Enzyme-Catalyzed Hydrolysis of Poly(ethylene terephthalate) Cyclic Trimer

Jacob Hooker,[1] David Hinks,[1] Gerardo Montero,[2] Magdelena Icherenska[1]

[1]*Department of Textile Engineering, Chemistry and Science, North Carolina State University, 2401 Research Drive, Raleigh, North Carolina 27695-8301*
[2]*Kenan Center for the Utilization of Carbon Dioxide in Manufacturing, Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27606-7565*

Received 6 December 2001; revised 2 August 2002; accepted 2 August 2002

ABSTRACT: Oligoesters present in poly(ethylene terephthalate) fibers are commonly extracted from the fiber during processing, such as aqueous-based dyeing. Aqueous, insoluble oligoesters, particularly cyclic trimers that precipitate on processing machinery and on the fiber surface are difficult to remove under benign conditions. Reported is a new method for the efficient removal of cyclic trimer by enzyme-catalyzed hydrolysis. Almost complete hydrolysis of trimer was accomplished at pH 8 and 60°C. Effects of time, agitation, surfactant, and enzyme and trimer concentrations on the efficiency of hydrolysis are reported. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 2545–2552, 2003

Key words: polyesters; oligomers; enzymes

INTRODUCTION

The polycondensation of terephthalic acid and ethylene glycol to form high molecular weight poly(ethylene terephthalate) (PET) also produces linear and cyclic oligomers. PET fibers contain 1–3% oligomer by weight, predominantly in the form of a cyclic trimer,[1] cyclo-*tris*-ethylene terephthalate (CTR), that can adversely affect various industrial processes, such as aqueous dyeing. The oligomeric particles of PET fibers migrate to the surface during high-temperature processing, form agglomerate crystals, and precipitate in aqueous baths.[2] The buildup of CTR deposits not only causes operation and maintenance problems in machinery,[3] but it also decreases the quality of processed PET fibers by a delustering affect that causes graying.

The degradation or removal of CTR is difficult because of its high aqueous insolubility and affinity to adhere to metallic and other surfaces. In severe cases, trimer buildup can reduce the efficiency of pumps and valves and limit flow rate. Hence, preventative maintenance is a requirement. The cyclic oligomers can be resistant to treatment unless relatively severe conditions are used, and these severe conditions can result in a significant loss of fiber material and produce effluent toxic to aquatic life. Furthermore, conventional removal of trimer is time and energy inefficient and may damage equipment.[4] Presently, PET oligomeric material is most commonly removed from equipment via high temperature aqueous hydrolysis involving strong alkali and cationic surfactants.[5]

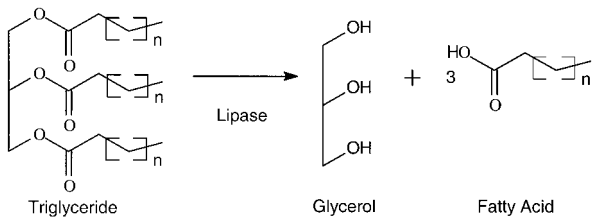
As technology in PET processing advances, the buildup of CTR in machinery continues to be problematic. Interestingly, the extraction and precipitation of PET oligomer on metal surfaces occurs in very hydrophobic media as well as in aqueous medium. For instance, dyeing PET in a medium of supercritical carbon dioxide ($SCCO_2$) also leads to significant oligomer extraction, contributing over time to trimer buildup in machinery.[6–10] Hence, new, more environmentally responsible methods for the removal of CTR are highly desirable. One such method that has been the subject of intense research for the processing of fibers during the last decade is in the area of enzyme-catalyzed reactions.

The high specificity and efficiency of enzymes coupled with their benign environmental impact have compelled research into the investigation of enzyme-catalyzed transformations as alternatives to conventional chemical reactions. In recent years, the development and utility of esterases has increased dramatically because of advances in the understanding of their molecular structure and mechanism of action. Lipolytic esterases, such as lipases and cutinases, are known in biological processes to catalyze hydrolysis reactions involving naturally occurring esters, as seen in Scheme 1. [11]Lipases have been demonstrated to be active not only at interfacial boundaries of oil and water, but also on plastic surfaces (or, in general, any interface of lower polarity than water).[12]

Among new areas of opportunity in the utilization of lipolytic esterases is the removal of CTR from PET

Correspondence to: D. Hinks (david_hinks@ncsu.edu).

Journal of Applied Polymer Science, Vol. 89, 2545–2552 (2003)



Scheme 1 Hydrolysis of triglycerides with lipases.

