Solubility and Degradation of Polyhydroxyalkanoate Biopolymers in Propylene Carbonate

Christopher W. J. McChalicher and Friedrich Srienc
Dept. of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455,
and Biotechnology Institute, University of Minnesota, St. Paul, MN 55108

Daniel P. Rouse
Biotechnology Institute, University of Minnesota, St. Paul, MN 55108

DOI 10.1002/aic.12087

New biobased materials and chemicals require different processing strategies than petroleum-derived commodities. The extraction and recovery of polyhydroxyalkanoate (PHA) biopolymers from the residual cellular biomass is particularly difficult because the polymer is accumulated within the cell. PHAs have low solubility in many classical polymer solvents and are most often dissolved using undesirable chlorinated solvents. The solubility kinetics is greatly diminished when these polymers are highly crystalline. Here, 1,2-propylene carbonate is used to dissolve highly crystalline polyhydroxybutyrate at ambient pressures and moderate temperatures. We have used kinetic studies of the dissolution of the crystalline material to determine the energy barrier for dissolution of the system. Further, the degradation of polyhydroxybutyrate and similarly prepared PHA block copolymers were studied during this extraction process using molecular weight characterization by gel permeation chromatography. Finally, we have used these findings to extract PHA block copolymers from dried biomass at the bench scale. © 2009 American Institute of Chemical Engineers *AIChE J,* 56: 1616–1625, 2010

Keywords: polymer processing, degradation biopolymers, PHAs, extraction, degradation, propylene carbonate

Introduction

Because of concerns about global warming, a firm belief in Hubbert's theory of peak oil,[1] or an increasing desire for general conservation, there has been a strengthening demand for green alternatives to current petroleum-derived products. In addition to the much discussed fuel industry, the materials industry and specifically the commodity plastics sector must undergo a drastic overhauling of technology to meet these requirements. Polyhydroxyalkanoates (PHAs) have been suggested as green alternatives to some conventional commodity plastics. In general, the PHA family of polymer has a wide set of compositions with more than 150 monomers identified.[2] These polymers have a wide range of performance characteristics and can be produced in various wild-type and recombinant microorganisms. Also, many PHAs can be made from various natural substrates and waste streams[3–5] and many are biodegradable.[6–9]

One hurdle in widespread commercialization of PHA biopolymers has been the efficient scale-up of separation processes, which remove the internally accumulated biopolymers from the cell debris. At the laboratory scale, volatile halogenated solvents (such as chloroform and 1,2,4-trichlorobenzene) are often used at reflux to extract gram quantities of PHAs from dried cell pellets. A nonsolvent, such as methanol, is often added to induce precipitation of PHAs suitable for chemical, mechanical, or physical characterization.[10] At

---

Correspondence concerning this article should be addressed to F. Srienc at srienc@umn.edu.

larger scales, these solvents become undesirable for a variety of perspectives, including cost, process safety, and residual solvent contamination of the end polymer.

Here, the underlying challenge is to overcome the latent energy associated with the crystallization of some of the most common varieties of PHAs, namely poly(3-hydroxybutyrate) (PHB). The solubility of PHB was calculated for 38 chemicals ranging from nonpolar hydrocarbons to acids and bases and to nitrogen- and sulfur-containing compounds.[11] These chemicals were classified as solvents, partial solvents, or nonsolvents. The ideal solvent would be useful for extraction at near-atmospheric pressures, dissolve PHAs well below the polymer autocatalytic degradation temperature, minimize the degradation of the polymer chains, be recyclable, and have low environmental risks.

Several alternatives to the volatile halogenated compounds have been suggested in the academic and patent literatures for a solvent extraction process. In the academic literature, designer solvents such as ionic liquids[12] have been suggested as useful for both PHA extraction as well as a medium for in vitro enzymatic PHA synthesis.[12] Ionic liquids are attractive because they have a low vapor pressure at typical process conditions and have been demonstrated to be environmentally benign. Unfortunately, it has also been demonstrated that small amounts of water contamination combined with the dense ionic matrix of these liquids often results in unwanted hydrolysis of the polymer chains at the extraction temperatures.

The patent literature often focuses on the use of traditional organic chemistry solvents at elevated pressures.[13–21,22] Common ingredients in these extraction cocktails include acetone, esters (such as ethyl acetate), alcohols (such as 1-propanol, 1-butanol, and 1-pentanol), and ketones (such as methyl ethyl ketone and methyl isobutyl ketone). Typical pressures of these systems range from 1 to 10 atmospheres so that these volatile organics can be used to extract PHAs at temperatures from 100 to 150°C. Like most patent literatures, these articles provide only a brief overview of the extraction systems and rarely consider the problems associated with the degradation of the polyester linkages in these solvent systems.

Additional methods have been demonstrated throughout the academic literature. Cell digestion methods focus on disrupting the integrity of the cells by either chemical (surfactants or alkaline treatment)[23] or biological (enzymatic)[24,25] means to liberate internally accumulated granules. Several mechanical disruption methods have also been discussed, including bead mills, high shear rates in fluid flows, and ultrasonication. After these disruption methods, a classification method, such as air flotation is used to separate PHA granules from the cell debris. Unfortunately, these methods often leave high costs associated with large volumes of solvent waste,[26] a high consumption rate of valuable surfactants or enzymes,[24,25] or the energy and water use associated with multiple passes of inefficient disruption or separation by water-based classifications.[27] Therefore, it seems likely that a commercial process which can provide PHAs at reasonable costs will probably focus, at least in part, on a solvent extraction-based recovery.

Fully defined investigations for PHA/solvent systems have rarely been reported in the literature. In addition to dissolution kinetics expressed as the concentration of PHA dissolved as a function of time and temperature, it is important to note the state of the polymer before dissolution. For example, a sample of PHB which has been resting for long time will be more crystalline, and thus harder to dissolve, than PHB freshly isolated from amorphous cell granules. Furthermore, without an indication of polymer molecular weight, the method of solubility is not clear. Polymers of lower molecular weight are generally more soluble than similar systems of higher molecular weight polymers. Therefore, if the polymers undergo hydrolysis, such as in the case for some ionic liquids, the apparent solubility will be supplemented by the degradation of the chains.

With these considerations in mind, we have recently reexamined a solvent extraction process based on 1,2-propylene carbonate. A thesis[28] and two patents[29,30] describe the high solubility of PHAs in propylene carbonate at temperatures from 120 to 150°C. In these studies, the molecular weight degradation of the system was determined by the measurement of intrinsic viscosity. However, the measurement of intrinsic viscosity is a bulk average measurement that results in a single viewpoint of the polymer chain distribution. Better methods are now available, such as gel permeation chromatography (GPC), which allow us to track the entire population of polymer chains during degradation.

From the view of green chemistry, propylene carbonate has many appealing characteristics. A high boiling point (240°C) when compared with our potential processing temperatures ensures a low risk of evaporative loss.[31] Also, the widespread of the boiling points of propylene carbonate and nonsolvents such as methanol suggest that these solvents can be easily separated and reused after recovery of the PHAs. Therefore, the majority of the separation could be potentially accomplished with recycled solvents, limiting the consumption and investment for new and expensive process fluids.

