Biodegradability of Diethylene Glycol Terephthalate and Poly(ethylene terephthalate) Fiber by Crude Enzymes Extracted from Activated Sludge

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ABSTRACT: In this study, diethylene glycol terephthalate (DTP) and poly(ethylene terephthalate) (PET) fiber were degraded by crude enzymes extracted from a strain that was isolated from activated sludge obtained from factories. The feasibility of the biodegradation of the PET fiber is discussed through the rule of the biodegradation of DTP, which was used as the simulacrum of the biodegradation of the PET fiber. We concluded that the proper conditions for the degradation of DTP with the crude enzyme were 30°C and a medium pH. Through the degradation kinetics, we determined that the crude enzyme was more capable of degrading DTP than was lipase. In addition, the crude enzyme also degraded the PET fibers. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 3855–3859, 2006

Key words: biodegradable; fibers; enzymes

INTRODUCTION

The biodegradation of poly(ethylene terephthalate) (PET) is one of the current hotspots of study in the world,[1–4] which can solve the environmental pollution problem of a great deal of white garbage induced by the increasing production of PET fibers. It is believed that PET fiber can be degraded into low-molecular materials that may be easily reclaimed. On the other hand, biotreatment of PET fibers may also improve their absorbance, dyeing, luster, drape, and hand.

Although there are a lot of ester bonds on PET fibers that can be easily hydrolyzed or biotreated, it is not favorable to react PET fiber with microorganisms or enzymes because of PET's compact structure. The structure of diethylene glycol terephthalate (DTP) is similar to that of PET fibers, so it may be used as a simulacrum in the study of the biodegradation behaviors of PET fibers. On the other hand, the biodegradation research of DTP can resolves pollution problems induced by DTP also.[5]

EXPERIMENTAL

Crude enzyme extraction

A strain was selected from the activated sludge of a wastewater pool in a chemical fiber plant (Tianjin Petrochemical Co., Ltd., Tianjin, China). DTP (5 g/L) was added to the culture medium[6] (see Table I), and the strain was incubated and domesticated at 30°C, with a shaking frequency of 120 rpm for 5 days. After that, the process was repeated.

After domestication, the culture liquid was centrifuged for 10 min (n = 10,000 rpm); the specificity solution was collected and defined as S1 (exoenzyme was included). Deposition was made at a 1 : 5 ratio of the suspended liquid to 0.02 mol/L K_2HPO_4–KH_2PO_4 phosphate buffer (pH = 7.5); then, the suspended liquid was intermittently fragmented by ultrasound (40 Hz, 100 W) in an ice bath five times (1 min each time) followed by centrifugation and collection of the specificity solution as S2 (endoenzyme was included).

Reagents

The reagents included E-3019 lipase (41 units/mg of solid, Sigma Co., Ltd.) and DTP (chromatographic pure, Shanghai, China).

Instruments

The instruments included a LabAlliance Series III high-performance liquid chromatograph, a LabAlliance model 500 detector, a LG10-2.4A centrifugal distilled machine for medicine (Factory of Medical Instrument, Beijing, China), a KQ218 ultrasonic cleaner (Factory of Ultrasonic Instrument, Kunshan, China), and a HZQ-C air bath oscillator (Factory of Dongming Medical Instrument, Haerbin, China).

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TABLE I
Composition of the Culture Medium

Component	Concentration (g/L)	Component	Concentration (μg/L)
NH_4Cl	1.0	H_3BO_3	0.5
$MgSO_4 \cdot 7H_2O$	0.25	$CuSO_4 \cdot 5H_2O$	40.0
KH_2PO_4	3.0	$FeCl_3 \cdot 6H_2O$	0.2
Na_2HPO_4	7.0	$MnSO_4 \cdot 5H_2O$	0.4
NaCl	0.5	$ZnCl_2$	0.4
Glucose	2.0	$(NH_4)_6Mo_7O_{24} \cdot 7H_2O$	0.2

Conditions of domestication: pH = 6.8; temperature = 30°C; time = 5 days; shaking frequency = 120 rpm.