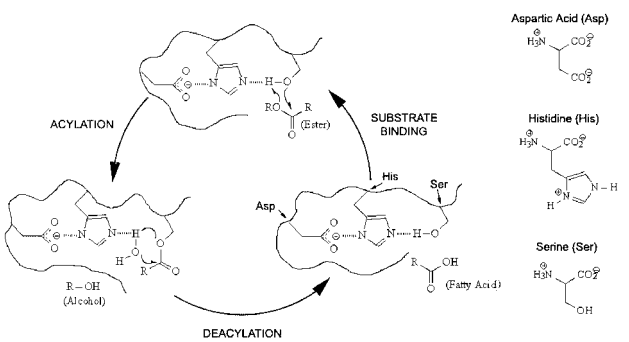
fibers and processing equipment, particularly with the enzyme class known as cutinase.[13] Cutinase is a serine esterase known to cause the hydrolytic degradation of cutin, the waxy-like biopolyester found on many types of plants.[14, 15] The catalytic triad of cutinase, involving histidine, aspartic acid, and serine, to hydrolyze ester bonds is shown in Scheme 2.

In the present work, a developmental enzyme was employed as a catalyst in the hydrolysis of CTR. This work may be the first application of what could become a versatile and promising new application of cutinases. The effects of key reaction conditions are described, and the potential effectiveness of enzyme-catalyzed CTR hydrolysis as a practical application in the textile industry is discussed. The potential cutinase-catalyzed degradation products of CTR are shown in Scheme 3.

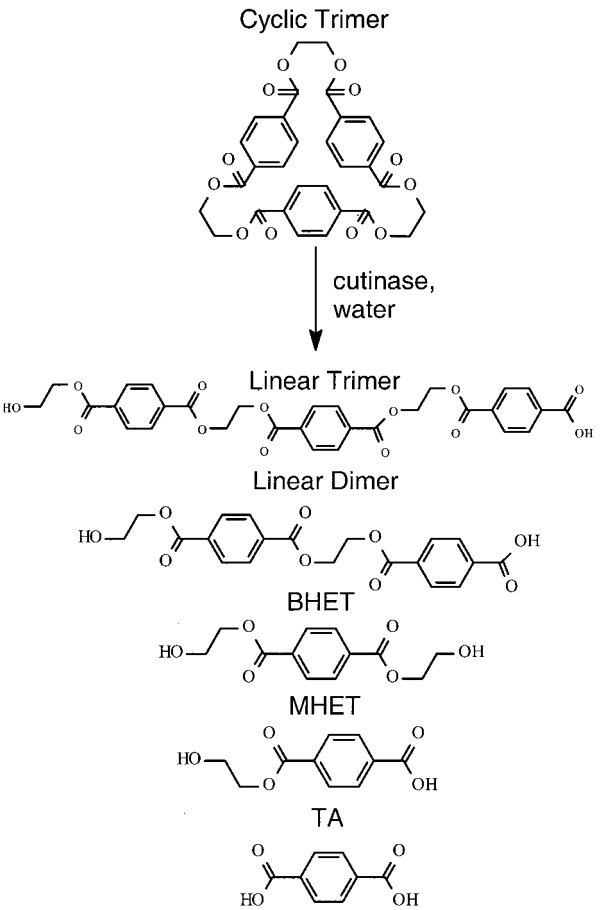
EXPERIMENTAL

Materials

The esterase used in the present study was a developmental cutinase provided by Novozymes North America, Inc. (Franklinton, NC). The cutinase was dosed in experiments on the basis of Lipase Units (LU), where one LU is the amount of enzyme that releases 1 μmol of titratable butyric acid per minute from tributyrin at 30° and pH 7.0. Acetonitrile, 1,4-dioxane, and water used for eluent dilution (all HPLC grade) were obtained from Fisher Scientific (Fair Lawn, NJ). Trifluoroacetic acid (spectrophotometric grade), glycine, terephthalic acid (TA), and bis(hydroxyethyl)terephthalate (BHET) (all reagent grade) were obtained from Sigma-Aldrich Chemical (Milwaukee, WI). Crude PET oligomer was obtained from a conventional aqueous PET package-dyeing machine located in Reidsville, NC (initially 58% CTR purified to 98%).



Scheme 2 Cutinase active sites and mechanism of hydrolysis.[14]



Scheme 3 Potential cutinase-catalyzed hydrolysis products of CTR (BHET = bis(hydroxyethylene) terephthalate; MHET = monohydroxyethylene terephthalate; TA = terephthalic acid).

