A Semiempirical Model for Predicting Biodegradation Profiles of Individual Polymers in Starch–Poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) Bioplastic

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ABSTRACT: Plastic prepared from formulations of cornstarch and poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (PHBV) biodegraded in tropical coastal waters. Biodegradation was monitored for 1 year. Starch—PHBV bioplastic appeared to lose weight in two overlapping phases until both biopolymers were entirely consumed. To examine the underlying degradation of starch and PHBV from biphasic weight-loss profiles, a semiempirical mathematical model was developed from which degradation profiles and lifetimes of the individual biopolymers could be predicted. The model predicted that starch and PHBV in the bioplastic had half-lives of 19 days and 158 days, respectively. Computed profiles also predicted that the starch in the composite would be completely degraded in 174 days, while residual PHBV would persist in the marine environment for 1107 days. The model further revealed that, for a 30% starch : 70% PHBV composite, PHBV degradation was delayed 46 days until more than 65% of the starch was consumed. This suggested that PHBV degradation was metabolically repressed by glucose derived from starch. Glucose repression of microbial PHBV degradation was substantiated in 91 of 100 environmental isolates. The validity of the elaborated model was proven when its revelations and predictions were later confirmed by chemical analysis of residual bioplastic samples. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 76: 1767–1776, 2000

Key words: starch; PHBV; biodegradable plastic; biopolymer degradation profiles; mathematical model; glucose repression

INTRODUCTION

There is growing public awareness and concern over environmental pollution caused by careless disposal of plastic materials into marine waters around the world. An international marine pollution treaty has been ratified that sets restrictions on plastic and garbage disposal from private vessels at sea. In support of the treaty, the U.S. Congress passed the Marine Plastic Pollution and Control Act (Public Law 100-220), which specifically prohibits overboard disposal of plastics anywhere in the world by U.S. Government vessels. The law required all federal agencies, including

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the Navy and Coast Guard, to be in compliance by 31 December, 1998. Consequently, increased attention has recently been given to replacing conventional plastics with biodegradable alternatives.[1–5]

New plastics prepared from biodegradable materials such as plant biopolymers and microbial polyesters have received considerable interest as natural alternatives to conventional synthetic plastics. Composite formulations of cornstarch and poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (PHBV) form bioplastics that have good mechanical properties and biodegrade at rates that are comparable to rates observed for the individual components.[6–9] Plastic composed of starch and microbial PHBV was expected to be highly biodegradable. However, composite starch–PHBV bioplastic that was submerged in tropical coastal waters for 1 year exhibited weight-loss vs. time profiles that were not predictable.[10]

Although starch and PHBV are biodegradable, the rate at which they degrade in a plastic composite is influenced by many factors, including surface area, temperature, microbial density and composition, enzyme percolation, microbe infiltration, glucose repression of enzymatic activity, etc. The manner in which these factors interact to produce the particular degradation profiles observed for starch–PHBV composites is not understood. Although composite bioplastics are attractive alternatives to synthetic plastics, their development is hampered by the absence of knowledge or theory about underlying behavior of the individual components. The goal of this research was to gain understanding of this underlying behavior from predictions and revelations obtained by fitting a semiempirical mathematical model to observed biodegradation profiles.

BACKGROUND

Starch–PHBV formulations produce bioplastics of acceptable quality that meet the biodegradability requirement set forth in the international marine pollution treaty. Several experimental bioplastic composites were tested for biodegradability in tropical coastal waters of Puerto Rico.[10] Biodegradability was measured by weight loss of specimens recovered from the water periodically. The degradation appeared to result from microbial consumption of starch and PHBV in the marine environment where starch degraders in the microbial population were much more prevalent than PHBV degraders. Accordingly, biodegradation of a given composite bioplastic correlated with the amount of starch present. However, starch–PHBV composites appeared to biodegrade in two overlapping phases until both starch and PHBV were entirely consumed.

In the absence of a known cause, the biphasic degradation was postulated to arise from two separate biodegradation processes in which starch and PHBV were consumed at different rates. To verify this hypothesis, bioplastic weight-loss data was fitted to a mathematical model that assumed the weight-loss curve over the period exposed to the marine environment could be exactly described as the sum of two independent sigmoidal exponential functions of time. The fitted mathematical model was then used to predict the underlying weight-loss curves, lifetimes, and other temporal biodegradation characteristics of the individual starch and PHBV components.