RESULTS AND DISCUSSION

Degradation of DTP with the crude enzyme

S1 or S2 (2 mL; or mixture of S1 and S2) was added to phosphate buffer (3 mL of K_2HPO_4–KH_2PO_4; pH = 7.8; 5 mg of DTP), and the solution was degraded for 15 h at 30°C and 120 rpm. The residual concentration of DTP was determined by HPLC, and the degrading capabilities of the endoenzyme and exoenzyme were compared.

Table II shows that the degrading capability of the endoenzyme was 10 times higher than that of the exoenzyme, and the endoenzyme played the major role in the degradation of DTP.

Effect of the temperature on the degradation of DTP

DTP (25 mg) and 5 mL of a mixture of the endoenzyme and exoenzyme (1 : 1) were added to a 25-mL measuring flask, and the solution was degraded for 15 h (120 rpm) each at 20, 30, and 40°C. The measuring flask was then put into boiling water for 5 min to inactivate the enzymes. The residual concentration of DTP was determined with HPLC, and the liquid was volume-fixed with 80% methanol; the results are shown in Figure 1.

Figure 1 indicates that the optimum temperature for the crude enzymes to degrade DTP was 30 °C. Reduced degradation was noticed when temperatures were below or above 30°C.

Effect of the ph on the degradation of DTP

DTP (25 mg) and 5 mL of a mixture of the endoenzyme and exoenzyme (1 : 1) were added to a 25-mL measuring flask, and the solution was degraded for 15 h (120 rpm) with different pH values. The measuring flask was then put into boiling water for 5 min to inactivate the enzymes. The liquid in the 25-mL measuring flask was volume-fixed with 80% methanol, and the residual concentration of DTP was determined with HPLC; the results are presented in Figure 2.

As shown in Figure 2, the perfect pH for the degradation of DTP was 7–7.5; a higher or lower pH resulted in adverse effects.

Kinetics of the degradation of DTP with E-3019 lipase

DTP (5, 25, 50, and 100 mg, added separately) was added to phosphate buffer (5 mL of K_2HP0_4–KH_2P0_4; pH = 8; 0.1 g/L E-3019 lipase), and the solution was degraded for 12 h at 30°C (120 rpm). The yield of terephthalate acid (TA) was measured with HPLC[7] (Fig. 3). The relationship between the original yielding velocity of TA (the tangent at the beginning of the degrading curve) and the original concentration of DTP is shown in Figure 4.

The Michaelis–Menten equation $\{V = V_m[S]/(K_m + [S])\}$ was modified as $[S]/V = K_m/V_m + [S]/V_m$. Then, a Hanes–Woolf curve was made with $[S]V \approx [S]$, and

TABLE II
Biodegradation of DTP with the Crude Enzyme

Crude enzymes	DTP concentration (g/L) Original	Residual	Average (g/L)	DTP degradation ratio (%)	Average %
		0.976		2.37	
Exoenzyme	1	0.959	0.969	4.10	3.13
		0.971		2.92	
		0.684		31.65	
Endoenzyme	1	0.679	0.669	32.07	
		0.644		35.59	33.10

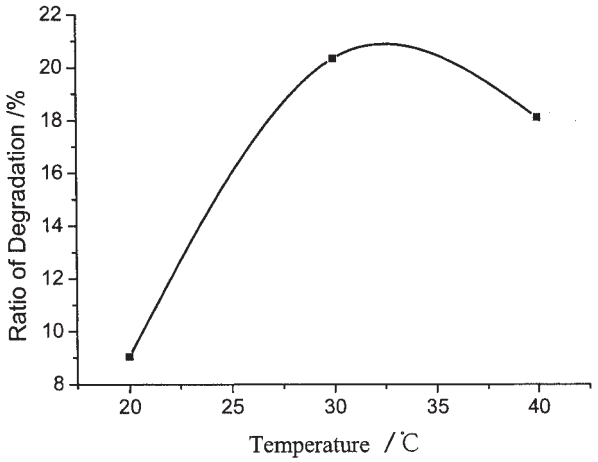

Figure 1 Effect of temperature on degradation of DTP with crude enzymes.

the linear equation was $Y = 112.5911 + 14.3986X$. Where V is the rate of the reaction of enzyme, V_m is the maximum of V, [S] is the concentration of DTP and K_m is the coefficient of Michaelis'.