The toxicity data are also promising.[32] Although there is justifiable concern over the presence of residual solvents in plastics, propylene carbonate has already been approved for use in many sensitive applications such as cosmetics. Studies have shown that propylene carbonate is not readily absorbed through the skin, and is therefore used in a variety of cosmetics at concentrations up to 5%. The LD_{50}-value of a single dose in rats in a short-term study was shown to be 29.1 g propylene carbonate per kg of rat body weight. A long-term study consisting of up to 5000 mg doses of propylene carbonate per kg of rat body weight per day showed no effects after 90 days. Although these studies are not conclusive, they suggest that propylene carbonate may be suitably used for sensitive applications such as food-grade plastics.[32]

In this study, we have examined the solubility kinetics of highly crystalline PHB in propylene carbonate at various temperatures. We have also examined the rates of degradation of PHB with respect to time and temperature and analyzed the resulting molecular weight using high-temperature GPC. The study of polymer degradation is extended toward PHB-*b*-PHBV block copolymer synthesized in *Cupriavidus necator*. Finally, a model is proposed to determine the properties and characteristic constants for the scalable extraction of PHAs from biomass using propylene carbonate.

Experimental

Polymers

Several experiments used a stock of powdered Biopol® polymer received by our laboratory in 1995 from Monsanto

Corporation (St. Louis, MO). Freshly prepared PHB homopolymer and PHB-*b*-PHBV bioblock copolymer were also used. These polymers were synthesized using a two-step cultivation method described previously.[33]

Determination of dissolution kinetics

The stocks of Biopol® powder suspended in 1,2-propylene carbonate (0.5 g PHB per 5 mL solvent; Sigma, St. Louis, MO) was prepared so that the final concentration was 100 g/L. This stock was then split three ways into open top glass tubes and used for replicates of a single-temperature experiment. The tubes were placed into a mineral oil bath held at constant temperature by use of feedback control. Samples were mixed before sampling by bubbling air through a glass Pasteur pipette. The samples were withdrawn at defined times and diluted with same-temperature propylene carbonate so that the undissolved fraction was less than 2 g/L. These dilutions were analyzed by spectrophotometry at 400 nm to determine the percent transmission. Standard solutions were analyzed by the sample method to determine a suitable calibration curve.

Determination of maximum solubility

Various concentrations (between 1 and 350 g/L) of Biopol® in 1,2-propylene carbonate were used to determine the maximum solubility of PHB in the solvent at temperatures ranging from 100 to 140°C. Five milliliters of each concentration was incubated at the desired temperature in a glass test tube for 15 min. The extent of dissolution was determined by the spectrophotometric method described earlier.

Kinetics of molecular weight degradation

Freshly prepared PHB homopolymer and PHA block copolymer were used for studies investigating degradation kinetics. The freshly prepared PHAs were initially extracted using chloroform at reflux. Rather than precipitating the polymer, 3 mL of the chloroform solution (approximately 11 g/L PHA) was allowed to evaporate from glass test tubes at 80°C, leaving behind film coatings of PHA. This method provided a consistent background to compare the effect of temperature on the degradation of polymer chain molecular weight. The PHA polymer film that results from the evaporation redissolves quickly and provides a more suitable starting point for kinetic analysis. Propylene carbonate (2 mL) was added to each tube. These tubes were transferred to a mineral oil bath held at the degradation temperature of interest. This procedure was repeated for temperatures ranging from 120 to 150°C. The samples (100 μL) were withdrawn at defined time points and immediately diluted 10-fold with tetrahydrofuran (THF) to a final volume of 1 mL.

Analysis of polymer molecular weight

Samples were analyzed by GPC using THF as the mobile phase at 35°C. An HPLC system with RID and UV detectors (Shimadzu, Kyoto, Japan) was fitted with a PLgel Mixed-C column (Polymer Labs/Varian, Palo Alto, CA). The flow rate of the mobile phase was 1 mL/min. A universal calibration curve was generated using polystyrene (PS) standards. Molecular weight calculations are reported as PS equivalents.

Dissolution kinetics of PHAs from dried biomass

Results from the controlled dissolution and degradation experiments were used to develop a PHA extraction strategy from dried biomass. Crushed, freeze-dried biomass containing 72% (by weight) PHA block copolymer at concentrations of 15 or 30 g/L total biomass was incubated in propylene carbonate at 120°C for 15 min (0.3 g and 0.6 g in 20 mL solvent). Biomass composition was determined using gravimetric analysis and gas chromatography of a propanolysis product as discussed previously.[33] Samples (0.5 mL) were periodically withdrawn and filtered through glass wool in a Pasteur pipette to remove the residual solids (cell debris and undissolved polymer). PHAs were precipitated from 100 μL of the filtered propylene carbonate through the addition of 2 mL of methanol (Sigma). The PHA/propylene carbonate/methanol mixture was incubated overnight at 4°C to allow full precipitation and agglomeration. The precipitate was recovered by centrifugation and washed twice with 0.5 mL of cold 1-propanol. The precipitates were derivatized by a propanolysis reaction (1-propanol), and the products were analyzed by gas chromatography.

Solvent recovery

Filter permeates containing propylene carbonate and methanol were recovered using a rotary evaporator (rotovap). The boiler was placed in a water bath at 90°C, and then vacuum was applied using a vacuum aspirator. Cold tap water was passed through the condensing stage to recover the methanol. A propylene carbonate-rich phase was recovered from the boiler.

Analysis of polymer content and concentration

A gas chromatography method was used to determine PHA concentration for the final recovery experiment. PHA polymer was derivatized by propanolysis as described previously.[33] The derivatized products were analyzed on a Shimadzu GC-17A equipped with a DB-WAX column and a flame ionization detector. An internal standard of benzoic acid and a calibration curve of known concentrations of PHB in 1,2-dichloroethane were used to build the calibration curve. Gas chromatography was used to determine the concentrations in the controlled dissolution and degradation experiments, as well as the downstream experiment to determine the dissolution of PHAs from dried biomass.

Results and Discussion

Determination of solubility kinetics

The kinetics of dissolving highly crystalline Biopol® PHB powder in 1,2-propylene carbonate was investigated at various temperatures. In this system, a spectrophotometer was used at 400 nm to determine the extent of dissolution for this polymer powder by measurement of the residual turbidity of the solution. This rapid assay permitted frequent and reliable sampling, which resulted in detailed monitoring of the dissolution process. Figure 1 illustrates the absorbance at several wavelengths (400, 500, and 600 nm) for PHB powder suspended in cold propylene carbonate (Panel A) and PHB powder fully dissolved in hot propylene carbonate (Panel B).