Equipment

Reactions were performed with a Precision Reciprocal Shaking Bath (Model 50). High-performance liquid chromatography (HPLC) separations were carried out with a Waters 1525 Binary HPLC Pump with an All-

TABLE I
HPLC Gradient

Time (min)	AcN (%)
0	10
2	20
10	40
20	90
33	95

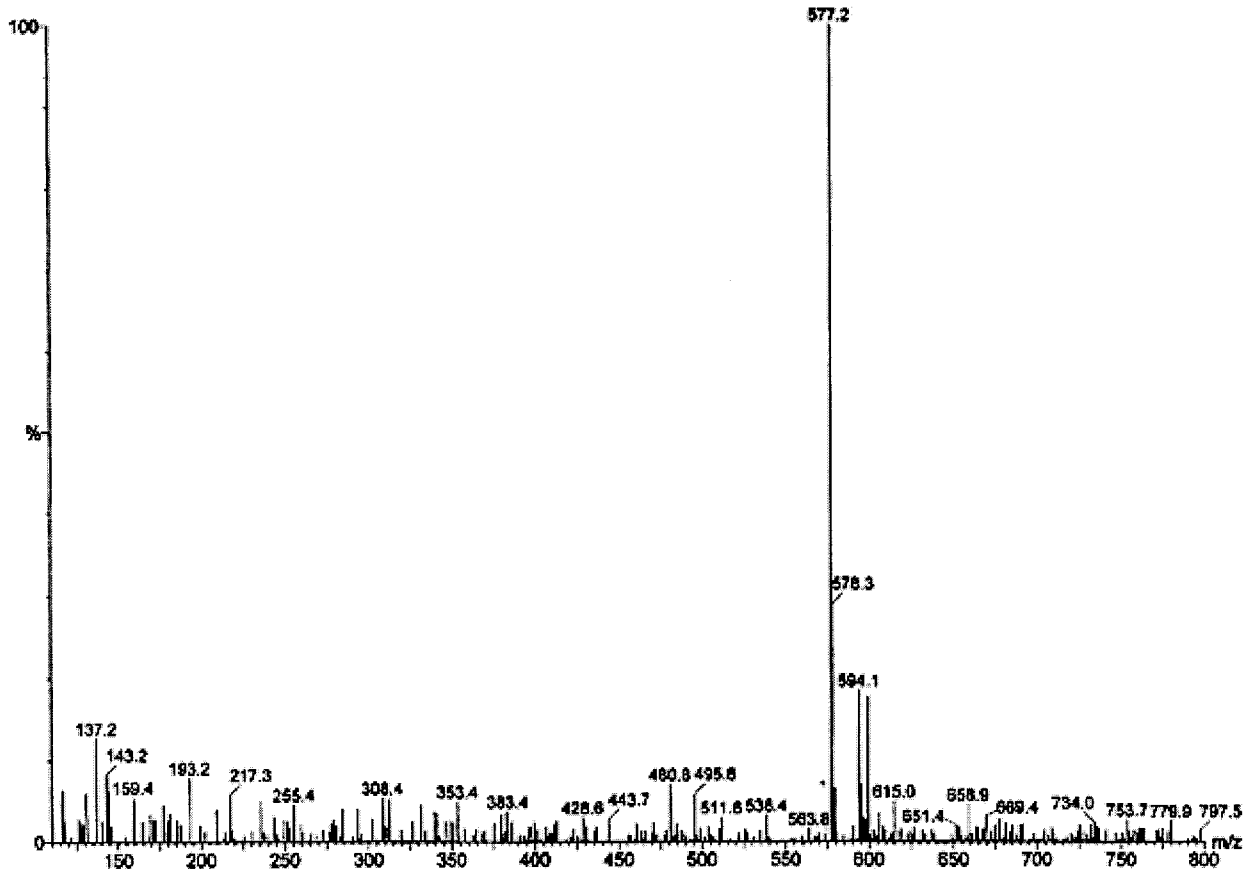


Figure 1 Mass spectrum of CTR.

tech C18 reversed-phase column (250 mm length, 4.6 mm diameter). Detection was accomplished with a Waters 2487 Dual λ Detector set at 254 nm.

Methods

Hydrolysis of a cyclic trimer

Hydrolysis of a cyclic trimer (CTR) was carried out in aqueous dispersions of various compositions. Aqueous CTR dispersions were prepared in 250-mL conical flasks by the addition of 0.005 M CTR in 1,4-dioxane solution to deionized water. A buffer of 0.2 M glycine, brought to pH 8 with the addition of 1 M NaOH, was added to the dispersions. In one experiment, to determine the effect of a nonionic surfactant on the hydrolysis of CTR, a 10% solution of Triton X-100 (octyl phenoxy polyethoxy ethanol) was added to the reaction mixture to produce a final concentration of 1 g/L. The conical flasks were capped with glass stoppers and then placed in a reciprocating shaker bath at 60°C. The reaction dispersions were equilibrated at 60°C, while reciprocating at 150 rpm. Agitation was halted while enzyme, measured according to enzyme activity, was added with a volumetric syringe. The reaction proceeded at 60°C and 150 rpm for a predetermined amount of time. Deactivation of the enzyme was accomplished with a 1:2 dilution of the reaction mixture with 1,4-dioxane. Samples were stored at 10°C to ensure enzyme deactivation.