The intercept on the Y axis of the line was $K_m/V_m = 112.5911$, the intercept on the X axis was $-K_m = -7.8196$, the slope was $1/V_m$ was 14.3986, and the correlative coefficient (R) was 0.98611.

Therefore, the Michaelis–Menten coefficient for the degradation of DTP with E-3019 lipase was $K_m = 4.7 \times 10^{-2}$ (mol/L).

Kinetics of the degradation of DTP with the crude enzyme

DTP (20, 25, 30, 40, 50, and 80 mg, added separately) and a 5-mL mixture of the endoenzyme and exoenzyme (1 : 1) were added to a 25-mL measuring flask and were degraded at 30°C (120 rpm). The measuring flask was then put into boiling water for 5 min to inactivate the enzymes. The yield of TA was determined with HPLC after the liquid in 25-mL measuring flask was volume-fixed with 80% methanol. The kinetic properties of the degraded DTP were studied through the yield of TA (Fig. 5).

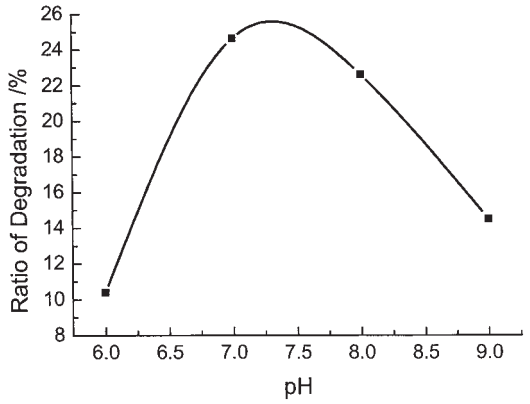

Figure 2 Effect of the pH on the degradation of DTP with crude enzymes.

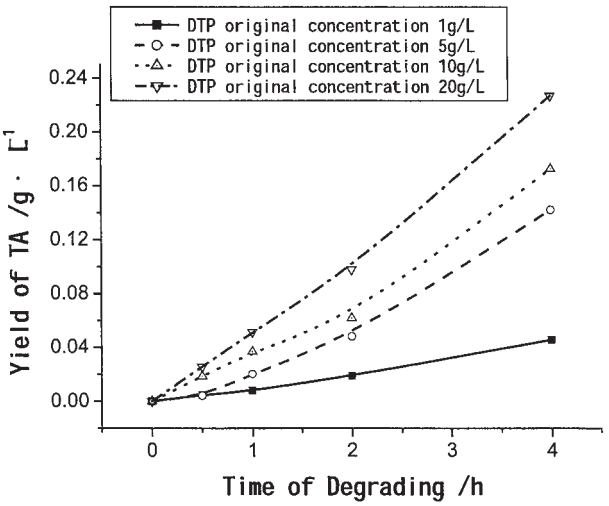

Figure 3 Change in TA in the degradation process with E-3019 lipase.

Figure 5 illustrates that the yield of TA increased with prolonged degradation time, which showed that the crude enzymes degraded DTP to some extent. However, the yield of TA showed no distinct change with increased original concentration of DTP.

A series of original reaction velocities (the original tangent equation on the curve) is shown in Figure 5. The curve in Figure 6 was plotted with reaction velocity versus the substrate concentration.

Figure 6 indicates that at very low substrate concentrations, the reaction rate increase was proportional to the substrate concentration; thus, the rate here was first order with respect to the substrate concentration.

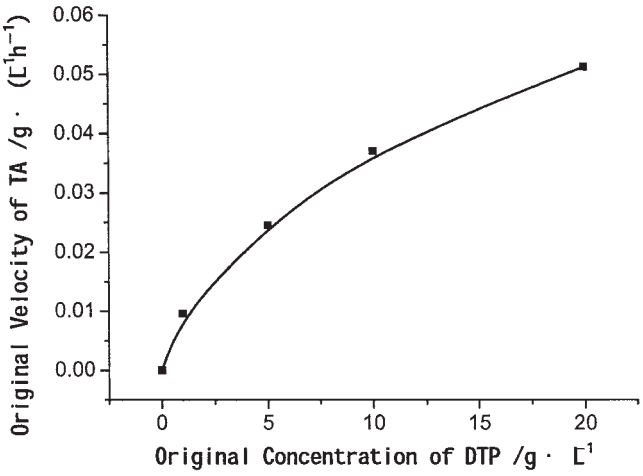

Figure 4 Original yielding velocity of TA in the degradation process with E-3019 lipase.

