PS degraded it slightly with 14.1% weight loss after 240 h (Fig. 2). In addition, PES containing ethylene and succinate unit was little degraded by lipase PS.

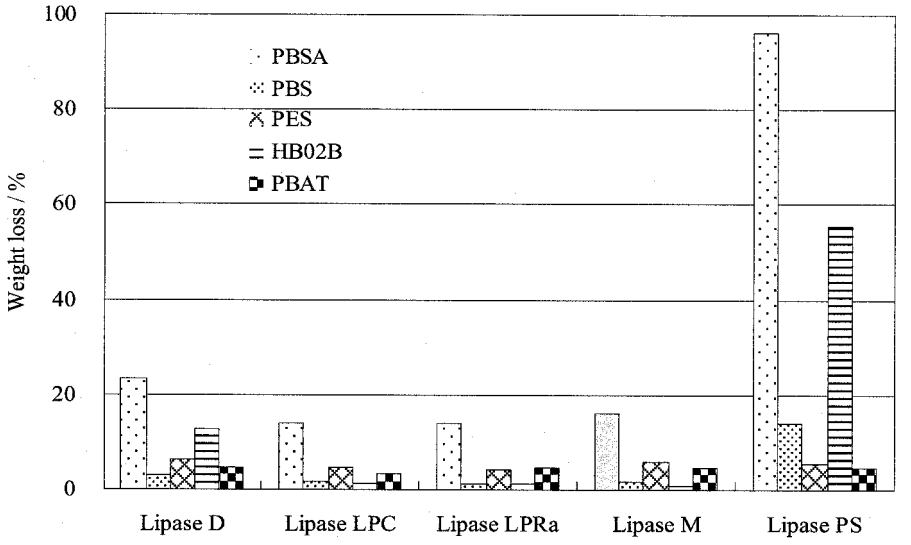


Fig. 2. Weight loss of biodegradable polyesters after enzymatic degradation (5 U/mg polymer) in phosphate buffer (pH 7) at 37°C for 240 h; Lipase D: from *Rhizopus delemar*, Lipase LPC: from *Candida cylindracea*, Lipase LPRa: from *Rhizopus arrizus*, Lipase M: from *Mucor javanicus*, Lipase PS: from *Pseudomonas* sp.

From these results, it can be said that polyester having relatively longer methylene chain (PBSA) was readily degraded by lipases such as lipase PS and lipase D. Degradation rate of the PBS/PCL blend (HB02B) was four times faster than that of PBS. This result indicates that the weight loss of HB02B by lipase PS was caused by much degradation of PCL and little degradation of PBS. Though PBAT, which contained aromatic ring, had butylene and adipate unit, the large weight loss recorded in the degradation of PBSA by lipase PS was not observed in the degradation of PBAT by lipase PS. It is considered that degradation of PBAT was inhibited by terephthalate unit. Effect of pH and temperature on lipase A, lipase AK and lipase PS were investigated in enzymatic degradation. Figs. 3 and 4 show the result of the effect of pH. PES was little degraded by lipase A and lipase PS in phosphate buffer (pH 7), whereas the weight loss of PES degraded by lipase A in citrate buffer (pH 4) was 30.3 % after 240 h (Fig. 3). Similarly, little degradation of PBSA was caused by lipase A at pH 7 after 240 h, while the weight loss at pH 4 was 62.0 % (Fig. 4). Figs. 3 and 4 show that the degradability by lipase PS at pH 7 was almost identical to that at pH 4, and that the enzymatic activity of lipase A from *Aspergillus niger* was higher at lower pH.

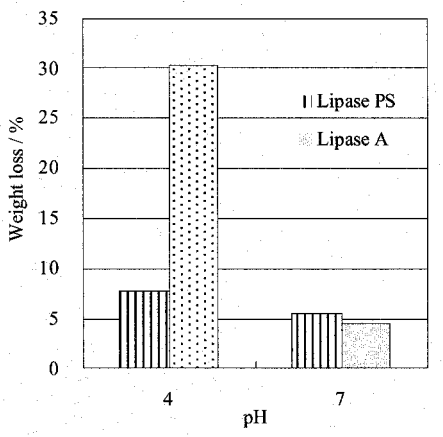


Fig. 3. Effect of pH on enzymatic degradation of PES after 240 h.