THEORY

A realistic mathematical model of starch–PHBV biodegradation must take into account the following conditions from obvious facts and logic:

1. Two separate and temporally different biodegradation processes occur simultaneously.
2. Neither of the two components requires the presence of the other to biodegrade.
3. Degradation of the composite bioplastic, as measured by weight loss, is nonlinear with time.
4. Degradation of either one or both of the components must be nonlinear.
5. Total weight loss from the composite bioplastic at any given time is the sum of the losses from the two components. Equivalently, component weights must add up to the total remaining at any time.
6. Weight loss from each component at any given time must be less than or equal to its original weight in the composite.
7. Weights of degraded and undegraded components at any time must sum to the original weight of the composite.
8. Complete biodegradation of both components will occur in bioplastic exposed to the environment for an infinite time.
9. Degradation of each component can reach a maximum equal to its original concentration in the bioplastic in any finite time.

10. All degradation curves are obliged to pass through the origin; i.e., weight loss is zero at zero time.
11. Underlying degradation functions can be monotonic, but must have sufficient flexibility to model possible delayed accelerations (S-shaped curves) in the biodegradation of either component.
12. Monotonicity is possible but not required in the composite sum of the underlying degradation functions.
13. Slopes of weight-loss curves must be positive at all times or zero, because weight gain from degradation is physically impossible.
14. Negative values for component concentrations or negative weight losses at any time are forbidden.

A reasonable mathematical model can also take into account the following assumptions that are supported by observation and experience:[6–10]

1. Degradation of each component at the microscopic level in a given instant, the weight differential, is directly proportional to the weight of the component remaining exposed at the given time.
2. Underlying degradation functions are monotonic, possibly sigmoidal, and their degradation rates are independent of each other. These are corollaries of the assumed proportionality of weight differentials to component weights at the microscopic level.
3. No obligatory mathematical interaction exists between components; i.e., component weight losses are inherently additive but no multiplicative, logarithmic, or other relation exists between them.

Adherence to the above factual conditions and assumptions is crucial to construct a semiempirical mathematical model of the temporal behavior of starch–PHBV composites during biodegradation. No *a priori* theoretical information about the chemistry or physics of the bioplastic is included in the model. Instead, these conditions and assumptions are built into the model as quasi-theoretical rules that the model must obey as it is statistically fitted to experimental data. Hence, the model is semiempirical, a kind of hybrid in the area between theory and statistics, which can provide knowledge and insight into otherwise hidden or unmeasurable phenomena.

Construction of the Model

To build a mathematical model based on the above conditions and assumptions we designate the underlying profiles as $w_1(t)$ for starch biodegradation and $w_2(t)$ for PHBV biodegradation. These are the unknown weight-loss functions of time t. Either one or both of the functions $w_1(t)$ and $w_2(t)$ must be nonlinear because their known sum is nonlinear, but their shapes are unknown. To model the shapes of the underlying functions, we invoke our assumption of proportionality of the weight differential, which can be defined mathematically by an exponential function of time as shown below:

Consider the infinitesimal changes in the weight of a microscopic volume of bioplastic available during degradation. If the available weight of bioplastic is halved, the potential weight loss in the diminished volume will also be halved. Thus the weight-loss differential dw in a given instant of time dt is directly proportional to w, the weight of bioplastic available at the time, i.e., $dw/dt \propto w$. Therefore,

$$\frac{dw}{dt} = -aw \tag{1}$$

where a is a proportionality constant. The negative sign is introduced because weight decreases as time increases. Integration of eq. (1) over the time span t gives the weight loss due to biodegradation. Transposing the variables gives:

$$\int_{w_0}^{w} \frac{dw}{w} = -a \int_{0}^{t} dt \tag{2}$$

where w_0 is the original weight at zero time, and w is the weight at time t.

Solving, we obtain a simple exponential function

$$w = w_0 e^{-at} \tag{3}$$

that describes how the bioplastic weight changes with time under our assumption of proportionality; the process is driven by exponential events at the microscopic level.