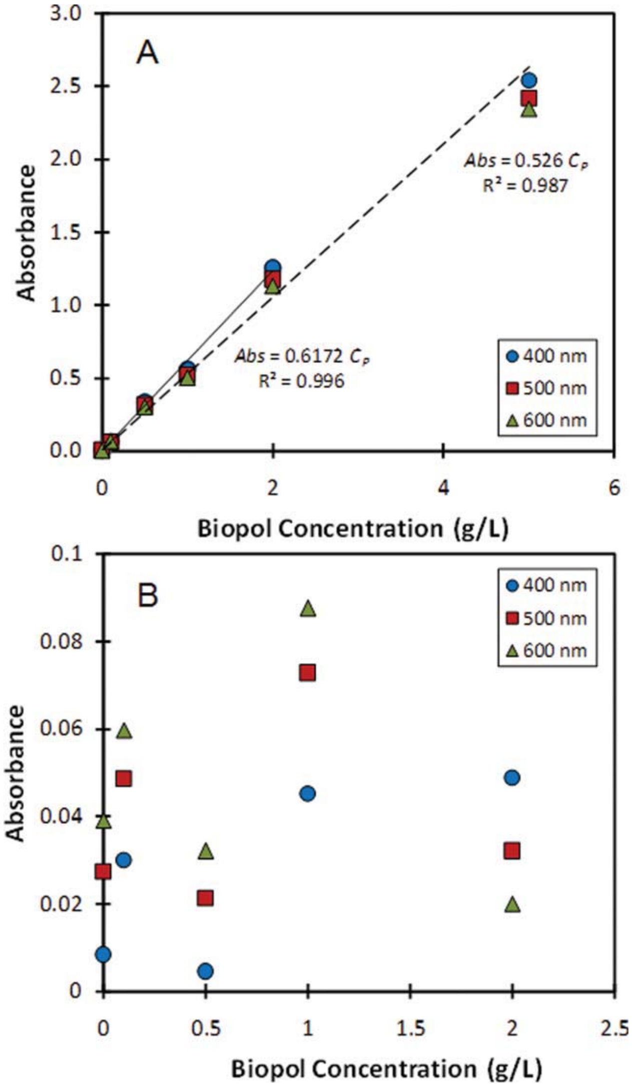

Figure 1. Absorbance of undissolved and dissolved Biopol® powder in 1,2-propylene carbonate at various wavelengths as determined by residual turbidity measurements.

(A) Calibration curve for the residual turbidity measurements for various wavelengths for undissolved PHA biopolymer suspended in propylene carbonate. (B) Residual turbidity for PHA polymers dissolved in propylene carbonate measured by various wavelengths. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In Panel A, we see that a linear dependence exists between absorbance and the concentration of the PHB powder for the three wavelengths. Also, we observed that above concentrations of 2 g/L, this dependence is no longer linear. Linear regressions are presented for the calibration curve from 0 to 2 g/L and from 0 to 5 g/L concentrations. Inclusion of the 5 g/L data point decreases the fitness of the regression and results in a bias of the residual errors (systematic under calibration) at lower concentrations. Therefore, samples were diluted so that the nondissolved fraction was less than 2 g/L in the sample during analysis to ensure that the sample readings were within the linear region. The corresponding dilution factor was used to determine the real concentration of solid-phase PHB at that time point. Furthermore, Panel B illustrates a low-level background for dissolved polymers at these concentrations. From this data, we chose to use 400 nm as our analysis wavelength because of the generally lower background readings for the dissolved polymer and because of the more sensitive slope for the solid-phase PHB particulates.

The panels of Figure 2 represent the dissolution kinetics of PHB vs. time for several temperatures. Panel A–D contain

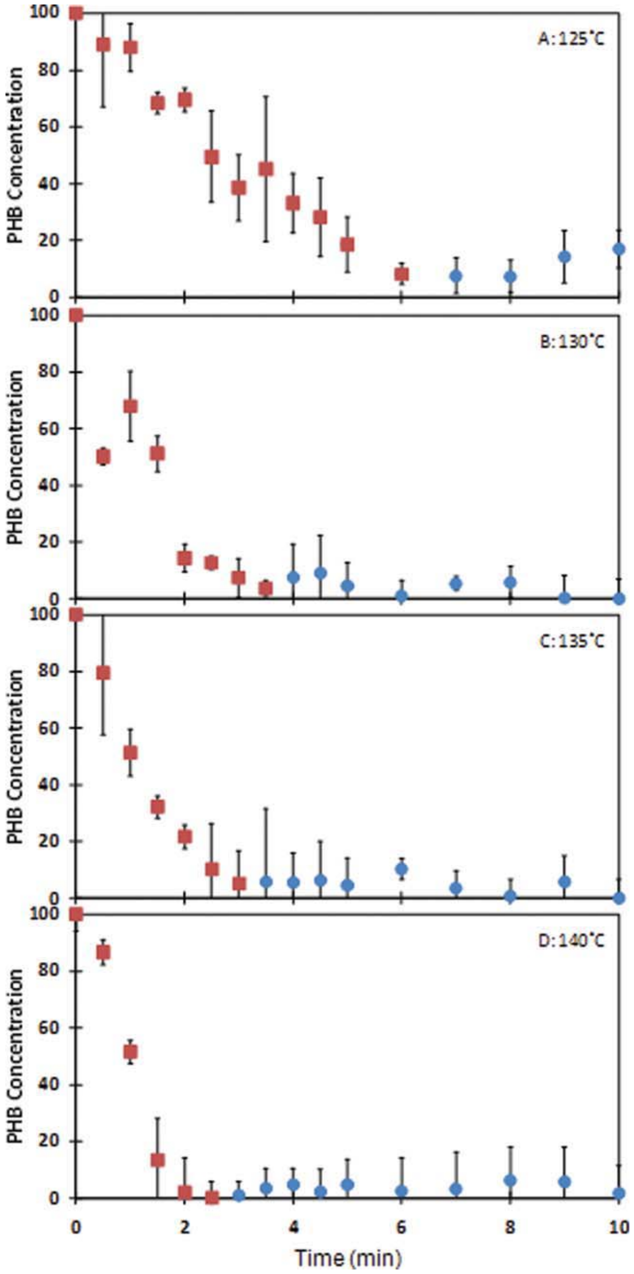

Figure 2. Dissolution kinetics of crystalline PHB powder in 1,2-propylene carbonate at several temperatures as determined by residual turbidity measurements.

Error bars represent standard deviation of three replicates. (A) 125°C; (B) 130°C; (C) 135°C; (D) 140°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table 1. Dissolution of Crystalline PHB in 1,2-Propylene Carbonate at Various Temperatures

Temperature (°C ± 2°C)	Last Concentration Dissolved (g PHB L^{-1})	First Concentration Not Fully Dissolved (g PHB L^{-1})	Approximate Solubility Limit (g PHB L^{-1})
100	N/A*	N/A	–
110	N/A	N/A	–
115	1	10	5
120	10	25	15
125	100	150	130
130	250	300	280
140	350	–	>350

*N/A indicates that no dissolution was observed for any concentration.

results for temperatures of 125, 130, 135, and 140°C, respectively. The amount of solid-phase PHB remaining as determined by the spectrophotometric method is plotted vs. time in minutes. Each sample was initially charged with 100 g/L Biopol® PHB in 1,2-propylene carbonate. Each curve represents an average of three runs, and the vertical error bars represent the standard deviation for each sample time. For all temperatures, full dissolution was observed within 6 min. Full dissolution is marked in Figure 2 by the transition from data points marked by red squares (still dissolving) to blue circles (fully dissolved) in the time courses. Error bars are reported, and they are the standard deviations of three repetitions.