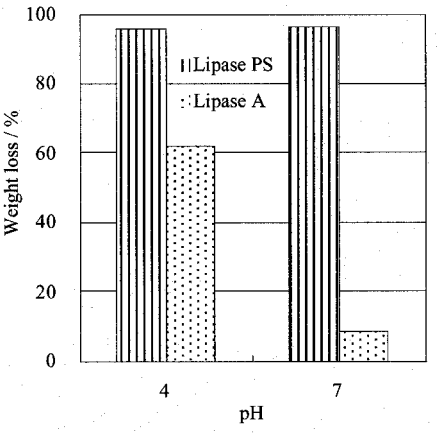


Fig. 4. Effect of pH on enzymatic degradation of PBSA after 240 h.

The effect of degradation temperature on lipase PS and lipase AK is shown in Figs. 5 and 6. For enzymatic degradation of HB02B and PBSA by lipase AK, the degradation rate at 45 °C was twice higher than at 37 °C. HB02B and PBSA were completely degraded by lipase PS at 45 °C after 220 h and 40 h, respectively. The results show that enzymatic activity of lipase AK and lipase PS from *Pseudomonas* sp. increased at 45 °C (mesophile temperature).

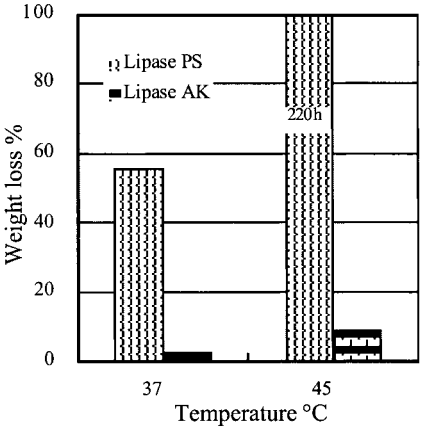


Fig. 5. Effect of temperature on the enzymatic degradation of HB02B after 220 h.

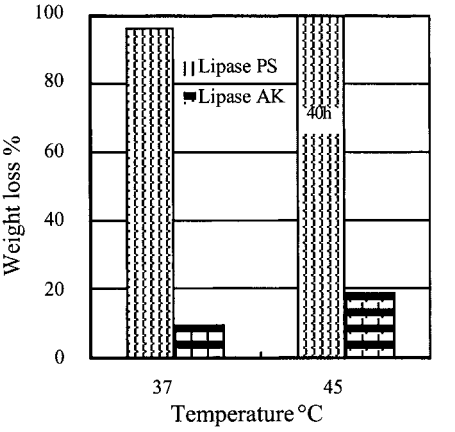


Fig. 6. Effect of temperature on the enzymatic degradation of PBSA after 40 h.

To investigate some properties before and after enzymatic degradation, composition, molecular weight and thermal property of PBSA degraded by lipase PS were measured by ^{1}H-NMR, SEC and DSC, respectively (Table 2, Fig.7, Fig.8). Weight remaining of PBSA after 240 h was 3.9%, but composition was rarely changed even after 240 h. Molecular weight of PBSA ($6.4 \cdot 10^4$) was decreased to $5.5 \cdot 10^4$ after 240 h. Changes of T_m and T_g were hardly observed before and after enzymatic degradation, whereas ΔH_m, which indicates crystallinity, increased from 70.3 J/g to 87.1 J/g. It is suggested that crystallinity region is extended with the progress of degradation. It was proved that noncrystalline region was at first degraded by lipase PS as it is generally observed in enzymatic degradation. Although degradation of PBSA by lipase PS occurred at the surface of film as shown in Fig. 9, its molecular weight and composition did not change much after degradation.

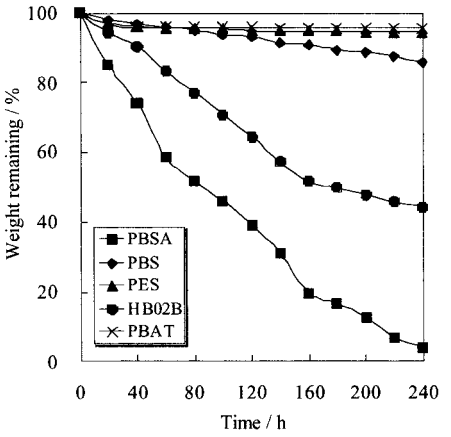


Fig. 7. Enzymatic degradation of biodegradable polyesters by lipase PS in 50 mM phosphate buffer (pH 7) at 37 °C.