HPLC analyses

HPLC analyses were used to determine the composition of the hydrolysis reaction of diluted samples. The mobile phase used was an acetonitrile (AcN)/0.1% trifluoroacetic acid (TFA) linear gradient with a flow rate of 0.8 mL/min (see Table I). Samples were prepared by diluting with 1,4-dioxane to give CTR in a concentration range from 0.05 to 0.7 mM. Separation was carried out at ambient temperature, and a linear calibration curve (R^2 = 0.9985) was used for the conversion of CTR peak area to concentration.

Purification of CTR

Purification of CTR was accomplished by heating an impure sample in boiling 1,4-dioxane with magnetic stirring. The solution was filtered hot to remove insoluble impurities, and solvent was removed by rotary evaporation. The white crystalline product was washed with boiling water, filtered, washed with acetone, and dried. The final purity of the solid recovered was determined

TABLE II
Summary of Reaction Conditions

Experiment	CTR (mL) (0.005 M)	H_2O (mL)	Buffer (mL) (0.2 M)	Triton X-100 10% (g)	Enzyme (LU/mL)	Reaction time (h)	Initial [CTR] (mM)[a]	Final [CTR] (mM)[b]	Percent hydrolysis
Time dependence	1.3	10	1.2	—	100	1	0.1733	0.1355	21.81
	1.3	10	1.2	—	100	2	0.1733	0.1272	26.60
	1.3	10	1.2	—	100	4	0.1733	0.0895	48.36
	1.3	10	1.2	—	100	8	0.1733	0.0774	55.34
	1.3	10	1.2	—	100	16	0.1733	0.0395	77.21
	1.3	10	1.2	—	100	32	0.1733	0.0219	87.36
	1.3	10	1.2	—	100	64	0.1733	0.0074	95.73
CTR concentration dependence	0.5	10	1.5	—	100	24	0.0694	0	100.00
	1.0	10	1.5	—	100	24	0.1333	0.0173	87.02
	1.5	10	1.5	—	100	24	0.1923	0.0585	69.58
	2.0	10	1.5	—	100	24	0.2469	0.1026	58.44
	2.5	10	1.5	—	100	24	0.2976	0.1287	56.75
	3.0	10	1.5	—	100	24	0.3448	0.2386	30.80
	3.5	10	1.5	—	100	24	0.3889	0.2994	23.01
	4.0	10	1.5	—	100	24	0.4301	0.3124	27.37
	0.5	10	1.5	—	100	48	0.0694	0.0000	100.00
	1.0	10	1.5	—	100	48	0.1333	0.0000	100.00
	1.5	10	1.5	—	100	48	0.1923	0.0092	95.22
	2.0	10	1.5	—	100	48	0.2469	0.0528	78.61
	2.5	10	1.5	—	100	48	0.2976	0.0971	67.37
	3.0	10	1.5	—	100	48	0.3448	0.2079	39.70
	3.5	10	1.5	—	100	48	0.3889	0.2997	22.94
	4.0	10	1.5	—	100	48	0.4301	0.3176	26.16
Surfactant dependence	2.5	10	1.5	0.140	100	24	0.2976	0.1449	51.31
	3.0	10	1.5	0.145	100	24	0.3448	0.1850	46.35
	3.5	10	1.5	0.150	100	24	0.3889	0.2038	47.60
	4.0	10	1.5	0.155	100	24	0.4301	0.2492	42.06
	4.5	10	1.5	0.160	100	24	0.4688	0.3413	27.20
	5.0	10	1.5	0.165	100	24	0.5051	0.3715	26.45
Enzyme activity dependence	2.0	10	1.5	—	75	24	0.2469	0.0663	73.15
	2.0	10	1.5	—	100	24	0.2469	0.0406	83.56
	2.0	10	1.5	—	125	24	0.2469	0.0219	91.13
	2.0	10	1.5	—	150	24	0.2469	0.0058	97.65
	2.0	10	1.5	—	175	24	0.2469	0	100.00
	2.0	10	1.5	—	200	24	0.2469	0	100.00

[a] Calculated based on homogeneous dispersion accounting for the final 1:2 dilution.
[b] Calculated based on HPLC peak area calibration data.

by HPLC to be 98%. Mass spectral analysis of the product (Figure 1) confirmed the following major fragments: $[M+H]^+$ 577.2, $[M+H_2O]^+$ 594.1. The pure trimer was employed for all hydrolysis experiments.