We also investigated the maximum solubility of Biopol® PHB in 1,2-propylene carbonate. Table 1 shows the solubility range for temperatures ranging from 110 to 150°C. Several concentrations were tested at each temperature to determine the range of the maximum solubility. The temperature of the solvent, the highest concentration of Biopol® PHB which fully dissolved, and the lowest concentration which failed to fully dissolve are reported in Table 1. The maximum solubility at a given temperature is therefore bounded by those concentrations and is estimated in Table 1 based on the observations of the undissolved sample.

Estimation of scalable solubility parameters

A model can be developed to describe the solubility of the Biopol®/propylene carbonate system using a mass balance approach. This system is characterized by small particles of highly crystalline polymer suspended in an isothermal solvent. Because solution occurs at the interface between the crystalline polymers and the solvent, it is reasonable to characterize the system as a surface-driven phenomenon. Therefore, a mass balance is used over the total mass of polymer in granule form at a given time, $M_P(t)$, as shown in Eq. 1. In this equation, $k_M(T)$ is the first-order dissolution rate at a given temperature per unit surface area. A driving force term is included to represent the surface area of the system by analysis of the surface area of individual granules, A_P. A distribution function, $P = f(A_P)dA_P$, is used to define the fraction of a particle population, which occurs between particles with area A_P and area $A_P + dA_P$. The first moment of this function represents the average area per granule. This moment is calculated by integrating the product of the distribution function with the granule area over the range of particle areas from zero ($A_P = 0$) to an area of infinity ($A_P = \infty$). This integration is then scaled by the number of particles in the system, which is equal to the number density of particles, n, multiplied by the volume of the system, V_S. Finally, the mass balance is completed by including the concentration gradient driving force. $C_S(t)$ and C_S^* are the concentrations of polymer in the solvent at a given time and at maximum solubility, respectively.

$$\frac{d}{dt}M_P(t) = k_M(T)nV_S\int_0^\infty A_P f(A_P)dA_P[C_S(t) - C_S^*]V_S \quad (1)$$

Here, the integration of the surface area probability over all the available surface areas leads to a term representing the total surface area in the system when multiplied by the proper scaling factors. This equation is simplified by recognizing that the integration in Eq. 1 results in the calculation of the average surface area of an individual particle. Furthermore, the average surface area is characterized by the corresponding radius of a granule, $\hat{R}_P$, when the assumption of spherical particles is made. These manipulations are shown in Eq. 2. In Eq. 3, we also represent the total mass of the system as a function of the radius corresponding to the average surface area of a particle. We represent the particle mass as the product of the density of the polymer in the particle phase, ρ_P, and the geometric expression for the volume of a sphere.

$$\int_0^\infty A_P f(A_P)dA_P = \overline{A}_P(t) = 4\pi\hat{R}_P^2(t) \quad (2)$$

$$M_P(t) = nV_S\hat{M}_P(t) = nV_S\rho_P\left[\frac{4}{3}\pi\hat{R}_P^3(t)\right] \quad (3)$$

Equation 3 can be easily rearranged to solve for the characteristic radius, corresponding to the average particle surface area, as a function of the total particle phase mass, $M_P(t)$. This radius definition can be used in Eq. 2. This result can be subsequently substituted for the total area in the original mass balance, Eq. 1. We arrive at a more functional form of the original mass balance in Eq. 4 after some reduction and canceling of terms.

$$\frac{d}{dt}M_P(t) = k_M(T)\left(36\pi\frac{nV_S}{{\rho_P}^2}\right)^{\frac{1}{3}}M_P^{\frac{2}{3}}(t)[C_S(t) - C_S^*]V_S \quad (4)$$

The final assumption in deriving the form of our mass balance is to assume that $C_S(t)$ is much less than C_S^*. This assumption can be made because (i) in these experiments, the value of $C_S(t)$ is limited to the initial amount of polymer distributed in the solvent, and (ii) at short times, when our implicit assumptions about the constant number of particles is valid, most of the polymer remains in the solid granule phase. The reduced form of the mass balance is expressed in Eq. 5.

$$\frac{d}{dt}M_P(t) = -k_M(T)\left(36\pi\frac{nV_S}{{\rho_P}^2}\right)^{\frac{1}{3}}M_P^{\frac{2}{3}}(t)C_S^*V_S \quad (5)$$

The dependent variable is separated from the independent variable and constants, and the equation is integrated using M_P^0 as the initial amount of polymer in the particle phase.

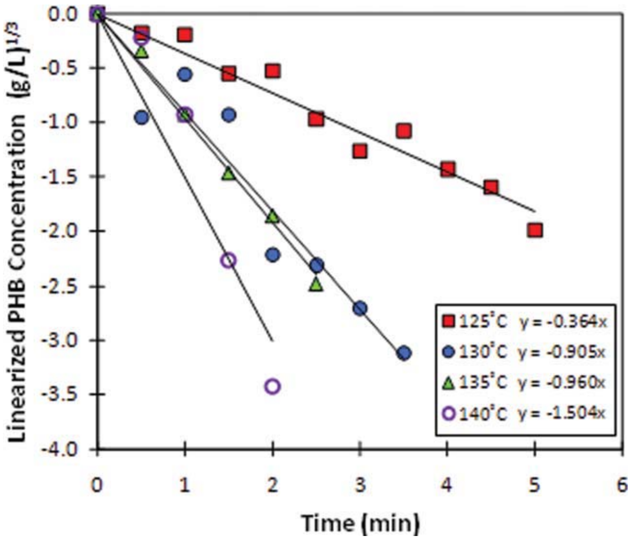

Figure 3. Linearized PHB concentration vs. time to find dissolution rates.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

$$\int_{M_{\mathrm{P}}^{0}}^{M_{\mathrm{P}}(t)} \frac{dM_{\mathrm{P}}(t)}{M_{\mathrm{P}}^{\frac{2}{3}}(t)} = 3M_{\mathrm{P}}^{\frac{1}{3}}(t) - 3M_{\mathrm{P}}^{0\frac{1}{3}} = -k_M(T)\left(36\pi\frac{nV_{\mathrm{S}}}{\rho_{\mathrm{P}}{}^2}\right)^{\frac{1}{3}} C_{\mathrm{S}}^{*} V_{\mathrm{S}} t \qquad (6)$$

The mass of the polymer in the particle phase can be expressed as the concentration by dividing by V_{S} to arrive at the final integrated version of the mass balance.

$$C_{\mathrm{P}}^{\frac{1}{3}}(t) - C_{\mathrm{P}}^{0\frac{1}{3}} = -k_M(T)\left(\frac{4\pi n}{3\rho_{\mathrm{P}}{}^2}\right)^{\frac{1}{3}} C_{\mathrm{S}}^{*} V_{\mathrm{S}} t \qquad (7)$$

The solubility data have been linearized according to Eq. 7 and are shown in Figure 3. Here, the linearized concentration is plotted against time in minutes along with a linear regression of each data set. The resulting slopes from these regressions can be found in the legend next to the appropriate symbols. These slopes, m_T, where T is the temperature of the curve of interest, represent the product of the physical terms and constants on the right-hand side of Eq. 7. The physical constants (such as polymer density), system constants (such as particle number density), and geometric factors can be clustered together for brevity into a constant, C_0. The term for the temperature-dependent mass transfer coefficient can be modeled using an Arrhenius form using a pre-exponential factor, C_1, and a term for the energy barrier for solvation, ΔE_{S}. The Boltzmann constant, k_{B}, and temperature, T, are also used in the Arrhenius model. These modifications are represented in Eq. 8.