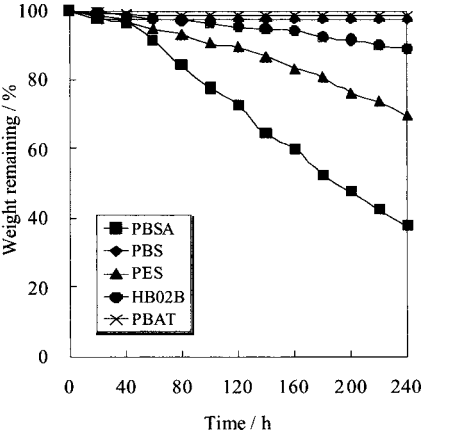
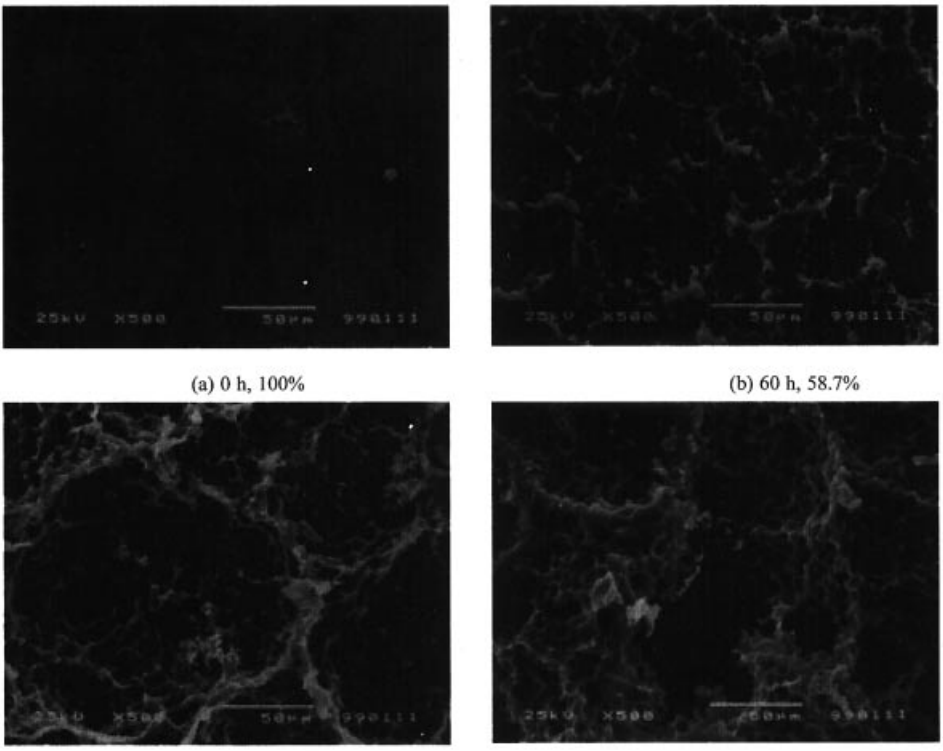


Fig. 8. Enzymatic degradation of biodegradable polyesters by lipase A in 50 mM citrate buffer (pH 4) at 37 °C.

Table 2. Changes in properties of PBSA before and after enzymatic degradation by lipase PS.

Time	R.W.	Comp. (B/S/A) [a]	M_n [b]	M_w/M_n [b]	T_m [c]	ΔH_m [c]	T_g [c]
h	%	mol %	$\cdot 10^4$		°C	J/g	°C
0	100	50/40/10	6.4	1.72	91.0	70.3	- 42.2
60	58.7	50/40/10	6.3	1.75	90.9	75.4	- 42.3
140	31.1	50/41/9	6.1	1.76	90.5	81.8	- 42.6
240	3.9	50/42/8	5.5	1.81	90.1	87.1	- 42.8

Ezymatic degradation of PBSA was carried out in phosphate buffer (pH 7) with lipase PS concentration of 5 U/mg polymer at 37 °C. R.W.: Residual weight. Comp.: Composition.
[a] Determined by ^{1}H-NMR (400 MHz). B: Butylene; S: Succinate; A: Adipate. [b] Determined by SEC. [c] Determined by DSC.



