Enzymatic Degradation of Poly(D,L-lactide) and Its Blends with Poly(vinyl acetate)

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ABSTRACT: The enzymatic degradation of poly(D,L-lactide) (PLA) was investigated using two different lipases, Novozym 435 and Lipolase. The optimum temperature was 50°C for the enzymatic degradation of PLA. The effect of various solvents on the degradation of PLA was investigated at 50°C using Novozym 435, and toluene was found to be the best solvent among the solvents investigated. The enzymatic degradation of the blends of PLA and PVAc was investigated at 50°C in toluene. The enzymatic degradation of the blends of PLA and PVAc showed that there is an interaction between the polymers during degradation, which results in the reduction of degradation rate of both polymers in the blend. A continuous distribution model was used to determine the rate coefficients for polymer degradation. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 675–680, 2006

Key words: enzymatic degradation; poly(D,L-lactide); poly(vinyl acetate); continuous distribution kinetics; lipases

INTRODUCTION

Polymers like poly(3-hydroxybutyrate) (PHB), poly(lactide), poly(lactic acid), poly(ε-caprolactone) (PCL), and poly(vinyl acetate) (PVAc) have attracted much attention recently because of their biodegradability. Because of the poor mechanical strength and thermal properties of these polymers, blending of these polymers with biocompatible polymers has attracted much attention.[1–3] Several investigations have been carried out on the miscibility and biodegradability of PHB with different polymers.[4–7] When PHB is blended with ethyl cellulose (EtC),[4] poly(1,4-butylene adipate),[5] and poly(vinyl acetate),[5] the degradation rate of PHB decreased. The reduction in degradation rate was attributed to changes in surface hydrophobicity, disfavoring enzyme binding. However, when PHB was blended with poly(ethylene oxide)[6] and PCL,[7] enhancement in the degradation of PHB was observed and was attributed to enhancement in stable binding sites to the enzyme. Many investigations have also been conducted on the biodegradability of blends of PCL with poly(D,L-lactide) (PLA),[8,9] starch,[10] low-density polyethylene,[11] polypropylene,[11] nylon 6,[11] poly(vinyl alcohol),[12] and poly(lactic acid).[13,14] Several factors influence the rate of degradation, such as the mode of blending, the weight percentage of polymers, the enzyme, and the environmental conditions. Recently, enzymatic degradation studies have also been investigated in nonaqueous medium.[15] In the degradation of polymer in solution, the solvents play a major role because the polymers and enzymes have different conformations for different properties of the solvent (temperature, pH, viscosity, and polarity).[16,17] It was reported that the degradation of PLA and PCL were by specific chain-end scission. However, no interaction was observed for the blend of these polymers. The enzymatic degradation of PVAc[18] and its blend with PCL[19] indicated that there is a strong interaction of PCL with PVAc, resulting in rapid reduction in the degradation of PVAc. The interaction of the blends of PCL and PVAc is different for different modes of degradation. Reports on oxidative[20] and thermal[21] degradation of these polymer blends indicate that the degradation rate of PVAc increased while the degradation rate of PCL decreased. Thus, studies on the degradation kinetics of polymer blends show that the polymers either interact positively (degradation decreases), negatively (degradation increases), or have no interaction, depending on the nature of the polymer and the modes of degradation. Although there is no interaction of PCL with PLA,[15] PCL interacts strongly with PVAc,[18] and therefore, it would be interesting to investigate the enzymatic degradation behavior of the blends of PLA and PVAc. The individual polymers undergo biodegradation by different mechanisms.[15,20] PLA undergoes degradation by cleavage at the main chain acetate group,[15] whereas PVAc undergoes degradation by hydrolysis of the

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pendant acetate groups in the side chain of the polymer.[20]

In the present study, the effect of various solvents, enzymes (Novozym 435 and Lipolase), and temperatures on the enzymatic degradation of PLA and its blends with PVAc has been investigated. A continuous distribution kinetic model with interaction between the polymers was used to determine the rate coefficient for the enzymatic degradation of PLA and its blends with PVAc.