$$m_T = -k_M(T)\left(\frac{4\pi n}{3\rho_{\mathrm{g}^2}}\right)^{\frac{1}{3}} C_{\mathrm{S}}^{*} V_{\mathrm{S}} = -k_M(T) C_0 = -C_1 e^{\frac{-\Delta E_{\mathrm{S}}}{k_{\mathrm{B}}T}} C_0 \qquad (8)$$

Applying a logarithm results in:

$$\ln(-m_T) = \ln(C_0 C_1) - \frac{\Delta E_{\mathrm{S}}}{k_{\mathrm{B}}T} \qquad (9)$$

In this format, the slopes found from the data analysis using Eq. 7 can be further used to find the solvation energy from the Arrhenius relationship. In Figure 4, the value $\ln(-m_T)$ is plotted vs. $1/T$ to determine the value of ΔE_{S}, the energy barrier associated with solvation. Using this analysis, an energy barrier for solvation for the crystalline PHB and propylene carbonate system is determined to be 118 kJ/mol.

In previous work,[28] a first-order rate of solubility was used to model the solubility kinetics. However, the parameters considered did not take into account the effects of surface area, and thus the parameter is reported as a macroscopic rate constant rather than the area-specific constant found here. Also, in this previous work, a maximum velocity was observed with increasing temperature. This same rate constant for dissolution, 22 min^{-1}, was observed at 120 to 140°C. Because the previous system used extraction from dried cells to determine the solubility kinetics, it is likely that at these elevated temperatures, the rate of dissolution was limited by the diffusion of the chains from the cell material into the bulk solvent phase. At the low end of this temperature range, we have observed an overall rate constant of 22 min^{-1} at 125°C. This is identical to the limiting rate of reaction for the previous work. At 140°C, we have observed an overall rate constant of 90 min^{-1}. Because our system has not used polymer trapped within cellular materials and should not be limited by diffusion, our rate constant continues to increase with increasing temperature.

Degradation of PHAs in propylene carbonate

A chief concern for any PHA extraction process is the effect of the process on the character of the polymer. The uncontrolled or unanticipated degradation of polymer chains can result in the production of polymers that do not meet the quality requirements of an intended application. We have

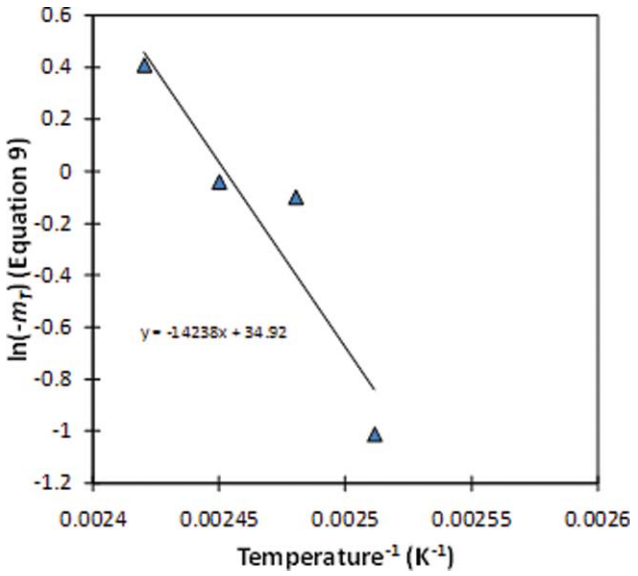

Figure 4. Arrhenius plot to determine rate constants for the dissolution of crystalline PHB in 1,2-propylene carbonate.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

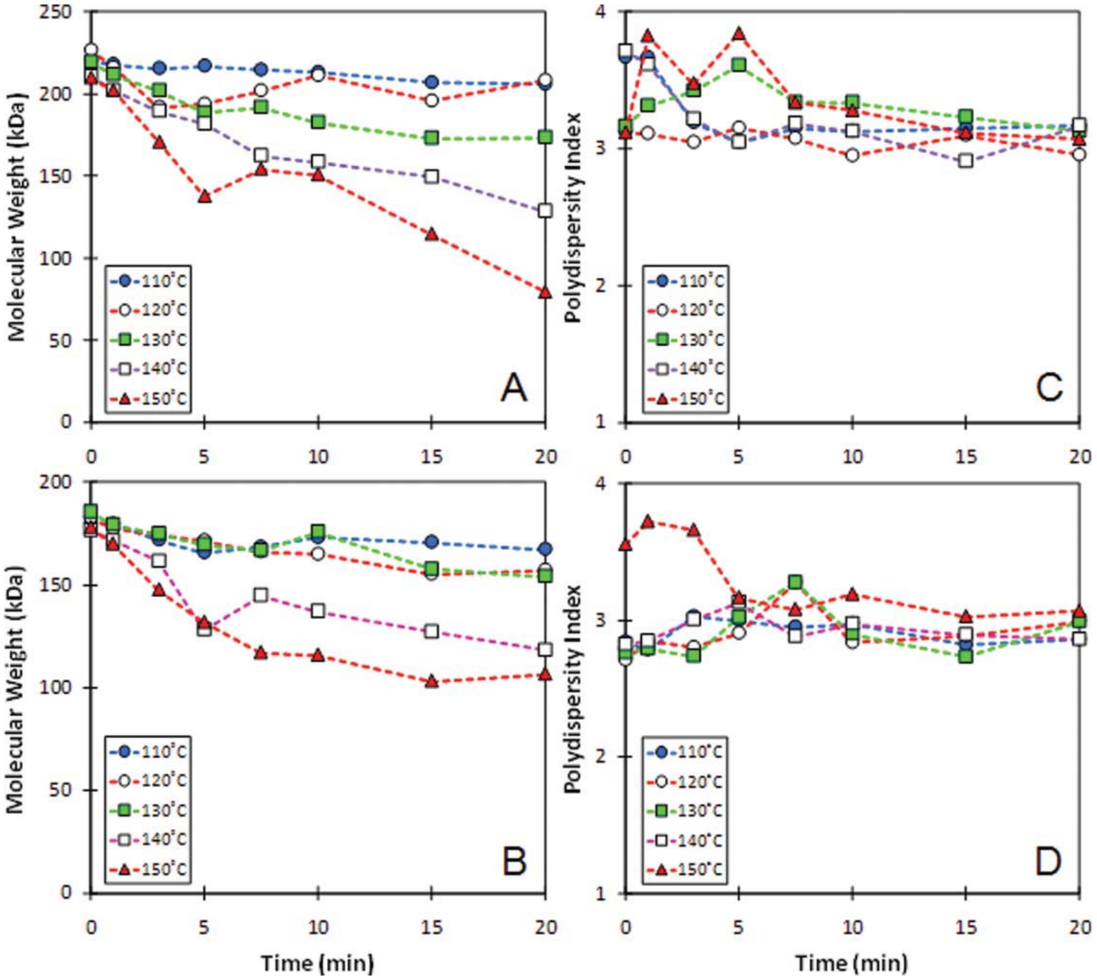

Figure 5. Number-average molecular weight (in kDa) vs. time for dissolved PHB homopolymer and PHA block copolymer at several temperatures as determined by GPC.