However, to meet all of the factual conditions listed above at the more complex macroscopic level, the model must be more flexible and less constrained than this simple exponential function. In particular, one of the factual conditions requires a function that can model possible lag times or delayed accelerations in the underlying degradation curves that would appear more or less S-shaped. For this, we propose a sigmoidal exponential function $w(t)$ that has the form

$$w(t) = \frac{w_0}{(a_1 + a_2 e^{-a_3 t})} \tag{4}$$

where the a's are positive constants. This function, eq. (4), is our generalization of the sigmoid transfer function, which is commonly used as a special case in artificial neural networks.[11,12] It can fit data with an extended lag time, or no lag time at all, whichever the case may be. It can be used as is to represent a rather stiff sigmoidal exponential, or it can be further modified for more flexibility. Therefore, to meet the flexibility condition we add two more exponential terms giving

$$w(t) = \frac{w_0}{(a_1 + a_2 e^{-a_3 t} + a_4 e^{-a_5 t^2} + a_6 e^{-a_7 t^3})} \tag{5}$$

It is necessary to limit the number of exponential terms to three, as in a third-degree polynomial, to preserve the required monotonicity. Also, the negative signs in the exponents force the slopes of the underlying degradation curves to be positive at all times, as required.

The function $w(t)$ in eq. (5) is now capable of all behavior needed to model an underlying degradation curve except for two final conditions still to be satisfied: zero weight loss at zero time, and maximum weight loss by infinite time. To meet the zero-weight condition, we subtract from the function $w(t)$ in eq. (5) its constant value when $t = 0$. Thus,

$$w(t) = w_0 \left[\frac{1}{(a_1 + a_2 e^{-a_3 t} + a_4 e^{-a_5 t^2} + a_6 e^{-a_7 t^3})} - \frac{1}{(a_1 + a_2 + a_4 + a_6)} \right] \tag{6}$$

and the underlying weight loss now becomes zero at zero time, regardless of the size of the constants.

To satisfy the maximum-weight condition we divide the function $w(t)$ in eq. (6) by the difference between the reciprocals of the denominator terms in the same function when $t = \infty$. Thus, upon dividing eq. (6) by the difference $[1/a_1 - 1/(a_1 + a_2 + a_4 + a_6)]$ and simplifying terms we have

$$w(t) = \left(\frac{w_0 a_1}{a_2 + a_4 + a_6} \right) \times \left(\frac{a_1 + a_2 + a_4 + a_6}{a_1 + a_2 e^{-a_3 t} + a_4 e^{-a_5 t^2} + a_6 e^{-a_7 t^3}} - 1 \right) \tag{7}$$

which reaches a maximum weight loss w_0, equal to its original weight, at infinite time. Whatever its shape, $w(t)$ is now obliged both to pass through the origin and to reach a virtual maximum in real time. Moreover, $w(t)$ cannot exceed w_0 or drop below zero weight loss at any time.

This function, eq. (7), is also driven by exponential events at the microscopic level, but unlike eq. (3), it can model all possible macroscopic behavior of either of the two underlying degradation processes required by the factual conditions and assumptions listed above. It is sufficiently flexible to fit any monotonic, nonlinear degradation profile with precision.

The function $w(t)$ in eq. (7) can be used for either starch biodegradation $w_1(t)$ or PHBV biodegradation $w_2(t)$. When used for $w_1(t)$, the constants w_0, a_1, a_2,. . . . and a_7 of $w(t)$ are labeled w_{10}, a_{11}, a_{12}, . . ., and a_{17}, respectively, but when used for $w_2(t)$ the same constants of $w(t)$ are labeled w_{20}, a_{21}, a_{22},. . ., and a_{27}, respectively. The new subscripts merely denote the starch constants differently from the PHBV constants. In our model w_{10} is the known original concentration (%) of starch in the composite bioplastic, while $w_{20} = 100 - w_{10}$ is the known original concentration of PHBV in the composite. Hence, there are seven unknown parameters in the starch function and seven unknown parameters in the PHBV function.

Convoluting the starch function $w_1(t)$ with the PHBV function $w_2(t)$ by addition, we obtain a resulting sum that contains 14 unknown parameters. This sum, which we will designate as $W(t)$, is the weight-loss function of the composite starch–PHBV bioplastic, i.e.,

$$W(t) = w_1(t, w_{10}, a_{11}, a_{12}, \ldots, a_{17}) + w_2(t, w_{20}, a_{21}, a_{22}, \ldots, a_{27}) \tag{8}$$

where the component weight-loss functions $w_1(t, w_{10}, a_{11}, a_{12}, \ldots, a_{17})$ and $w_2(t, w_{20}, a_{21}, a_{22}, \ldots, a_{27})$ both have the form of eq. (7) and are, therefore, explicitly defined.