EXPERIMENTAL

Materials

Poly(D,L-lactide) (PLA) (intrinsic viscosity of 0.55–0.75; M_v 1790–2850) was purchased from Sigma–Aldrich (India). The commercial vinyl acetate monomer was obtained from Aldrich Chemicals. The solvents (THF, toluene, and benzene) were obtained from S. D. Fine Chemicals. The solvents were distilled and filtered prior to use. Benzoyl peroxide (S. D. Fine Chemicals) was purified by dissolving it in chloroform, followed by precipitating in methanol.

Poly(vinyl acetate) synthesis

Inhibitors from the vinyl acetate monomer was removed by caustic wash (5%), followed by washing with water, and finally by distillation at low temperature. To remove the remaining monomers, the obtained mass was dissolved in acetone, followed by the precipitation with hexane, and drying at lower temperature over calcium carbonate. The obtained polymer was dissolved in THF and analyzed in gel permeation chromatography (GPC) to obtain the molecular weight distribution (MWD). The number–average molecular weight and the polydispersity of the obtained PVAc was 160,000 and 1.7, respectively.

Degradation experiments

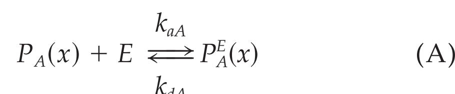
PLA solution of 2 kg/m^3 was prepared in three different solvents (toluene, benzene, xylene and dichlorobenzene). Fifteen milliliters of each solution was taken in different screw cap culture tubes, and 0.015 g of Novozym 435 was added to each tube and sealed properly. These tubes were placed in an incubator shaker water bath maintained at (50 ± 0.1)°C with PID controller. The shaker speed was kept at 50 rpm to maintain the polymer solution concentration uniform throughout the reactor volume. Aliquots of 200 μL were collected at regular intervals and analyzed in GPC. The enzyme was removed from the sample by centrifugation before analysis. Control experiments were carried out in the absence of enzyme, and no degradation was observed. Many experiments were repeated thrice, and the standard deviation in rate coefficients was less than 2%.

GPC analysis

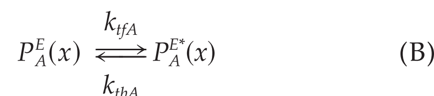
The GPC(Waters, USA) consists of isocratic pump (Waters 501) with automated gradient controller, size exclusion columns (300 mm × 7.5 mm, Styragel HR 5E, HR 3, and HR 0.5), differential refractometer (Waters R401), and a data acquisition system in series. Samples were injected in a Rheodyne valve, with a sample loop of 50 μL, and the refractive index was continuously monitored using a differential refractive index detector and stored digitally. The chromatograph was converted to molecular weight distribution, using a universal calibration curve determined using polystyrene standards (Polymer Lab, UK).

Theoretical model

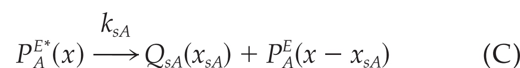
The model is similar to that developed earlier.[15] It is assumed that the molecular weight of polymer, x, is a continuous variable. P_A and P_B represent the polymer molecules of species A and B, respectively, while E refers to the enzyme. The mechanism for P_A is described later; similar mechanism is valid for P_B as well, as given in the following reactions:



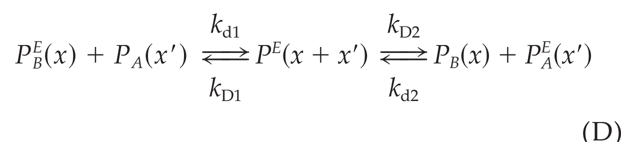
$$P_A(x) + E \underset{k_{dA}}{\overset{k_{aA}}{\rightleftharpoons}} P_A^E(x) \quad (A)$$



$$P_A^E(x) \underset{k_{tbA}}{\overset{k_{tfA}}{\rightleftharpoons}} P_A^{E^*}(x) \quad (B)$$



$$P_A^{E^*}(x) \xrightarrow{k_{sA}} Q_{sA}(x_{sA}) + P_A^E(x - x_{sA}) \quad (C)$$



$$P_B^E(x) + P_A(x') \underset{k_{D1}}{\overset{k_{d1}}{\rightleftharpoons}} P^E(x + x') \underset{k_{d2}}{\overset{k_{D2}}{\rightleftharpoons}} P_B(x) + P_A^E(x') \quad (D)$$

Reaction (A) represents the reversible adsorption of enzymes over the polymer. where $P_A^E(x)$ is the adsorbed polymer substrate of the species A. Reaction (B) represents a reversible reaction where the adsorbed polymer substrate undergoes transition to an activated complex, $P_A^{E^*}$. Reaction (C) represents the irreversible chain end scission of the activated complex to a smaller polymer chain and a specific product, Q_{sA}. In reaction (D), the adsorbed enzyme-polymer B intermediate, $P_B^E(x)$, combines with polymer A to form an intermediate complex that undergoes transforma-

tion to polymer B and an intermediate, $P_A^E(x)$. The coefficient k_{d1} is the interaction coefficient for polymer A with transition complex of B, and k_{D1} is the interaction coefficient for polymer and transition complex B. The rate coefficients are multiplied by the activity (a) of the enzyme, which decreases with time, as the enzyme deactivates. The population balance for polymer A can be represented as follows:

$$\frac{\partial p_A}{\partial t} = -k_{aA}p_A e + k_{dA}p_A^E - k_{d1}p_A p_B^{E,(0)} + k_{D1}\int_x^\infty p^E(x')(1/x')\,dx' \quad (1)$$

Similarly, the population balance equation for polymer B, the transition complexes, and other species are described elsewhere.[15] Applying the moment operation $f^{(j)}(t) = \int_0^\infty x^j f(x,t)\,dx$ on the population balance equations, the following ordinary differential equations for species A and the transition complex (Reaction (D)) are obtained:

$$\frac{dp_A^{(j)}}{dt} = -k_{aA}p_A^{(j)}e + k_{dA}p_A^{E,(j)} - k_{d1}p_A^{(j)}p_B^{E,(0)} + \frac{k_{D1}p^{E,(j)}}{j+1} \quad (2)$$

$$\frac{dq_{sA}^{(j)}}{dt} = k_{sA}ax_{sA}^j p_A^{E*,(0)} \quad (3)$$

$$\frac{dp_A^{E*,(j)}}{dt} = k_{tfA}p_A^{E,(j)} - k_{tbA}p_A^{E*,(j)} - k_{sA}ap_A^{E*,(j)} \quad (4)$$

$$\frac{dp_A^{E,(j)}}{dt} = k_{aA}p_{aA}^{(j)}e - k_{dA}p_A^{E,(j)} - k_{rfA}p_A^{E,(j)} + k_{rbA}p_A^{E*,(j)} + k_{sA}a\sum_{i=0}^{j} {}^jC_i(-x_{sB})^i p_A^{E*,(j-i)} - k_{d2}p_B^{(0)}p_A^{E,(j)} + \frac{k_{D2}p^{E,(j)}}{j+1} \quad (5)$$

$$\frac{dp^{E,(j)}}{dt} = k_{d2}\sum_{i=0}^{j} {}^jC_i p_A^{E,(i)}p_B^{(j-i)} + k_{d1}\sum_{i=0}^{j} {}^jC_i p_B^{E,(i)}p_A^{(j-i)} - (k_{D1}+k_{D2})p^{E,(j)} \quad (6)$$

Similar moment equations are obtained for species B.[15]

