MODELING AND SIMULATION OF BIODEGRADATION OF XENOBIOTIC POLYMERS BASED ON EXPERIMENTAL RESULTS

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Abstract: Biodegradation of polyethylene glycol is studied mathematically. A mathematical model for depolymerization process of exogenous type is described. When a degradation rate is a product of a time factor and a molecular factor, a time dependent model can be transformed into a time independent model, and techniques developed in previous studies can be applied to the time independent model to determine the molecular factor. The time factor can be determined assuming the exponential growth of the microbial population. Those techniques are described, and numerical results are presented. A comparison between a numerical result and an experimental result shows that the mathematical method is appropriate for practical applications.

1 INTRODUCTION

Biodegradation is an essential factor of the environmental protection against undesirable accumulation of xenobiotic polymers. It is particularly important for water soluble polymers, because they are not suitable for recycling nor incineration. It is also important for water-insoluble polymers, so-called plastics, because they are not completely recycled nor incinerated, and a significant portion of products remains in the environment after use. Microbial depolymerization processes are generally classified into either one of two types: exogenous type or endogenous type. In an exogenous depolymerization process, monomer units are separated from the terminals of molecules stepwise. The β-oxidation of polyethylene (PE) is an example of exogenous depolymerization process. Microbial depolymerization processes of PE are based on two primary factors : the gradual weight loss of large molecules due to the β-oxidation and the direct consumption or absorption of small molecules by cells. On the other hand, one of characteristics of endogenous depolymerization processes is the rapid breakdown of large molecules due to internal separations to yield small molecules. The enzymatic degradation of polyvinyl alcohol (PVA) is an example of endogenous depolymerization process. Mathematical models for those depolymerization processes have been proposed, and those models are analyzed to study the biodegradation of the xenobiotic polymers.

In this paper, the study of exogenous depolymerization processes is continued to cover the biodegradation of polyethylene glycol (PEG). PEG is one of polyethers which are represented by the expression $HO(R\text{-}O)_nH$, *e.g.*, PEG: $R = CH_2CH_2$, polypropylene glycol (PPG): $R = CH_3CHCH_2$, polytetramethylene glycol (PTMG): $R = (CH_2)_4$ (Kawai, 1993). Those polymers are utilized for constituents in a number of products including lubricants, antifreeze agents, inks, and cosmetics. They are either water soluble or oily liquid. Some portion of products are eventually discharged through sewage to be processed, while some others enter streams, rivers, and coastal areas. and therefore it is especially important to evaluate their biodegradability. PEG is produced more than any other polyethers, and the major part of production is consumed in production of nonionic surfactants. PEG is depolymerized by releasing C_2 compounds, either aerobically or anaerobically (Kawai, 1995; Kawai, 2002; Kawai and Xenobiotic Polymers, 2002) (Figure 1).

High performance liquid chromatography (HPLC)

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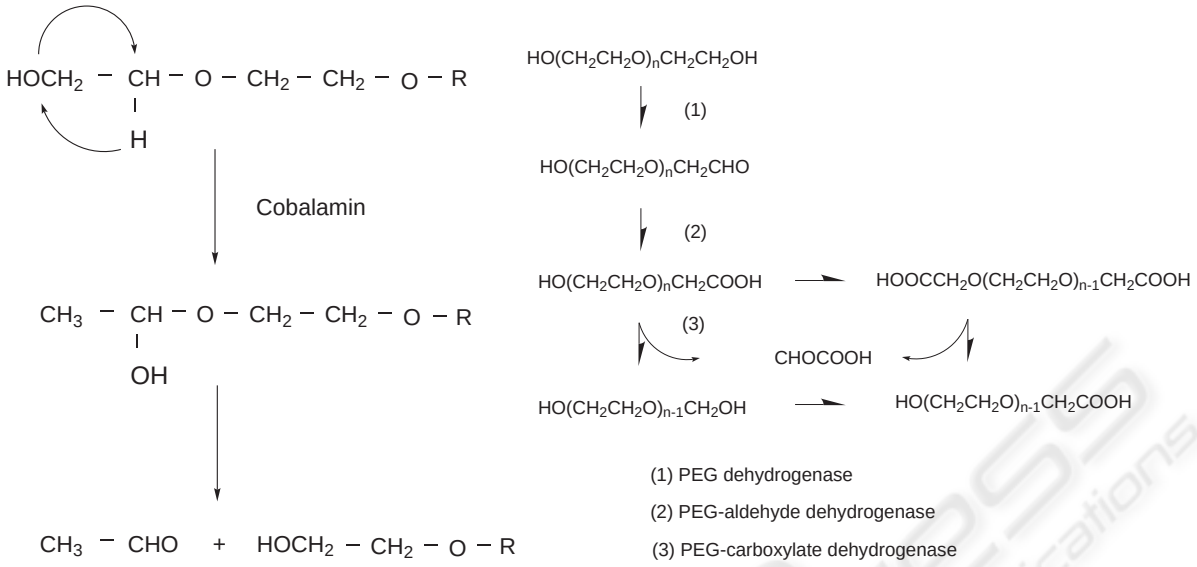