Hence, eq. (8) can be used to deconvolute the weight-loss profile of the starch–PHBV composite described by the experimental data into the desired biodegradation profiles of starch and PHBV.

Solution of the Model

Given the model of eq. (8), we solve for the parameters that best fit the experimental data and thereby predict the underlying biodegradation profiles. Using observed data $\hat{w}_i$ for $i = 1,2,3,\ldots,n$ weight-loss measurements over time t the parameters are predicted by regression of the $\hat{w}_i$ onto t, where the overlay diacritical mark indicates measured rather than true weight loss. The unknown parameters $a_{11}, a_{12}, a_{13}, \ldots, a_{17}$ and $a_{21}, a_{22}, a_{23}, \ldots, a_{27}$ are obtained statistically by minimizing the residual errors e in the equation $\hat{w} = W(t) + e$ to give the maximum likelihood fit of the model to the data. Thus, we solve the constrained nonlinear regression problem:

$$\underset{a_{11}\cdots a_{27}}{\text{minimize}} \sum_{i=1}^{n} [[w_1(t, w_{10}, a_{11}, a_{12}, \ldots, a_{17}) + w_2(t, w_{20}, a_{21}, a_{22}, \ldots, a_{27})]_i - \hat{w}_i]^2$$

$$\text{subject to: } a_{11}, a_{12}, a_{13}, \ldots, a_{17} \geq 0$$

$$a_{21}, a_{22}, a_{23}, \ldots, a_{27} \geq 0$$

where w_{10} = original starch concentration (%); $w_{20} = 100 - w_{10}$ = original PHBV concentration (%); $\hat{w}_i$ = weight losses (%) from $n \geq 14$ measurements.

It is important to note that nonlinear regression methods[13–15], which can be used to perform the above minimization, require at least as many different measurement times as unknown parameters. Otherwise, the system of equations is underdetermined, and the regression process will not converge to a unique solution. This condition is not usually encountered in practice.[16] However, a convenient, albeit risky, technique for dealing with underdetermined systems is to generate as many additional measurements as needed by interpolation between a number of measurement times using cubic spline[17] or Savitsky-Golay[18] smoothing. It can be shown via computer simulation that the regression error introduced by nonlinear interpolation is negligible when the measurements used for smoothing are carefully selected.

It is also worth noting that each of the parameters in the function $w(t)$ has a specific and predictable influence on the behavior of the model. This means it is possible to control the model behavior to a large extent by placing tight constraints on the range known or expected for particular parameters in the solution process. For example, the parameter a_1 in $w(t)$ greatly affects the slope of the S-shaped curve. So, if it is known that PHBV exhibits very slow weight loss under certain environmental conditions, we can effect this behavior in the model by constraining a_{21} in eq. (8) to low values, say $0 < a_{21} < 1$. The regression algorithm will then adjust all of the parameters until a_{21} settles at a value between 0 and 1, and the maximum likelihood fit of the model under the given conditions is found. On the other hand, if nothing is known or expected for PHBV behavior under the given conditions, we can simply allow the model freedom to behave at will in the regression by removing the upper bound of the constraint on a_{21}. This is the situation usually met in practice for both starch and PHBV. Therefore, we let the model attain a maximum likelihood fit controlled only by its built-in conditions and the experimental data.

EXPERIMENTAL

Description of the Data

Full details of the study including materials, sample preparation, marine stations, determinations of biodegradation, and physical properties of the bioplastic before and after exposure to marine environments are presented elsewhere.[10] Briefly, bioplastic was prepared by blending formulations of cornstarch and PHBV, which were then extruded as ribbons and annealed. Test samples were cut, weighed, and transported to test stations where they were exposed to tropical marine environments. Samples composed of 100% starch and other samples composed of 100% PHBV were used for comparative purposes. Biodegradation was periodically measured by weight loss of samples recovered from the marine environments for 1 year. Experimental errors in the gravimetric weight measurements were considered to be negligible.