RESULTS AND DISCUSSION

The hypothesized interaction of the polymer and enzyme-polymer species can be verified with limiting cases. When $k_{d1} = k_{d2} = k_{D1} = k_{D2} = 0$, the two polymers undergo degradation independently. The degradation rates for a polymer in a binary mixture may increase, decrease,[19–21] or show no change,[18] depending on the interaction of the polymers. Thus, the rate coefficient for the chain end scission of PLA is a function of the concentration of PVAc in the blend. The degradation rate of individual polymer can be obtained by setting the concentration of the other polymer to zero. For example, to get the degradation kinetics of polymer A (PLA) in the absence of polymer B (PVAc), one can set $p_B^{(0)} = p_B^{E,(0)} = 0$, and solve the system with a simplified exponential model to account for the deactivation, $a(t) = \exp(-k_{enz}t)$, where k_d is the specific deactivation rate. The rate of change of mass of specific product A is given as follows:

$$\frac{dq_{sA}^{(1)}}{dt} = k_A \exp(-k_{enz}t)x_{sA}p_A^{(0)} \quad (7)$$

where $k_A = \frac{k_{sA}k_{aA}}{k_{dA}}\frac{k_{tfA}}{(k_{sA}+k_{tbA})}e$. Solving eq. (7) with the initial condition $q_{sA}^{(1)}(t = 0) = 0$ yields the following equation:

$$q_{sA}^{(1)} = \frac{k_A x_{sA}p_{A0}^{(1)}}{k_{enz}M_{nA0}}(1-\exp(-k_{enz}t)) \quad (8)$$

where x_{sA}, represents the molecular weight of the specific product A, $p_{A0}^{(1)}$ represents the mass concentration of polymer A, and M_{nA0} is the initial molecular weight of polymer A. As $t \to \infty$, the enzyme is no more functional, and eq. (8) changes as follows:

$$q_{ssA} = q_{sA}^{(1)}(t \to \infty) = \frac{k_A x_{sA}p_A^{(1)}}{k_{enz}M_{nA0}} \quad (9)$$

The rationalized mass fraction of specific product formed can be obtained from eqs. (8) and (9):

$$q_r(t) = \frac{q_{sA}(t)}{q_{sA}(t \to \infty)} = 1 - \exp(-k_{enz}t) \quad (10)$$

Equation (10) indicates that a semilogarithmic plot of $1 - q_r(t)$ with time will be linear with a slope of k_{enz}. Equation (9) can then be employed to get k_A. The saturation degradation values for the polymers were measured after 15 days, when the enzyme almost becomes inactive. The effect of solvents on the degradation of PLA was investigated using four different solvents (toluene, benzene, xylene, and 1,2-dichlorobenzene) on the basis of their viscosity difference. The degradation rate coefficient decreased with increase in solvent viscosity (Figs. 1 and 2). This behavior is attributed to the decreasing diffusivity of the enzymes on to the surface of the polymers with increase in viscosity. The dependence of degradation rate coefficient on viscosity is similar to that reported earlier.[15] Because the maximum degradation rate was observed

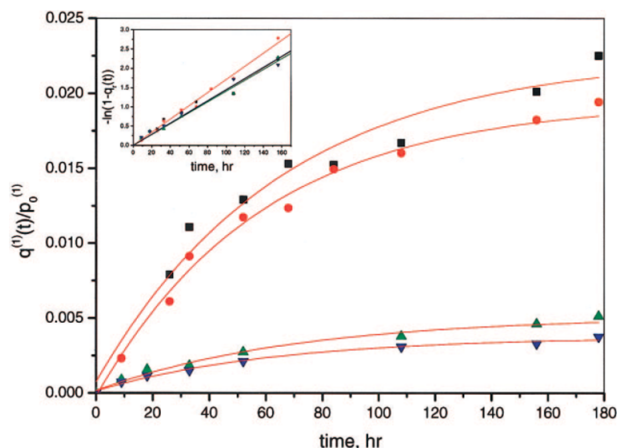


Figure 1 Variation of the mass fraction of the specific product with time by Novozym 435 at 50°C in different solvents for the degradation of PLA. The lines are fit by the model. Legends: ■, Toluene; ●, Benzene; ▲, Xylene; ▼, 1,2-Dichlorobenzene. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