Figure 1: Anaerobic metabolic pathway (left) and Aerobic metabolic pathway (right) of PEG.

patterns were introduced into analysis of an exogenous depolymerization model to set the weight distribution of PEG with respect to the molecular weight before and after cultivation of a microbial consortium E1 (Figure 2).

In the previous studies (Watanabe and Kawai, 2004), the degradation rate was assumed to be independent of time. The time dependent degradation rate was considered in a recent study assuming a logistic growth in a microbial population (Watanabe and Kawai, 2005), and using a cubic spline to take the change of microbial population into considerateion (Watanabe and Kawai, 2007). In this paper, the mathematical study of biodegradation of PEG is continued with the time dependent degradation rate incorporated into the exogenous depolymerization model. A change of variable reduces the model into the one for which the degradation rate is time independent. The techniques developed previously were applied to solve an inverse problem to determine the time independent degradation rate for which the solution of an initial value problem satisfies not only the initial weight distribution but also the weight distribution after cultivation. The time factor was determined by assuming the exponential growth of the microbial population. Once the degradation rate was found, the transition of the weight distribution was simulated by solving the initial value problem numerically.

2 MODEL WITH TIME DEPENDENT DEGRADATION RATE

The PE biodegradation model (1) is based on two essential factors: the gradual weight loss of large molecules due to terminal separations (β-oxidation) and the direct consumption of small molecules by cells (Kawai et al., 2002; Watanabe et al., 2003; Kawai et al., 2004).

$$\frac{dw}{dt}(t,M) = -\alpha(M)\,w(t,M) + \beta(M+L)\,\frac{M}{M+L}\,w(t,M+L)\,. \tag{1}$$

Here t and M represent the time and the molecular weight respectively. Let a M-molecule be a molecule with molecular weight M. Then $w(t,M)$ represents the total weight of M-molecules present at time t. Note that $w(t,M)$ is a function of time variable t, and that it also depends on the parameter M. The parameter L represents the amount of the weight loss due to the β-oxidation. The variable y denotes $w(t,M+L)$, and it is the total weight of $(M+L)$-molecules present at time t. The function $\alpha(M)$ denotes $\rho(M)+\beta(M)$, where the function $\rho(M)$ represents the direct consumption rate, and the function $\beta(M)$ represents the rate of the weight conversion from the class of M-molecules to the class of $(M-L)$-molecules due to the β-oxidation. The left-hand side of the equation (1) represents the rate of change in the total weight