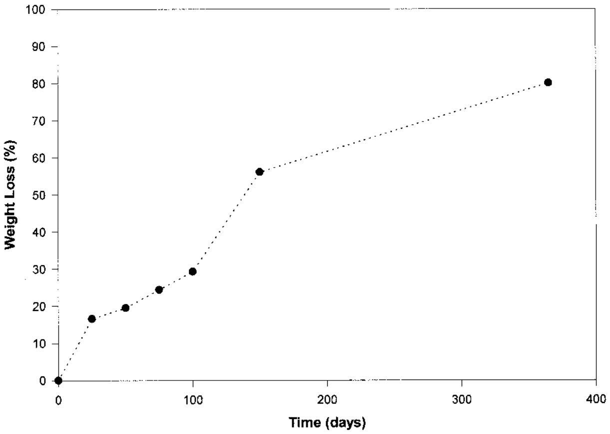

Figure 1 Weight loss from degradation of a 30 : 70 starch–PHBV bioplastic submersed in water at edge of a tropical mangrove stand.

One bioplastic was a 30% starch–70% PHBV composite that was evaluated for degradability by submersion in a mangrove on the southwest coast of Puerto Rico. Test samples were retrieved from the water at 25-day intervals during the first 150 days. A final sample was retrieved after 365 days. The 30 : 70 starch–PHBV composite was selected for this modeling study because of its curiously pronounced biphasic degradation profile that represented a characteristic phenomenon observed to some degree in all composites. As shown in Figure 1, the weight-loss profile exhibits two conspicuous steps that appear to arise from separate overlapping phases in the degradation process.

Pretreatment of the Data

For this study, only seven weight-loss measurements were available from samples, while, in principle, our model requires at least 14 measurements for solution. Therefore, seven additional data points were generated by computer interpolation between selected measurements (three points at a time) using a cubic spline smoothing routine in SigmaPlot software (Jandel Scientific, Inc.). Figure 2 shows the "dummy" points, each interpolated within 5 days of an actual weight-loss measurement.

Kinetic Parameter Estimation and Deconvolution

The model of eq. (8) was used as the objective function to estimate the unknown kinetic parameters and deconvolute the weight-loss profile of the 30 : 70 starch–PHBV composite by solving the constrained nonlinear regression problem stated above. Sums of squared residual errors in fitting the model to the experimental data of Figure 2 were minimized using a Marquardt-Levenberg[19,20] curve-fitting algorithm supplied with SigmaPlot software. Loose inequality constraints, ($a_{11}, a_{12}, a_{13}, \ldots, a_{17} \geq 0$ and $a_{21}, a_{22}, a_{23}, \ldots, a_{27} \geq 0$) were placed on all unknown parameters. The original starch and PHBV concentrations were set at their known percentages, $w_{10} = 30$ and $w_{20} = 70$. Depending on the starting approximations for the unknown parameters, which in this software required much trial and error, the minimization process converged in 100–200 iterations at the solution which best fit the 14 data points according to a least-squares criterion (norm = 1.3681). The software returned positive values predicted by the model for the 14 unknown parameters.

Deconvolution of the composite weight-loss profile of Figure 2 into the underlying weight-loss profiles for starch and PHBV was achieved by substituting the predicted parameter values in eq. (8) and plotting $W(t)$, $w_1(t, w_{10}, a_{11}, a_{12}, \ldots, a_{17})$, and $w_2(t, w_{20}, a_{21}, a_{22}, \ldots, a_{27})$ separately.

Component Weight Loss by Chemical Extraction

Individual losses of starch and PHBV from partially degraded bioplastic were determined by extracting residual PHBV with dichloromethane. Soluble PHBV was thereby separated from insoluble carbohydrate. The separated contents were recovered, dried, and weighed, and final individual biopolymer concentrations were calculated as percentage of starch and percentage of PHBV in

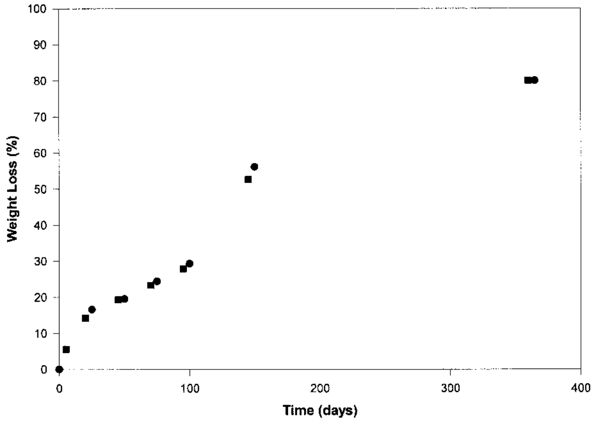

Figure 2 Interpolated biodegradation data from cubic spline smoothing between selected weight-loss data points from Figure 1. Measured points (●) and interpolated points (■).