(a) 0 h, 100%

(b) 60 h, 58.7%

(c) 140 h, 31.1%

(d) 240 h, 3.9%

Fig. 9. Scanning electron micrographs of the surfaces of PBSA films (a) before and (b) to (d) after enzymatic degradation by lipase PS at 37 °C.

Chemical Degradation

Chemical degradation of biodegradable polyester was conducted in sodium hydroxide solution and was compared with enzymatic degradation. Fig. 10 shows the results of chemical degradation in 1.0 N NaOH solution at 37°C. The degradation rate of PES was extremely high in comparison with other polyesters (PES >> HB02B > PBSA > PBS > PBAT), PES disappeared completely after 20 h (Data not shown), and it was rapidly degraded in 0.01 N NaOH solution. Its residual weight was 21.8 % after 240 h (Fig. 10). Degradation rate of PBAT containing aromatic ring in polymer chain was the lowest in 1.0 N NaOH solution and its residual weight was 78.6 % after 240 h. This shows that PBAT is difficult to degrade in sodium hydroxide solution in analogy with the enzymatic degradation by lipases.

Chemical degradation of PBSA in 1.0 N NaOH solution was compared with the enzymatic degradation by lipase PS at 5 U/mg polymer (Fig. 11). Although the chemical degradation was carried out in rather concentrated NaOH solution (1.0 N), the ratio of chemical degradation rate to enzymatic degradation rate was 1 : 16 after 240 h. These results make it clear that the enzymatic degradation by lipase PS is more efficient and environmentally safer than the chemical degradation in sodium hydroxide solution.

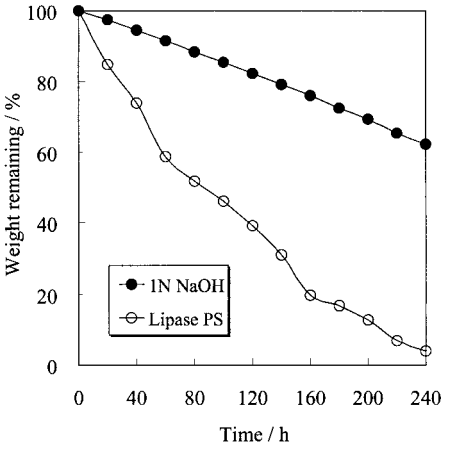


Fig. 10. Chemical degradation of biodegradable polyesters (thickness of ca. 0.1 mm) by 1.0 N NaOH solution at 37°C.

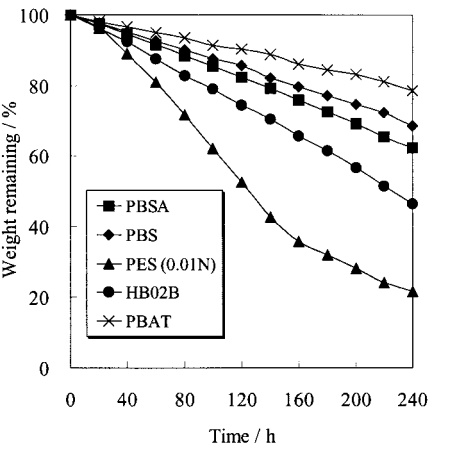


Fig. 11. Comparison of the enzymatic and chemical degradation of PBSA at 37°C .

Conclusion

For enzymatic degradation, 1) aliphatic polyesters containing 1,4-butylene units were readily degraded by lipase PS. 2) In enzymatic degradation by lipase A, PES and PBSA were faster degraded in citrate buffer at pH 4 than in phosphate buffer at pH 7. 3) The enzymatic degradation rate of lipase AK and that of lipase PS for aliphatic polyesters at 45 °C were both higher than at 37 °C. 4) The enzymatic degradation rate of PBSA by lipase PS was 16 times higher than the chemical degradation rate after 240 h.

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