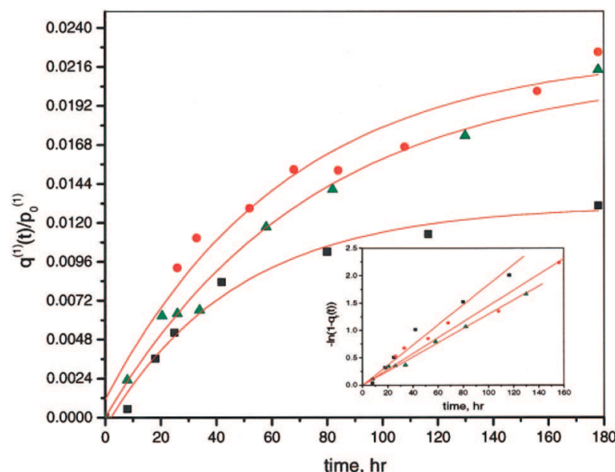


Figure 3 Variation of the mass fraction of the specific product with time in Toluene for PLA using Novozym 435. The lines represent the model fits. Legends: ■, 40°C; ●, 50°C; ▲, 60°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

in toluene, it was chosen as the solvent for rest of the experiments. The effect of temperature on the enzymatic degradation of PLA was investigated at 40, 50, and 60°C in toluene, using two different lipases (Novozym 435 (Fig. 3) and Lipolase [Fig. (4)]. The solid lines shown are model prediction by eq. (10). The effect of temperature on the degradation rate coefficients of polymers was determined from the plot of k_A/k_{enz} with temperature. The optimum temperature for degradation was 50°C (Fig. 5), which is similar to that reported earlier.[15,20] The biodegradation of PVAc was also investigated in toluene at 50°C, using Lipolase. The degradation of PVAc is less, compared with that of PLA (Fig. 6).

We next investigate the degradation of the blends. Using the zeroth moment equations, excluding the scission coefficient of the individual polymers, and using the result that the molar concentrations of the pure polymers are constant, the molar rate of formation of the specific product A is given as follows:

$$\frac{dq_{sA}^{(1)}}{dt} = k_A k_{\mathrm{int},B} x_{sA} p_A^{(0)} a \qquad (11)$$

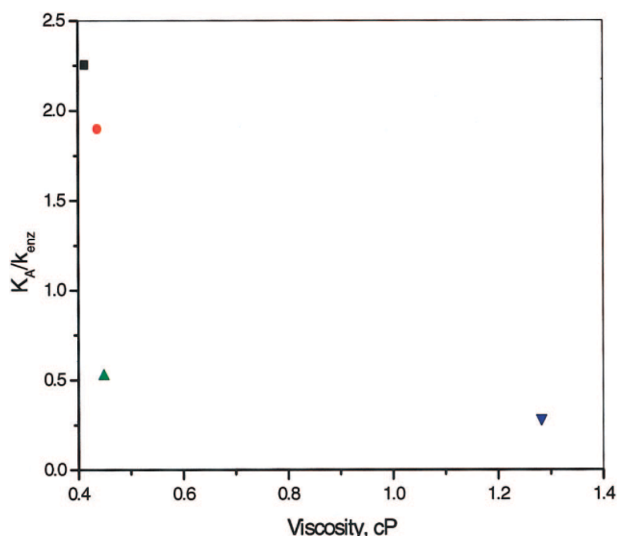


Figure 2 Dependence of rate coefficient K_A/k_{enz}, of the degradation of PLA on the viscosity of the solvent, using Novozyme 435, at 50°C in various solvents. See Figure 1 for Legends. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