(A) PHB homopolymer and (B) PHA block copolymer. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

investigated the use of 1,2-propylene carbonate as a suitable solvent by measuring the kinetics of chain degradation as a function of time and temperature. In Figure 5, the number-average molecular weight (M_{n}) is plotted vs. time for temperatures ranging from 110 to 150°C. These experiments have been conducted for PHB homopolymer (Panel A) and a PHA block copolymer (Panel B) materials. In these panels, the number-average molecular weight (expressed as PS equivalent units) is plotted vs. time. The initial number-average molecular weight was 218 ± 7 kDa for the PHB homopolymer and 181 ± 4 kDa for the block copolymer samples, respectively. The average polydispersity index (PDI) of these initial points are 3.4 and 2.9, respectively. The PDI of the degradation experiments is also presented in Panel C (PHB homopolymer) and Panel D (PHA block copolymer). The PDI remains stable throughout the degradation process within a polymer type.

The temperature of the system greatly affects the degree of chain length degradation. At 110°C, the two systems degrade less than 10% after 20 min of incubation. As the temperature increases, the degree of degradation also increases. At 130°C, the polymers experience 21% and 17% reductions in number-average molecular weight for the PHB and PHA block copolymer systems, respectively. As the temperature increases to 150°C, the molecular weights of the polymer are reduced by 62% and 41%. Previous investigations of the degradation of PHAs at high temperatures (170–200°C) suggest that the degradation proceeds by a random chain scission mechanism.[34] Equation 10 shows the previously derived kinetic rate equation for the polymer chain random scission mechanism. In this equation, M_{n} and M_{n}^{0} are the number-average molecular weight at time t and at the initial time point, respectively.

$$\frac{1}{M_{\mathrm{n}}}-\frac{1}{M_{\mathrm{n}}^{0}}=kt \tag{10}$$

Equation 10 is used to linearize the data of Figure 5. The regression slopes in Figure 6 represent the kinetic rate constant for the degradation mechanism modeled by Eq. 10. An Arrhenius plot of these kinetic rate constants can be used to determine the Arrhenius prefactor and the activation energy required for PHA chain scission. The Arrhenius plot for

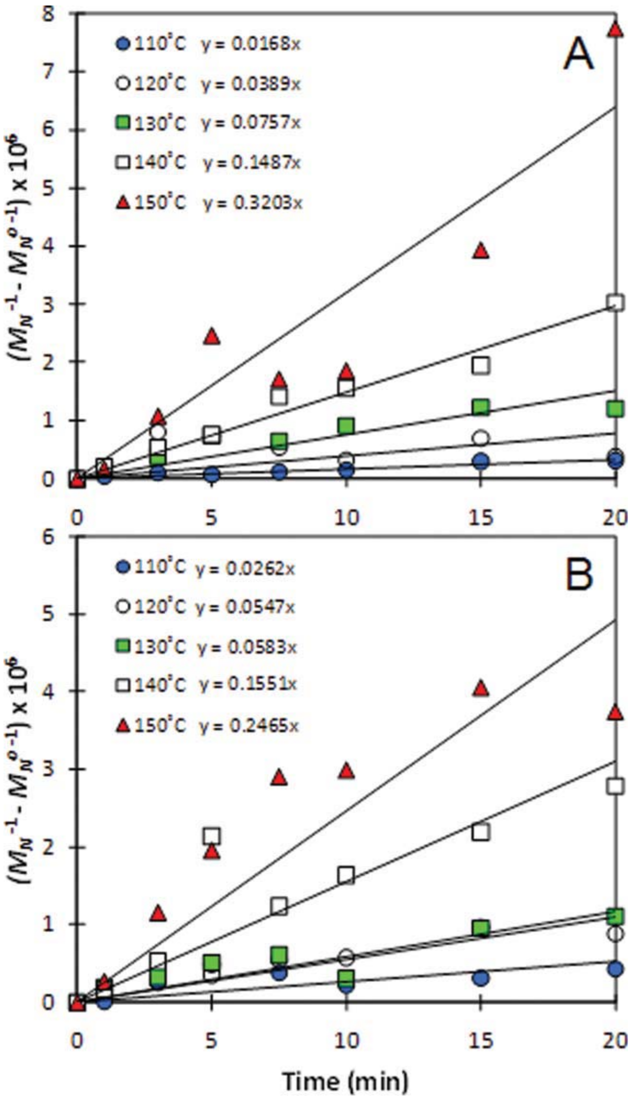

Figure 6. Linearized number-average molecular weight vs. time for dissolved PHB homopolymer and PHA block copolymer to find rates of degradation at several temperatures.

(A) PHB homopolymer and (B) PHA block copolymer. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

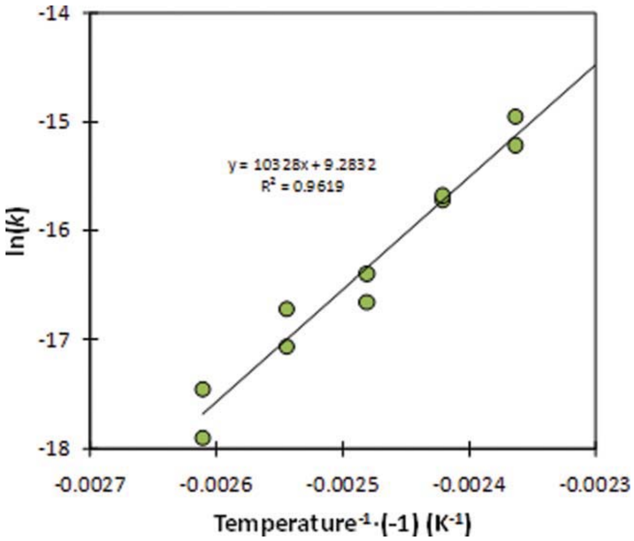

Figure 7. Arrhenius plot to determine rate constants for the degradation of PHAs in 1,2-propylene carbonate.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

chain degradation is shown in Figure 7. The data for both the degradation of PHB homopolymer and PHA block copolymer are used to calculate the Arrhenius parameters. An activation energy of 86 kJ/mol and an Arrhenius prefactor of 11,000 scissions per monomer unit per minute were determined from this analysis.

These data can be compared with two sets of previous work.[28,34] In the earlier model, a zero-order kinetic model was used to describe the chain degradation of PHAs in 1,2-propylene carbonate within the same temperature range we have investigated in this study.[28] This analysis resulted in an activation energy of 54 kJ/mol (reported in thermal energy units as 6500 K). This compares favorably with the activation energy reported here of 86 kJ/mol, considering the difference of choice of model. Another analysis considers the degradation of PHB in a vacuum at elevated temperatures (170–200°C) using a random scission model.[34] In this analysis, the activation energy was found to be 247 kJ/mol. Similar behavior was observed when the experiments were repeated in both nitrogen and air atmospheres.