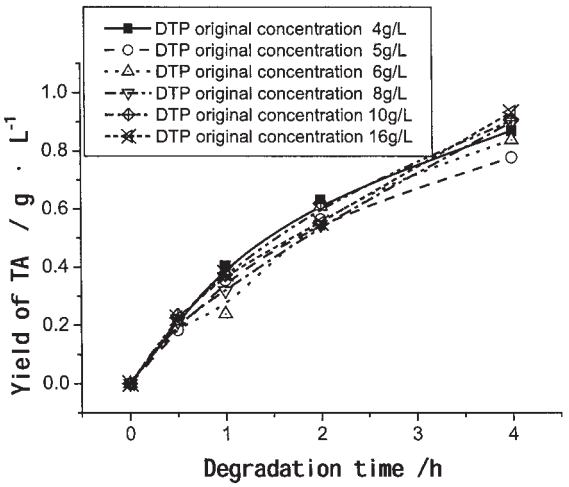

Figure 5 Change in TA in the degradation process with the mixture of crude enzymes.

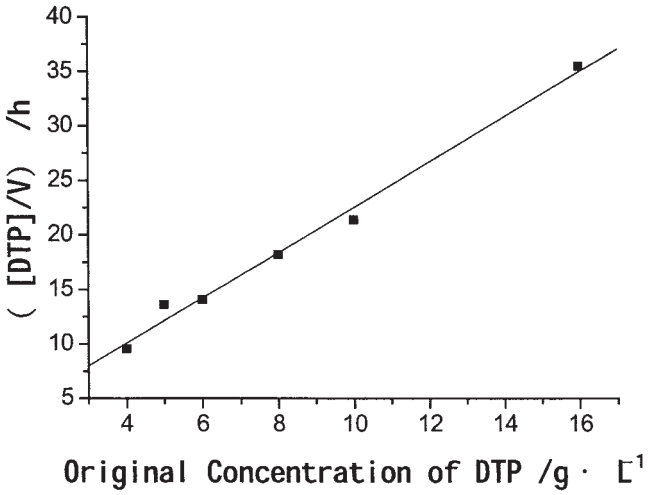

Figure 7 Hanes–Woolf curve in the degradation of DTP with crude enzymes.

As the substrate concentration increased, however, the rate did not follow in a proportional manner, which gave a mixed-order reaction. At higher substrate concentrations, the enzymes were saturated with substrate, and no further rate increase was expected. This was the maximum rate for the reaction, a so-called zero-order reaction.

A Hanes–Woolf curve was made with $[S]/V \sim [S]$ and the linear equation (Fig. 7) $Y = 1.29715 + 2.07159X$. The intercept on the Y axis of the line was $K_m/V_m = 1.29715$, the intercept on the X axis was $-K_m = -0.6262$, the slope was $1/V_m = 2.07159$, and the correlative coefficient (R) was 0.99537.

Therefore, the Michaelis–Menten coefficient for the degradation of DTP with the crude enzymes was $K_m = 3.8 \times 10^{-3}$ (mol/L).

K_m is a characteristic constant; its value is related to the enzyme properly, not the concentration. K_m represents the affinity between the substrate and the enzyme. A low value of K_m indicates a higher affinity; in this case, the maximum rate was reached at a relatively low substrate concentration. K_m of the crude enzymes extracted from the strain was 3.8×10^{-3}, less than the 4.7×10^{-2} of E-3019 lipase. Thus, we concluded that the crude enzymes were more suitable for the degradation of DTP.

There were no distinct differences among the curves in Figure 5, which showed that the substrate concentration used in the experiment was much great than K_m, which gave a reaction between mixed order and zero order. Because the substrate concentration had little effect on the degradation of DTP, much more enzyme had to be added to increase the degradation ratio of the substrates.