RESULTS AND DISCUSSION

In addition to its ability to hydrolyze lipid-type ester bonds, the cutinase used in the present study was also active on the aromatic ester of CTR. A further understanding of enzyme-catalyzed degradation of CTR was gained by exploring the variables affecting the hydrolysis of the aromatic ester with the developmental cutinase. To avoid competing alkali hydrolysis traditionally used for degradation of CTR, reactions were performed at pH 8 using a glycine buffer. In addition, to ensure little variability of its activity over time, the enzyme was stored at 0°C. Water used for hydrolysis was always provided in vast excess (i.e., anywhere from 500 to 2000 times the molar amount of CTR), therefore guaranteeing water was never the limiting reagent. Given that the enzyme catalysis necessarily occurs at the boundary of water and the aqueous insoluble CTR, homogenous dispersions were vigorously agitated in a reciprocating shaker bath to help promote the interfacial activity. Highly dilute aqueous dispersions of CTR were prepared by introducing the CTR as a dilute 1,4-dioxane solution. Although not ideal for a commercial application, this procedure facilitated accurate measurement of the initial concentration, ensured that a fine dispersion formed, and minimized enzyme use. HPLC analysis was employed to follow the course of the reactions, and absorption peaks were identified by spiking the reaction mixtures

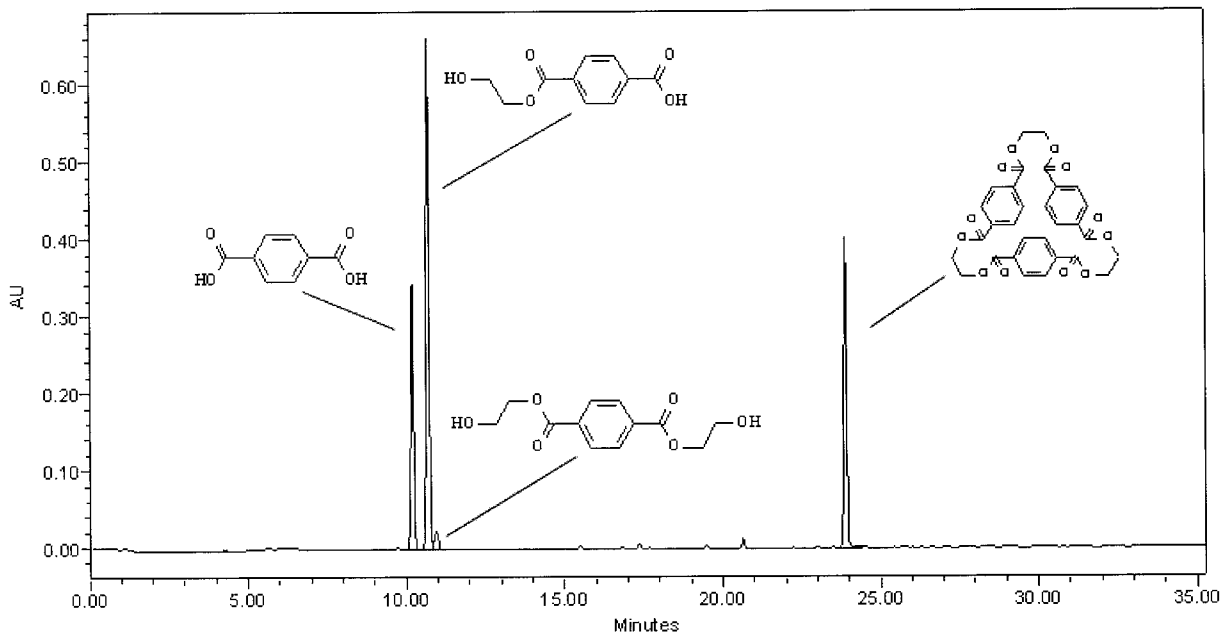


Figure 2 Typical HPLC chromatograms of CTR cutinase-catalyzed hydrolysis reaction (0.19232 mM CTR, 100 LU/mL cutinase, 60°C, pH 8, 150 rpm, 24 h).

with CTR, TA, and BHET prior to injection. By varying the reaction parameters, near complete hydrolysis of the CTR was possible. The various reaction conditions employed and a summary of the initial and final CTR concentrations are shown in Table II.

A typical HPLC chromatogram following cutinase-catalyzed hydrolysis of CTR is shown in Figure 2. Practically no linear trimer or dimer hydrolysis products were observed in any of the reactions performed. It is clear that, once the cyclic trimer is opened, rapid hydrolysis continues until almost all the ester bonds are hydrolyzed.

Effect of time

To determine the effect of time on the rate of cutinase-catalyzed hydrolysis of CTR, a series of identical reactions were performed but were terminated at increasing time intervals. The rate of cutinase-catalyzed hydrolysis was greatest on the initial injection and decreased as the reaction proceeded (Figure 3). CTR hydrolysis began to level off after ~16 h, with the first 8 h being the most effective. Conventional methods of hydrolysis via alkaline treatment at high temperatures are reported to take 60 min for a substantial reduction of CTR.[4] Hence, for a commercial application, optimization of reaction conditions would be required to improve the efficiency of the cutinase-catalyzed reaction. However, in conventional CTR treatments, it is common to allow the trimer to significantly build up on machinery prior to treatment. Hence, an alternative approach would be to employ the cutinase at the end of each processing step (e.g., after each dyeing) to prevent any significant buildup of trimer on machinery.