the degraded sample. Sample weight losses due to individual degradation of starch or PHBV were calculated as

$$w_{ct} = w_{co} - w_{ct}(1 - w_t/100) \qquad (9)$$

where w_{ct} is weight percent of the sample lost due to degradation of a particular biopolymer component c (either starch or PHBV) at time t, w_{co} is the weight percent of component c originally present in the undegraded sample, w_{ct} is the weight percent of component c remaining at time t in the degraded sample, and w_t is the measured weight loss (%) of the composite bioplastic.

PHBV Glucose Repression Assay (Microbiology)

Water samples were collected in randomly selected locations at four sites in tropical coastal waters off the south western coast of Puerto Rico. The location of these sites has been described earlier.[10] Sterile screw-capped bottles were taken to approximately 1.0 m depth, opened, allowed to fill, and the cap was replaced. The samples were immediately transported to the laboratory and 200 μL of each sample was plated out on MB agar (Difco Laboratories, Detroit, MI). The plates were incubated at 25°C for 24–48 h. Individual colonies from these plates were transferred to plates containing PHBV as a sole source of carbon and incubated at 25°C for 48–96 h. The composition of the medium has been described earlier.[10] Individual colonies showing clearing zones were transferred with sterile toothpicks to PHBV agar supplemented with 2% glucose. Growth on these plates was monitored for 48–120 h for signs of clearing zones.

RESULTS AND DISCUSSION

There were four objectives of this research: first, to construct a semiempirical mathematical model of the underlying degradation behavior of both starch and PHBV in composite bioplastic; second, to develop a numerical solution of the unknown kinetic parameters; third, to describe and interpret the underlying degradation behavior revealed by the model; and fourth, to verify the model and parameters by comparison with starch and PHBV degradation profiles obtained chemically.

Interest in the biphasic weight-loss profiles exhibited by starch–PHBV composites led to a postulation that biodegradation of composite bioplastic occurs in two overlapping phases. Based on this and a number of factual conditions and reasonable assumptions, a biodegradation model was elaborated in the Theory section (eq. (8)), which consists of two terms: a function for estimation of starch degradation behavior, and another for estimation of PHBV degradation behavior. Both functions are mathematically identical, having the sigmoidal exponential form of eq. (7), and differ only in the values of the parameters.

Table I Model Parameter Values Predicted for Biodegradation of a 30 : 70 Starch–PHBV Composite Exposed in a Tropical Mangrove Environment

Parameter[a]	Starch	PHBV
w_0	30	70
a_1	1697748.8876	1.5168
a_2	1.6361	16347.2916
a_3	0.0826	0.0620
a_4	1.3112	1.0292
a_5	1.2607e-4	4.1354e-6
a_6	5.2106e-3	0.7673
a_7	6.9998e-3	7.5779e-3

[a] The notation of eq. (7) is used for simplicity.

Computed Parameter Values

The model, eq. (8), was fitted to experimental weight-loss data from the 30 : 70 starch–PHBV composite, which was exposed to the tropical mangrove environment for 1 year. Fitted parameters from the solution of the constrained nonlinear regression problem are listed in Table I. These are the values predicted by the model for the 14 unknown parameters. They are all positive constants as required by the constraints embodied in the model but, what is more important, the corresponding parameters for starch and PHBV show considerable differences which reflect large dissimilarities in the degradation of the two biopolymers. For example, in Table I the value of the first fitted parameter a_1 is 1,697,749 for starch but only 1.5168 for PHBV. This fact enables us to predict a much larger biodegradation rate for starch than for PHBV in the early days of exposure. All of the fitted parameter values contain information about the degradation of starch and PHBV that can be deduced from the numbers alone.