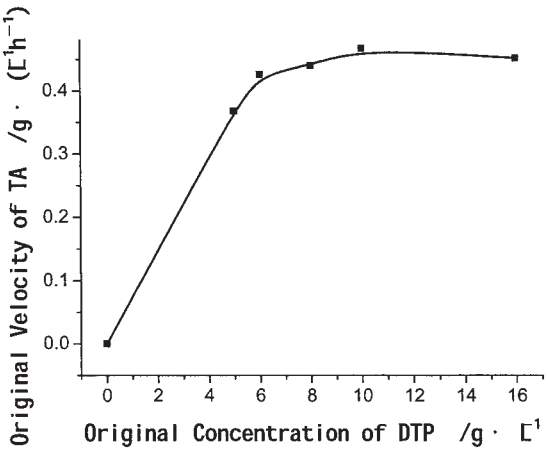

Figure 6 Original yielding velocity of TA in the degradation process with the mixture of crude enzymes.

Degradation of the PET fiber with a mixture of the crude enzymes

The PET fiber was degraded for 24 h with the crude enzymes.

Figure 8 shows the HPLC picture of the PET fiber degraded for 24 h with the crude enzymes. Low absorbance peaks of TA were noticed in only endoenzyme or exoenzyme solution; this may have contributed to some midst products (TA) produced in the incubation and domestication. A higher absorbance peak of TA was found in the degradation of the PET fiber; this indicated that the long molecule of the PET fiber were degraded by crude enzyme, and TA molecules were generated. So the crude enzymes extracted from the strain were proven effective in the degradation of the PET fiber.

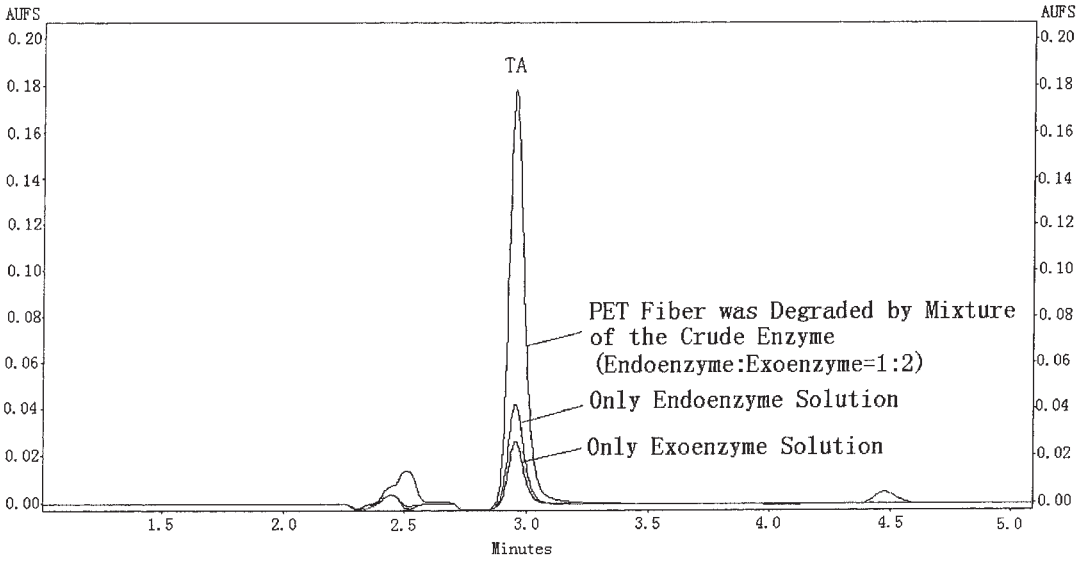

Figure 8 HPLC picture of PET fiber degraded for 1 day with the crude enzymes.

CONCLUSIONS

1. The crude enzyme could degrade DTP. The degradation capability of the endoenzyme was higher than that of the exoenzyme.
2. The optimum conditions for the degradation of DTP with the crude enzyme were 30°C, pH = 7–7.5, and shaking frequency = 120 rpm.
3. The degradation kinetics showed that the crude enzyme had a greater effect on the degradation of DTP than E-3019 lipase.
4. The crude enzyme, whose strain was incubated and domesticated by DTP, could also degrade the PET fiber. This means it is feasible that DTP is one of the best simulacrums for studying the biodegradation of PET fibers.

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