The data analysis shown here indicates much lower activation energy than the degradation analyzed in the polymer melt phase. The chemical pathway for random scission in

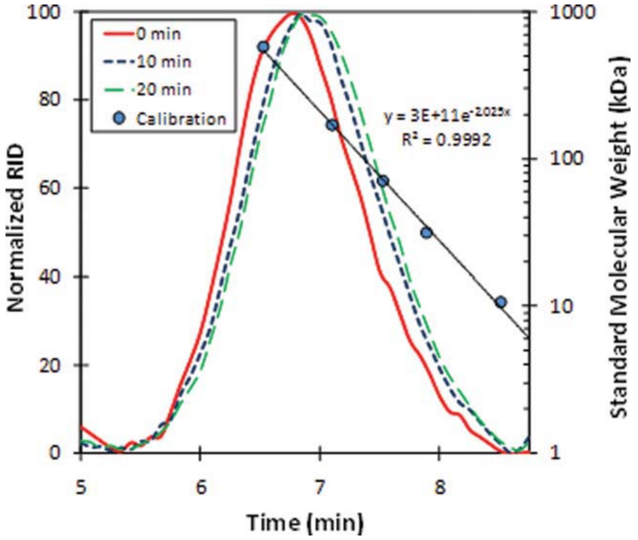

Figure 8. Molecular weight distributions of the degradation products of PHA block copolymer in 1,2-propylene carbonate at 150°C as determined by GPC.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

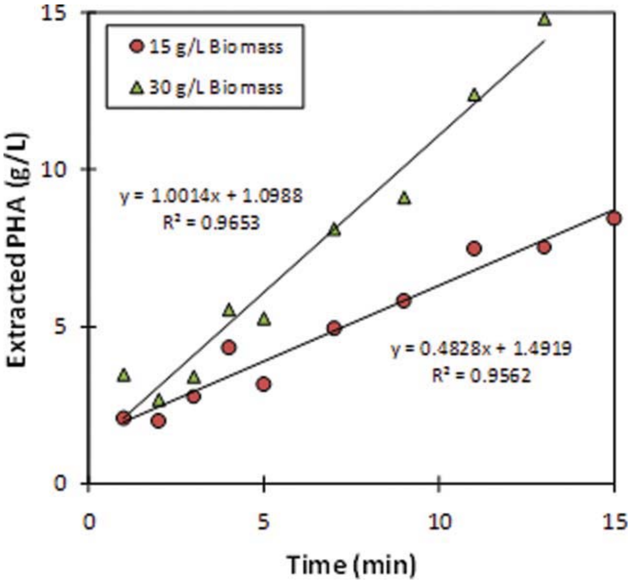

Figure 9. Extraction of PHA block copolymer from dried biomass using 1,2-propylene carbonate at 120°C.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PHB has been presented by an ester decomposition mediated by a six-membered ring intermediate.[34] In this chemical mechanism, degradation relies upon the free rotation of the polyester backbone to orient the participating atoms into the proper configuration for the degradation reaction to progress. In the melt phase, this rotation is strongly hindered, resulting in the higher activation energy. When the PHA is dissolved in 1,2-propylene carbonate, two contributing factors could result in the lower activation energy. First, dissolution in a suitable solvent may decrease the resistance for free rotation, increasing the likelihood that a given chemical unit will enter into the proper configuration. Second, the carbonate moiety in the solvent could potentially participate in a six-membered ring decomposition reaction. In this case, the higher concentration of the solvent and the relative ease of diffusion could significantly lower the activation energy of the decomposition phenomena. Since solvents such as chloroform do not result in significantly increased degradation kinetics, it is most likely that the decreased activation energy is due to the participation of 1,2-propylene carbonate in the chemical mechanism.

The use of GPC to analyze the degradation phenomena allows for observation of the complete molecular weight distribution of the sample. Figure 8 contains representative GPC traces for the degradation of PHA block copolymer in 1,2-propylene carbonate at 150°C. Traces for the 0-, 10-, and 20-min time points are shown as indicated by the legend. These traces have been normalized to the maximum peak height for each trace to make comparisons of trace shapes more convenient. The universal calibration curve prepared by the analysis of PS standards of known molecular weight is presented on the secondary ordinate axis. We see in this figure that the shape of the distribution does not change greatly over time. The initial PDI (at 0 min) of this distribution was 2.82 and the final PDI (at 20 min) was 2.87. The conservation of the distribution shape as the molecular weight degrades suggests a random degradation process with respect to molecular weight (short chains vs. long chains) and position along a chain (ends vs. middle). The negligible change in PDI agrees with the model of thermal degradation by means of random scission presented previously.[34]

Extraction of PHA from dried biomass

The previous data sets suggest that the extraction of PHAs from dried biomass should be efficient using the solvent system presented here. Figure 9 shows the recovery of PHA block copolymer from biomass vs. time at 120°C. Two concentrations of freeze-dried biomass were investigated, 15 g/L and 30 g/L. This biomass was determined to hold 72% PHA by weight, indicating that the polymer concentrations of these trials should be 10.8 g/L and 21.6 g/L, respectively. We have presented the kinetics of PHA extraction in Figure 9. After 15 min, nearly 75% of the PHA had been extracted from the cells. Because PHA block copolymer is less crystalline than the PHB powder studied earlier, a temperature of 120°C was sufficient to dissolve this material. As shown earlier, this temperature has been shown to have only a small degradation potential. Incubation at 120°C for 15 min should result in less than 15% degradation of the molecular weight of the polymer, as presented earlier.

Conclusions

We have investigated the use of 1,2-propylene carbonate for the use in PHA extraction and other processing occurring at ambient pressures. Highly crystalline PHB homopolymer powder was used to determine the dissolution kinetics of the PHA and 1,2-propylene carbonate system. The high crystallinity of this material presents a significant barrier to dissolution, and is therefore the correct choice to rigorously evaluate this solvent. More favorable kinetics should be obtained from less crystalline material (PHB or otherwise); however, systems designed with these parameters may not be robust in processing all grades of desired PHAs. We have shown that temperatures more than 125°C are required to dissolve this material. Once above 125°C, the crystalline PHB dissolves rapidly at high concentrations. Analysis of the kinetics using a surface-driven dissolution model and Arrhenius-type rate constants results in the calculation of an energy barrier for dissolution of 118 kJ/mol.

PHB homopolymer and PHA block copolymer, which have not been allowed to crystallize, were dissolved at temperatures as low as 110°C. Although we were unable to measure the kinetics of these phenomena, we were able to use these solutions to study the chemical degradation kinetics of the PHAs. Using a random chain scission model, an activation energy of 86 kJ/mol was determined. This value compares favorably to other systems involving PHAs dissolved in 1,2-propylene carbonate.[28] The activation energy of degradation found in these studies is lower than the activation energy (247 kJ/mol) found for melt-phase degradation of polymer chains.[34] We have hypothesized that this lower activation energy is due to the participation of propylene carbonate in a six-membered ring decomposition of the ester bonds along the polymer backbone.