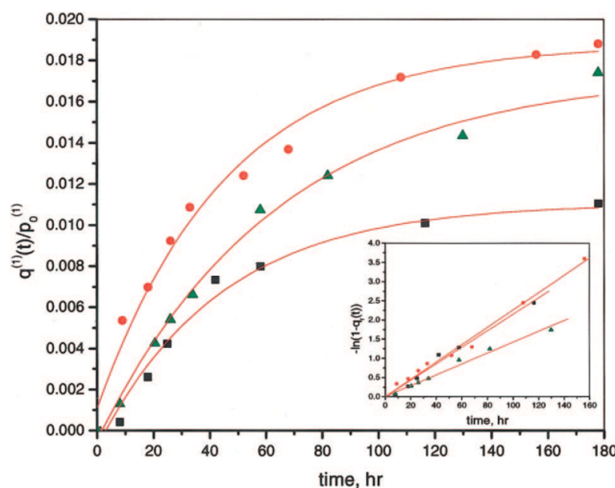


Figure 4 Variation of the mass fraction of the specific product with time in Toluene for PLA using Lipolase. See Figure 3 for legends. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

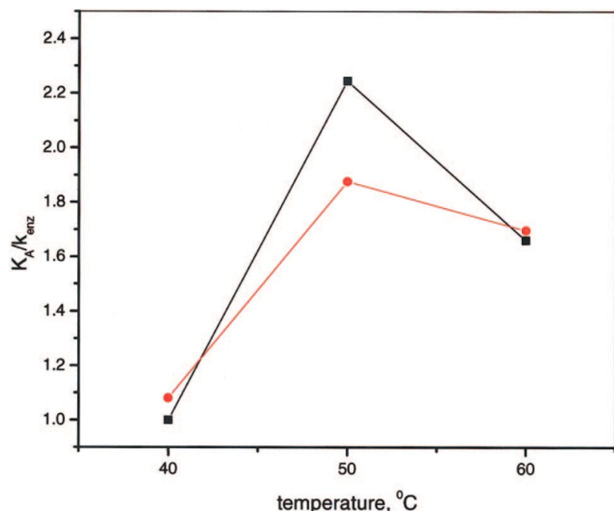


Figure 5 Variation of (K_A/k_{enz}) with temperature for enzymatic degradation of PLA in toluene. Legends: ■, Novozym 435; ●, Lipolase. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

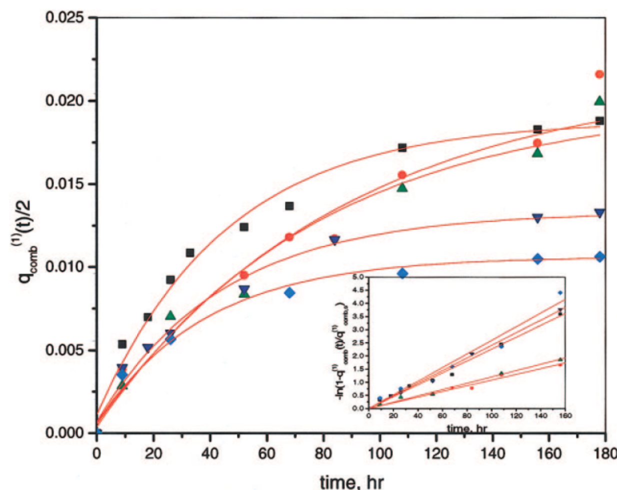


Figure 7 Effect of weight % of PVAc on the degradation of blends of PLA and PVAc in toluene, using Lipolase at 50°C. The lines represent the model fits. Legends: ■, 0% PVAc; ●, 30% PVAc; ▲, 50% PVAc; ▼, 70% PVAc; ♦, 100% PVAc. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