Effect of agitation

As with all heterogeneous reactions, agitation of the CTR reaction dispersion is essential in promoting enzyme-catalyzed hydrolysis. By applying one set of identical reaction conditions, except varying only the rate of agitation, there was substantial variation in the amount of CTR degraded. As seen in Table III, increasing the rate of agitation from 0 to 150 rpm increased the amount of hydrolysis by almost 10 times. Because the enzyme acts at the CTR–water boundary, delivery of the lipase to the interface is critical. Increased agi-

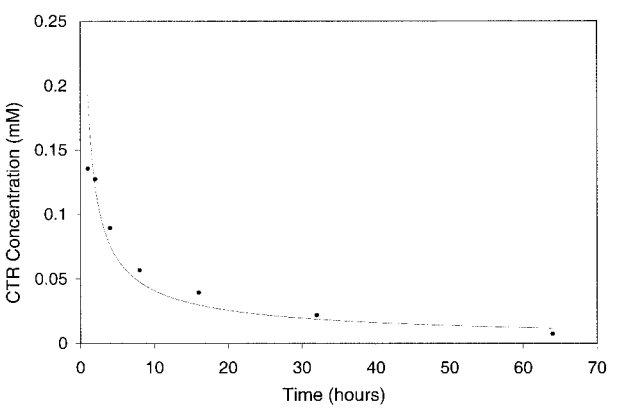


Figure 3 Time dependence of enzymatic hydrolysis of CTR (0.1733 mM CTR, 100 LU/mL cutinase, 60°C, pH 8, 150 rpm, 24 h).

TABLE III
Effect of Agitation on Trimer Hydrolysis[a]

Agitation rate (rpm)	Initial [CTR] (mM)[b]	Final [CTR] (mM)[c]	% Hydrolysis
0	0.2469	0.2247	8.99
75	0.2469	0.1119	54.66
150	0.2469	0.0406	83.55

[a] 100 LU/mL lipase, 60°C, pH 8, 24 h
[b] Calculated based on homogeneous dispersion accounting for the final 1:2 dilution.
[c] Calculated based on HPLC peak area calibration data.

tation promotes enhanced dispersion and therefore can decrease particle size allowing for greater enzyme delivery to the CTR particle surface. Industrial equipment set up for PET processing, such as aqueous dyeing, has considerable agitation capabilities to enhance enzyme-catalyzed hydrolysis. Increased agitation decreases the amount of enzyme required and reduces the amount of time necessary for complete hydrolysis.

Effect of CTR concentration and addition of surfactant

Given that lipases catalyze reactions at the interface of hydrophobic boundaries, the effect of increased CTR concentration on the amount of hydrolysis was investigated to ascertain the need for regular repetition of the process to prevent excessive CTR buildup. It is well established that CTR has a propensity to form agglomerates.[4,16,17] As the concentration of CTR increases, the tendency to form agglomerate crystals increases. Increasing CTR particle size and, therefore, decreasing surface area, in effect reduces the size of the interfacial boundary at which the lipase can act for hydrolysis. As seen in Figure 4, at concentrations >0.3 mM CTR, there was a substantial decrease in the amount of CTR hydrolyzed. At CTR concentrations >0.3 mM, the amount of hydrolysis is significantly less than that achieved at half of the CTR concentration.

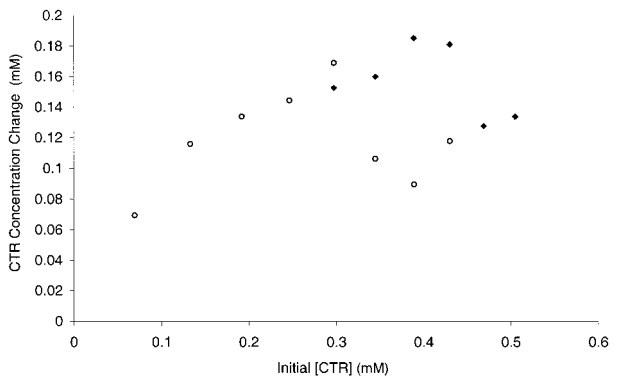


Figure 4 Concentration dependence of enzymatic hydrolysis of (○) CTR and (♦) after addition of 1 g/L of surfactant Triton X-100 (octyl phenoxypolyethoxy ethanol).