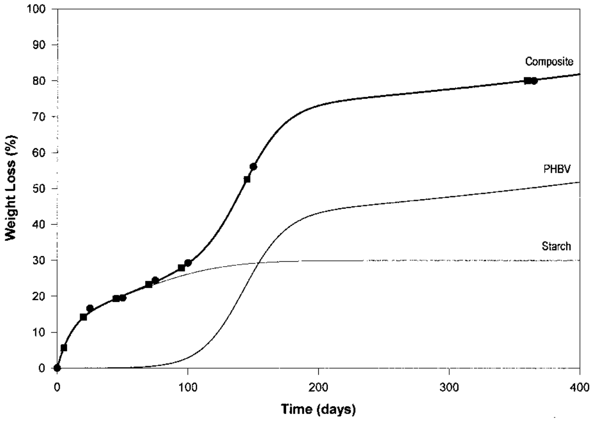

Figure 3 Weight loss profiles of the 30 : 70 starch–PHBV composite predicted from the biodegradation model (eq. (8)).

Predictions and Revelations of the Model

It is more informative to graphically represent the weight loss profiles predicted by the model, as shown in Figure 3. The upper curve represents an excellent fit of the model to the weight-loss data for the 30 : 70 starch–PHBV composite, while the underlying curves represent the starch and PHBV weight-loss profiles predicted by deconvolution of eq. (8).

The weight-loss profiles reveal that starch and PHBV degrade at dramatically different rates. Starch in the composite biodegraded much faster than PHBV during the first few days of marine exposure, and PHBV showed a delayed onset of degradation, or lag time, in the presence of the starch. Significant PHBV degradation was delayed for 46 days until the starch was more than 65% consumed, at which time measurable PHBV degradation occurred and then rapidly accelerated. The rate of starch degradation was maximum in the first few days of exposure. PHBV reached its maximum degradation rate 142 days later. The computed profiles predicted that starch in the 30 : 70 starch–PHBV composite would be, in practical terms, completely degraded by 174 days of exposure, while residual PHBV would persist in the marine environment for 1107 days. The model also predicted that starch and PHBV in the composite would have respective half lives of 19 days and 158 days. These predictions are summarized in Table II.

It is also instructive to complement numerical data with graphics from fitted equations, as shown in Figure 3. This not only visualizes underlying profiles, but it also suggests and guides calculation of useful information not discernable by visual inspection. In this work, the fitted model and parameters enabled calculation of the predictions listed in Table II. Using eq. (7) for each biopolymer the half life was calculated as the value of t at $w_0/2$; the lifetime was calculated as the value of t when the function reaches 99.9% of w_0; the maximum rate was calculated as the time at the point of inflection in the sigmoid function [when the second derivative of eq. (7) is zero]; and the onset of degradation (lag time) was calculated as the time when the function first rises (arbitrarily 0.1%).

One unexpected revelation, which became apparent after visualization of the deconvoluted PHBV degradation profile, was the extended lag period observed before the onset of PHBV degradation. This lag coincided with a period where, as depicted in Figure 3 by its individual degradation profile, the majority of starch was lost from the composite. Such coincidental profiles are reminiscent of those of classical glucose repression, which would be highly plausible given that starch is a polymer of glucose. To substantiate this revelation, PHBV degrading isolates were obtained from test station waters. The ability to degrade PHBV was repressed by the presence of glucose in all but 9 of 100 isolates tested. Furthermore, the isolates that did not exhibit glucose repression were all among the poorer PHBV degraders, as judged by the size and clarity of clearing zones surrounding individual colonies on PHBV-containing microbial plates.

Verification of the Model and Parameters

The biodegradation profiles of starch and PHBV predicted by the model were compared with experimental weight losses of the individual biopolymers determined by dichloromethane extraction of the same 30 : 70 starch–PHBV sam-

Table II Predicted Biodegradation Behavior for Starch and PHBV in a 30 : 70 Starch–PHBV Composite Exposed to a Tropical Mangrove Environment

Behavior	Starch (days)	PHBV (days)
Half life	19	158
Lifetime	174	1107
Maximum rate	1	142
Lag time	0	46