where $k_1 = 1 + \frac{k_d p_B^{(0)}}{k_{dA}}$, $k_2 = 1 + \frac{k_d p_A^{(0)}}{k_{dB}}$, $k_3 = \left[\left(\frac{k_{aB}k_{d1}}{k_2 k_{dB}}\right) + \left(\frac{k_{aA}k_{d2}}{k_1 k_{dA}}\right)\right] - \left[\frac{1}{(k_{D1}/k_2 + k_{D2}/k_1)}\right]$, and $k_{\mathrm{int},B} = \frac{[1 + (k_{D2}k_3/k_{aA})p_B^{(0)}]}{[1 + (k_{d2}p_B^{(0)}/k_{dA})]}$.

If the polymer B is absent, then $k_{\mathrm{int},B}$ reduces to unity, and eq. (11) reduces to the expression for the degradation of one polymer in the absence of the other. Similarly, the rate of change of specific products formed from PVAc (B) can be written as follows:

$$\frac{dq_{sB}^{(1)}}{dt} = k_B k_{\mathrm{int},A} x_{sB} p_B^{(0)} a \qquad (12)$$

In the chromatogram response from GPC, it is not possible to separate the specific products obtained from both the polymers (M_n '7e 450), and therefore, only combined response is obtained. Summing eqs. (11) and (12) followed by integration gives the following expression:

$$q_{\mathrm{comb}}^{(1)} = q_{sA}^{(1)} + q_{sB}^{(1)} = \left[\frac{k_A k_{\mathrm{int},B} x_{sA} p_A^{(0)} + k_B k_{\mathrm{int},A} x_{sB} p_B^{(0)}}{k_{\mathrm{enz}}}\right] \times [1 - \exp(-k_{\mathrm{enz}}t)] \qquad (13)$$

As $t \rightarrow \infty$,

$$q_{\mathrm{comb},s}^{(1)} = \left[\frac{k_A k_{\mathrm{int},B} x_{sA} p_A^{(0)} + k_B k_{\mathrm{int},A} x_{sB} p_B^{(0)}}{k_{\mathrm{enz}}}\right] \qquad (14)$$

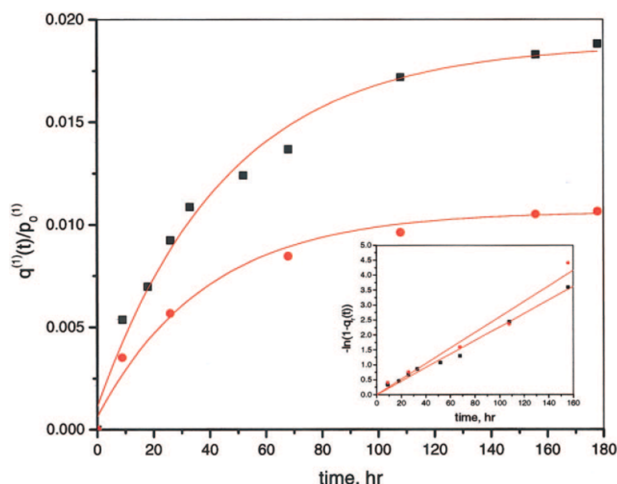


Figure 6 Comparison of the variation of the mass fraction of the specific product with time in toluene at 50°C, for PLA and PVAc, using Lipolase. The lines represent the model fits. Legends: ■, PLA; ●, PVAc. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The deactivation coefficient of the enzyme is obtained from the slope of the linear fit of $-\ln(1-[q_{\mathrm{comb}}^{(1)}/q_{\mathrm{comb},s}^{(1)}])$ with degradation time. The effect of PVAc on the biodegradation of the blends of PLA and PVAc was investigated at different concentrations (0, 30, 50, 70, and 100 wt %) of PVAc in the blend. Figure 7 indicates that the model appropriately fits the experimental data. The maximum specific product formed is determined from the saturation value. The rate coefficients, $k_{\mathrm{int},A}$ and $k_{\mathrm{int},B}$, are obtained by multivariable linear regression of the kinetic data obtained for different blend concentrations of PLA and PVAc at 50°C in toluene, using Lipolase. The parameters thus obtained are $k_{\mathrm{int},A} = 0.642$ and $k_{\mathrm{int},B}$

= 0.715. Since the interaction parameters are less than unity,[15] this indicates that there is interaction between the polymers, and the interaction leads to reduction in the degradation rate of both PLA and PVAc in the blend.