Finally, we have demonstrated the recovery of PHA block copolymer from dried biomass at 120°C. After 15 min, up to 75% of the PHA was extracted from the cells. These demonstrations along with the solubility and degradation studies shown here suggest that 1,2-propylene carbonate is a useful solvent for the recovery and processing of short-chain-length PHAs. For extraction purposes, balancing the rate of dissolution, the diffusion and movement of the PHA chains away from the bulk cellular material, and the rate of chain degradation is necessary in designing effective large-scale recovery of PHAs. Also, when combined with potential shear-induced degradation considerations, the dissolution kinetics and temperature-induced degradation are critical when designing PHA-processing operations. Understanding these principle phenomena ensures the design of robust unit operations, which result in the production of desirable PHA materials.

Acknowledgments

The authors thank the BioTechnology Institute, University of Minnesota, and the Initiative for Renewable Energy and the Environment (IREE), University of Minnesota, for funding this work.

Literature Cited

1. Hubbert MK. Nuclear Energy and the Fossil Fuels. *Drilling Production Practice*. Washington DC: American Petroleum Institute. 1956.
2. Steinbüchel A, Lütke-Eversloh T. Metabolic engineering and pathway construction for biotechnological production of relevant polyhydroxyalkanoates in microorganisms. *Biochem Eng J.* 2003;16:81–96.
3. Khardenavis AA, Suresh Kumar M, Mudliar SN, Chakrabarti T. Biotechnological conversion of agro-industrial wastewaters into biodegradable plastic, poly β-hydroxybutyrate. *Bioresour Technol.* 2007; 98:3579–3584.
4. Yu L, Dean K, Li L. Polymer blends and composites from renewable resources. *Prog Polym Sci.* 2006;31:576–602.
5. Satoh H, Iwamoto Y, Mino T, Matsuo T. Activated sludge as a possible source of biodegradable plastic. *Water Sci Technol.* 1998;38: 103–108.
6. Jo S, Maeda M, Ooi T, Taguchi S. Production system for biodegradable polyester polyhydroxybutyrate by *Corynebacterium glutamicum*. *J Biosci Bioeng.* 2006;102:233–236.
7. Tsuge T, Kikkawa Y, Doi Y. Microbial synthesis and enzymatic degradation of renewable poly[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyhexanoate]. *Sci Technol Adv Mater.* 2004;5:449–453.
8. Lee SY, Choi JI. Production of microbial polyester by fermentation of recombinant microorganisms. *Adv Biochem Eng Biotechnol.* 2001;71:183–207.
9. Lafferty RM, Korsatko B, Korsatko W. Microbial production of poly-β-hydroxybutyric acid. In: Rehm HJ, Gees G, editors. *Biotechnology*, Vol. 6b. Weinheim: VCH-Verlagsgesellshaft, 1988:135–176.
10. Baptist JN. Process for preparing poly-*b*-hydroxybutyric acid. U.S. Pat. 3,044,942, 1962.
11. Terada M, Marchessault RH. Determination of solubility parameters for poly(3-hydroxyalkanoates). *Int J Biol Macromol.* 1999;25:207–215.
12. Gorke JT, Okrasa K, Louwagie A, Kazlauskas RJ, Srienc F. Enzymatic synthesis of poly(hydroxyalkanoates) in ionic liquids. *J Biotechnol.* 2007;132:306–313.
13. Kurdikar DL, Strauser FE, Solodar AJ, Paster MD. High temperature PHA extraction using PHA-poor solvents. U.S. Pat. 6,087,471, 2000.
14. Kurdikar DL, Strauser FE, Solodar AJ, Paster MD. Methods of PHA extraction and recovery using non-halogenated solvents. U.S. Pat. 6,043,063, 2000.
15. Noda I, Schechtman LA. Solvent extraction of polyhydroxyalkanoates from biomass. U.S. Pat. 5,942,597, 1999.
16. Stageman JF. Extraction process. U.S. Pat. 4,562,245, 1985.
17. Barham PJ, Selwood A. Extraction of poly(beta-hydroxybutyric acid). U.S. Pat. 4,391,766, 1983.
18. Senior P, Wright LF, Alderson B. Extraction process. U.S. Pat. 4,324,907, 1982.
19. Walker J, Whitton JR. Extraction of poly(beta-hydroxy butyric acid). U.S. Pat. 4,358,583, 1982.
20. Martin DP, Peoples OP, Williams SF. Methods for isolating polyhydroxyalkanoates from plants. U.S. Pat. 6,709,848, 2004.
21. Blauhut W, Gierlinger W, Strempfl F. Process for obtaining a polyhydroxyalkanoate from the cell material of a microogranism. U.S. Pat. 5,213,976, 1993.
22. Traussnig H, Kloimstein E, Kroath H, Estermann R. Extracting agents for poly-D(–)-3-hydroxybutyric acid. U.S. Pat. 4,968,611, 1990.
23. van Hee P, Elumbaring ACMR, van der Lans RGJM, Van der Wielen LAM. Selective recovery of polyhydroxyalkanoate inclusion bodies from fermentation broth by dissolved-air flotation. *J Colloid Interface Sci.* 2006;297:595–606.
24. Kapritchkoff FM, Viotti AP, Zuccolo M, Pradella JGC, Maiorano AE, Miranda EA, Bonomi A. Enzymatic recovery and purification of polyhydroxybutyrate produced by *Ralstonia eutropha*. *J Biotechnol.* 2006;122:453–462.
25. Yasotha K, Aroua MK, Ramachandran KB, Tan IKP. Recovery of medium-chain-length polyhydroxyalkanoates (PHAs) through enzymatic digestion treatments and ultrafiltration. *Biochem Eng J.* 2006; 30:260–268.
26. Ramsay JA, Berger E, Ramsay BA, Chavarie C. Recovery of poly-3-hydroxyalkanoic acid granules by a surfactant-sodium hypochlorite treatment. *Biotechnol Tech.* 1990;4:221–226.
27. Jacquel N, Lo C, Wei Y, Wu H, Wang SS. Isolation and purification of bacterial poly(3-hydroxyalkanoates). *Biochem Eng J.* 2008;39:15–27.
28. Heinzle E. *Wachstum und Synthese von Poly-b-Hydroxybuttersäure (PHB) in Alcaligenes eutrophus, Extraktion des Polymeren sowie deren mathematische Modellbildung,* Ph.D. Thesis, Technical University Graz, Austria, 1978.
29. Lafferty RM, Heinzle E. Cyclic carbonic acid esters as solvents for poly-(.beta.-hydroxybutyric acid). U.S. Pat. 4,101,533, 1978.
30. Lafferty RM, Heinzle E. Cyclic carbonic acid esters as solvents for poly-(beta-hydroxybutyric acid). U.S. Patent 4,140,741, 1978.
31. U.S.Environmental Protection Agency. Environmental profile for propylene carbonate, Cincinnati, OH: National Risk Management Research Laboratory Office of Research and Development, U.S. Environmental Protection Agency, 1998.
32. CIR. Final report on the assessment of propylene carbonate. *J Am Coll Toxicol* 1987;6:23–51.
33. Pederson EN, Mcchalicher CW, Srienc F. Bacterial synthesis of PHA block copolymers. *Biomacromolecules.* 2006;7:1904–1911.
34. Grassie N, Murray EJ, Holmes PA. The thermal degradation of poly(-(D)-*b*-hydroxybutyric acid), Part 2: Changes in molecular weight. *Polym Degrad Stab.* 1984;6:95–103.

Manuscript received May 4, 2009, and revision received Aug. 20, 2009.