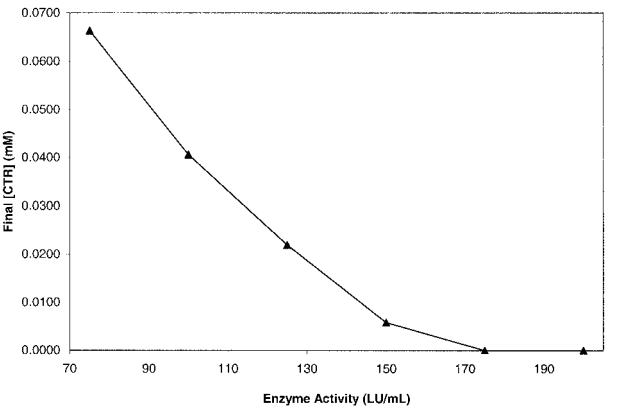


Figure 5 Effect of enzyme concentration on hydrolysis.

To substantiate the premise that agglomerate formation could be responsible for the observed reduction in hydrolysis with increased CTR concentration, a nonionic surfactant, Triton X-100 (octyl phenoxy polyethoxy ethanol), was added in an attempt to decrease particle size growth and enhance dispersion. Surfactant was effective in facilitating continued enzymatic activity beyond the initially observed limiting concentration, as shown in Figure 4. The addition of surfactant did not eliminate the phenomenon described, but instead increased the limiting concentration of maximum hydrolysis. It is likely, therefore, that regular preventative removal of CTR from processing equipment (i.e., before allowing excess buildup) would enhance enzymatic hydrolysis efficiency.

Results from the CTR concentration experiment carried out for 48 h follow the same trend as the 24-h reaction. As illustrated previously in Figure 3, there is only a slight increase in the amount of hydrolysis of the CTR after 24 h (see Table II).

Effect of enzyme activity

The effect of increased enzyme concentration on the amount of CTR hydrolysis was determined using enzyme concentrations from 75 to 200 LU/mL at 60°C and pH 8 (buffered), with agitation at 150 rpm. A direct correlation between the amount of enzyme and the extent of hydrolysis was found (Figure 5). Furthermore, it was observed that as the concentration of CTR approached zero, there was a shift in the relative ratio of MHET/TA formed (Figure 6), with more hydrolysis to TA. This result indicates that higher concentrations of the enzyme facilitated more complete continued hydrolysis of the ester bonds of MHET after all of the CTR had been hydrolyzed. Hence, higher concentrations of enzyme may be used to allow for the hydrolysis of higher concentrations of CTR.