CONCLUSIONS

The biodegradation of PLA was investigated in different solvents at different temperatures, using different enzymes (Novozym 435 and Lipolase). The best solvent for degradation was found to be toluene. The optimum temperature for degradation of PLA in toluene was determined to be 50°C, using both Novozym 435 and Lipolase. The effect of the weight percentage of PVAc in a blend of PLA and PVAc was also investigated in toluene at 50°C, using Lipolase. A continuous distribution model was used to model the enzymatic degradation. Based on this model, it was found that the two polymers interact, with one polymer reducing the degradation rate of the other polymer in the blend. The model was able to predict the experimental data satisfactorily.

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References

1. Meredith, J. C.; Amis, E. J Macromol Chem Phys 2000, 201, 733.
2. Broz, M. E.; VanderHart, D. L.; Washburn, N. R. Biomaterials 2002, 24, 4181.
3. Dell'Erba, R.; Groeninckx, G.; Maglio, G.; Malinconico, M.; Migliozzi, A. Polymer 2001, 42, 7831.
4. Finelli, L.; Scandola, M.; Sadocco, P. Macromol Chem Phys 1998, 199, 695.
5. Kumagai, Y.; Doi, Y. Polym Degrad Stab 1992, 36, 241.
6. Kumagai, Y.; Doi, Y. Polym Degrad Stab 1991, 35, 87.
7. Focarete, M. L.; Ceccorulli, G.; Scandola, M.; Kowalczuk, M. Macromolecules 1998, 31, 8485.
8. Li, S.; Liu, L.; Garreau, H.; Vert, M. Biomacromolecules 2003, 4, 372.
9. Gan, Z.; Xu, D.; Zhong, Z.; Liang, Q.; Jing, X. Polymer 1999, 40, 2859.
10. Ishiaku, U. S.; Pang, K. W.; Lee, W. S.; Mohd Ishak, Z. A. Eur Polym J 2001, 38, 393.
11. Iwamota, A.; Kokiawa, Y. Polym Degrad Stab 1994, 45, 205.
12. Takasu, A.; Aoi, K.; Tsuchiya, M.; Okada, M. J Appl Polym Sci 1999, 73, 1171.
13. Nagata, M.; Okano, F.; Sakai, W.; Tsutsumi, N. J Polym Sci Part A: Polym Chem 1998, 36, 1861.
14. Sheth, M.; Kumar, R. A.; Dave, V.; Gross, R. A.; McCarthy, S. P. J Appl Polym Sci 1997, 66, 1495.
15. Sivalingam, G.; Vijayalakshmi, S. P.; Madras, G. Ind Eng Chem Res 2004, 43, 7702.
16. Staahl, M.; Wistrand, U. J.; Maansson, M. O.; Mosbach, K. J Am Chem Soc 1991, 113, 9366.
17. Russel, A. J.; Klibanov, A. M. J Biol Chem 1988, 163, 11624.
18. Chattopadhyay, S.; Sivalingam, G.; Madras, G. Polym Degrad Stab 2003, 80, 477.
19. Sivalingam, G.; Chattopadhyay, S.; Madras, G. Chem Eng Sci 2003, 58, 2911.
20. Sivalingam, G.; Madras, G. Chem Eng Sci 2004, 59, 1577.
21. Sivalingam, G.; Madras, G. Ind Eng Chem Res 2004, 43, 1561.