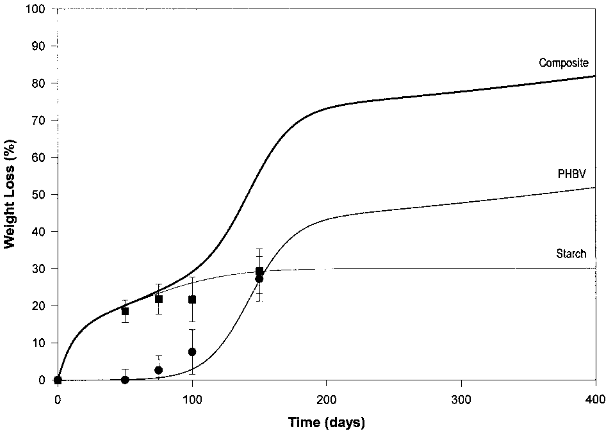

Figure 4 Deconvoluted weight-loss profiles of starch and PHBV in the biodegraded composite compared with weight losses of starch (■) and PHBV (●) determined by chemical extraction.

ples. Experimental weight-loss data resulting from dichloromethane extraction were calculated using eq. (9). The experimental data points are shown in Figure 4 superimposed upon the weight-loss profiles predicted for starch and PHBV by the model. Within experimental error, estimated from a single outlier (impossible weight-gain datum not shown), the predicted biodegradation profiles were in excellent agreement with the chemical extraction data (plotted in Fig. 4), which were obtained after the model predictions. This was particularly instructive in view of the somewhat arbitrary selection of interpolated measurements. That is, even greater accuracy would be expected had more samples been available. Ideally, many more than 14 samples would be exposed and weighed to eliminate the need for data interpolation and improve the reliability of the predictive model.

The computed profiles are good estimates of starch and PHBV degradation in the composite bioplastic because the chemically extracted weight-loss data support the predictions of the biodegradation model. This corroboration indicates that the above predictions and revelations can be accepted with confidence, and furthermore, that prediction by the model offers an advantage over prediction by plotting data from chemical extraction. Attempts at prediction by any feasible chemical method would require many more test samples than would be practical to adequately describe degradation profiles with the resolution and information that the model provides.

CONCLUSIONS

A semiempirical mathematical model was developed from which the underlying degradation profiles and other kinetic behavior of starch and PHBV in a composite bioplastic exposed to a tropical marine environment were accurately predicted. The model verified the hypothesis that biodegradation of a typical composite occurs in two overlapping phases, with starch and PHBV degrading in markedly different profiles. It also revealed a delayed onset or lag in PHBV degradation, which was presumably caused by the presence of the starch.

Composites of starch and PHBV are known to degrade at rates that are intermediate to those observed for bioplastic containing 100% starch or 100% PHBV.[10] However, the biphasic degradation (Fig. 3), reflecting the initial depletion of starch followed by PHBV loss, was not expected. This lag period can be attributed to the far greater number of starch-degrading microbes relative to PHBV degraders present in tropical waters, as well as to the fact that most PHBV-grown microbes also grow on starch. Glucose repression of PHA-esterase activity has been suggested as a possible explanation for the lag in PHBV degradation seen in the starch–PHBV composites.[10] Glucose (starch) repression of PHBV degradation is likely to be a salient phenomenon in a number of environments, a hypothesis that requires validation.

This is a plausible hypothesis that resulted from the insight and understanding gained from the predictions and revelations of the biodegradation model. It was possible to uncover the hidden and unmeasurable biodegradation profiles of both starch and PHBV from the weight-loss profile of the composite bioplastic. As though it were a kind of mathematical X-ray lens, the semiempirical model served to visualize and measure images of heretofore unseen degradation profiles of two components in the bioplastic, which were later confirmed by chemical extraction. The lag period was never anticipated or imagined before, yet the model predicted its existence and even measured the lag time. Thus, the usefulness of the model in this research has been proven. However, the present research is a special case. Generally, a semiempirical model would be used as a tool for comparing the relative behavior of different composites within a single experiment.

It is important to remember that new kinetic parameters for the model must be computed for each new material in each environment studied.

The parameters in Table I are not absolute predictions, and cannot be applied to different experiments. This is in the nature of semiempirical models; they are not strictly theoretical models, and should not be viewed as absolute. However, when used appropriately, as shown in this work, semiempirical models are much more informative than purely empirical ones. Therefore, by fitting the model of eq. (8) to biodegradation data from new bioplastics in the future it will be possible to make important conclusions and discoveries based on this research.

The degradation model will be another valuable tool for comparing the biodegradability of new composite bioplastics in the effort to meet the requirement of the international marine pollution treaty.

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