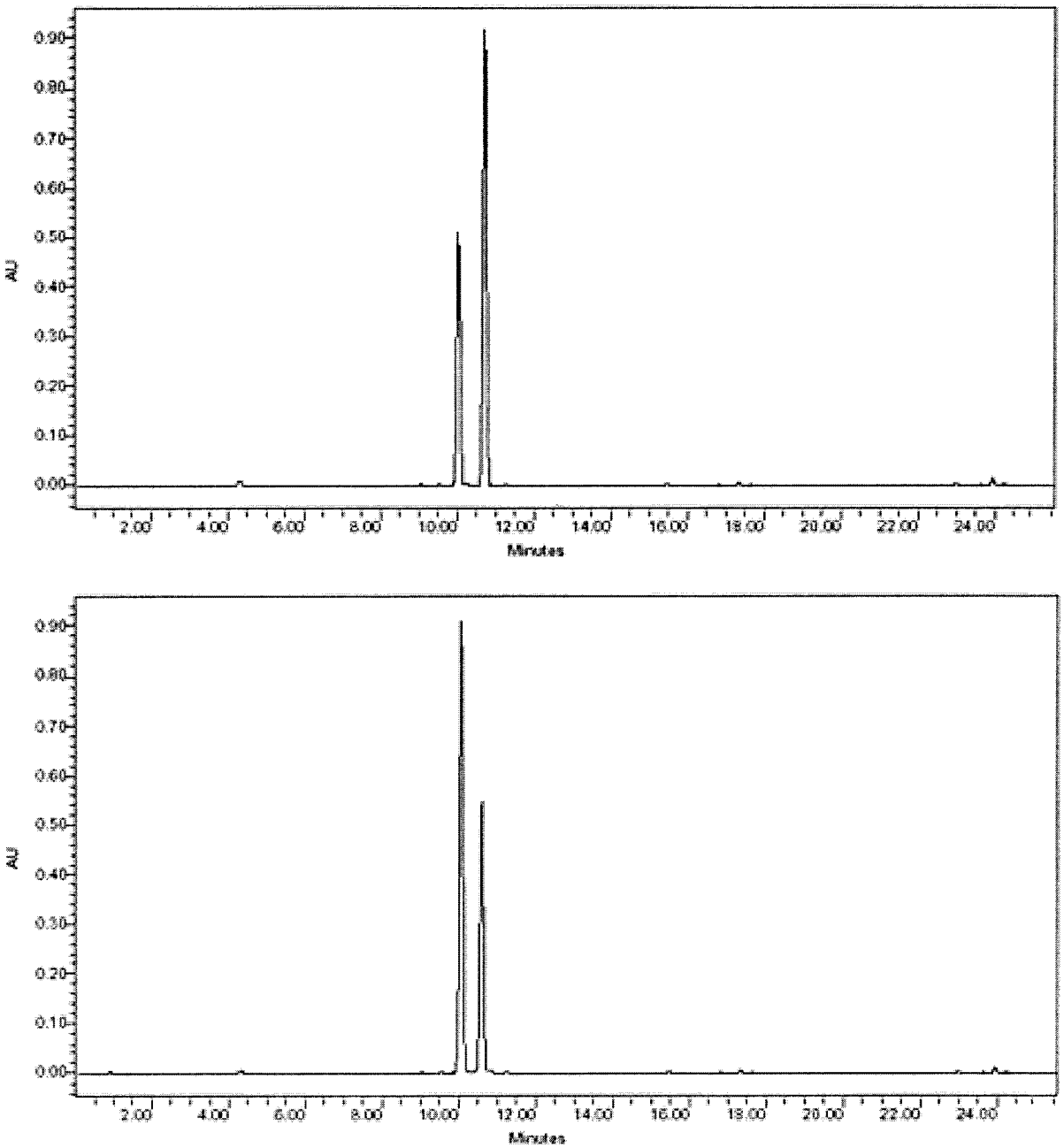


Figure 6 Chromatograms of CTR hydrolysis products using (top) 175 LU/mL enzyme (TA/MHET, 0.78:1) and (bottom) 200 UL/mL enzyme (1.64:1).

CONCLUSIONS

Cutinase-catalyzed hydrolysis of cyclic oligoesters was demonstrated using mild conditions and a simple but effective procedure. The enzymatic hydrolysis of CTR produces three significant hydrolyzed products, TA, BHET, and MHET. TA and MHET were the predominant products, and BHET was found in trace amounts. Regular preventative maintenance to limit the formation of CTR agglomerates, use of surfactant, vigorous agitation, and a highly active and specific enzyme will likely be required for complete enzyme-catalyzed hydrolysis of cyclic trimer to be achieved on large-scale PET processing machinery. Furthermore, development of an aqueous-based method without the incorporation of any organic solvent will be a requirement, and further work is required to address this need. The development of cutinase-catalyzed hydrolysis of highly insoluble cyclic trimer offers a new approach toward the removal and possibly even the use of oligomeric polycondensation byproducts.

The authors sincerely thank Henrik Lund, Sonja Salmon, Joe Jump, and Stefanie McCloskey of Novozymes North America, Inc., for very helpful discussions and suggestions. This

work was supported by the NCSU College of Textiles Novozymes Fellowship.

References

1. Cimeciouglu, A.I.; Zeronian, S.H.; Alger, K.W.; Collins, M.J. J Appl Polym Sci 1986, 32, 4719–4733.
2. Calhoun, F.S.; Bradford, K.C.; Holden, W.H. Text Chem Color 1975, 7(8), 19.
3. Drews, M.J.; Jordan, C. Text Chem Color 1998, 30, 6, 13–20.
4. Valk, G.; Loers, E.; Kueppers, P. Melliand Textilber 1970, 5, 504–508.
5. Yang,Y.; Shiqi, L. Text Chem Color Am Dyest Rep 2000, 32(10), 42–46.
6. Bach, E.; Cleve, E.; Schollmeyer, E.; Vardag, T.; Korner, P. Melliand Int 1999, 2, 165–168.
7. Montero, G.; Hinks, D. Proceedings of 6th Conference on Supercritical Fluids and Their Applications (Italy), Sept 9–12, 2001; pp. 571–578.
8. Bartle, K.; Boddington, T.; Clifford, A.; Cotton, N. Anal Chem 1991, 63, 2371–2377.
9. Küppers, St. Chromatographia 1992, 33, 434–440.
10. Cotton, N.; Bartle, K.; Clifford, A. J Chromatogr Sci 1993, 31, 157–161.
11. Johri, B.N.; Ahmad, S. Biotechnology 1999, 219–243.
12. Schmid, R.D. Angew Chem Int 1998, 37, 1608–1633.
13. Riegels, M.; Koch, R.; Pedersen, L.S.; Lund, H.(to Novo Nordisk A/S) U.S. Pat. 6,184,010 ().
14. Kolattukudy, P. Adv Biochem Eng Biotechnol 2001, 71(Biopolyesters), 1–49.
15. Longhi, S.; Cambillau, C. Biochim Biophys Acta 1999, 1441(2–3), 185–196.
16. Mohapatra, C.R. Textile Dyer Printer 1984, 17(24), 21–25.
17. Shah, D.L. Man-Made Text (India) 1981, 24(2), 81–84.