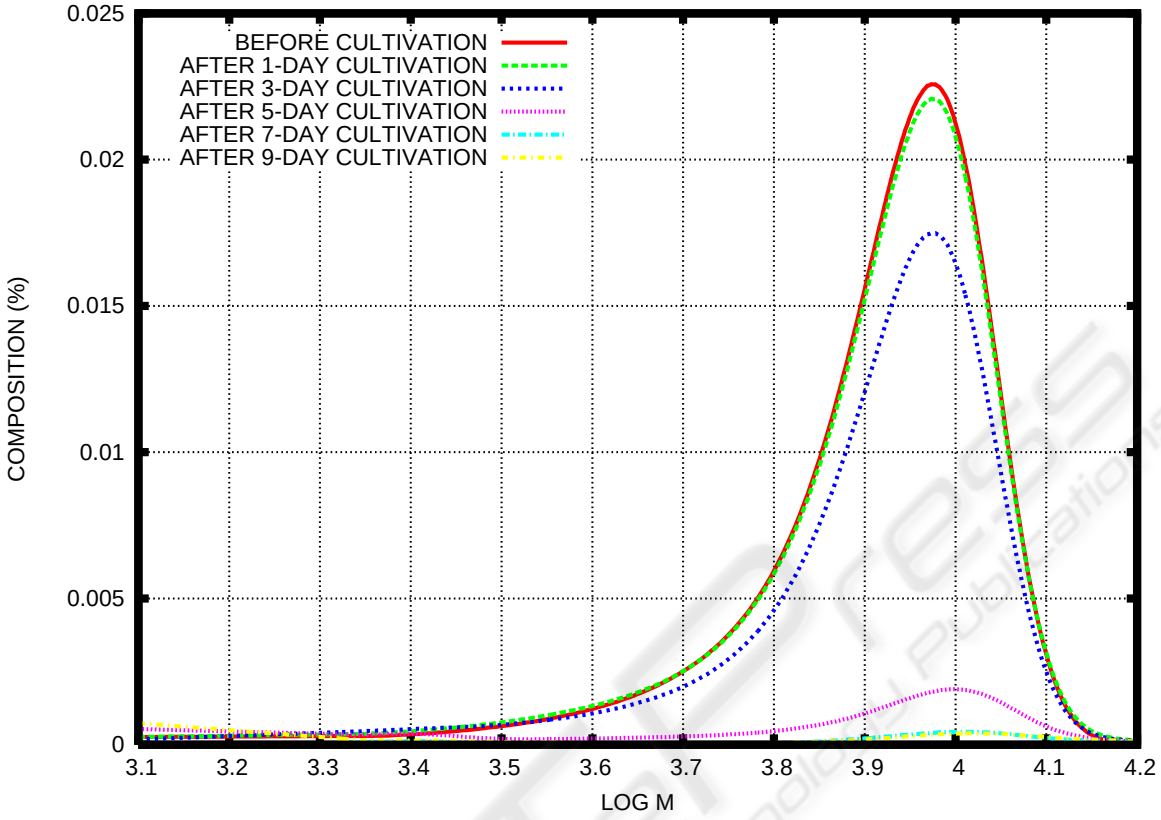

Figure 2: Weight distribution of PEG before and after cultivation of a microbial consortium E1 for one day and three days.

of M-molecules. The first term on the right-hand side of the equation (1) represents the amount lost by the direct consumption and the β-oxidation in the total weight of M-molecules per unit time, and the second term represents the amount gained by the β-oxidation of $(M+L)$-molecules per unit time. The mathematical model (1) was originally developed for the PE biodegradation, but it can also be viewed as a general biodegradation model involving exogenous depolymerization processes. In the exogenous depolymeization of PEG, a PEG molecule is first oxidized at its terminal, and then an ether bond is split. It follows that $L = 44$ (CH_2CH_2O) in the exogenous depolymerization of PEG. PEG molecules studied here are lagre molecules that can not be absorbed directly through membrene into cells. Then $\rho(M) = 0$, and $\alpha(M) = \beta(M)$.

The equation (1) is appropriate for the depolymerization processes over the period after the microbial population is fully developed. However the change of microbial population should be taken into consideration for the period in which it is still in a developing stage, and the degradation rate should be time dependent. Then the exogenous depolymerization model becomes

$$\begin{aligned}\frac{dw}{dt}(t,M) &= -\beta(t,M)\,w(t,M)\\ &\quad +\beta(t,M+L)\,\frac{M}{M+L}\,w(t,M+L).\end{aligned} \tag{2}$$

to model the change of weight distribution of PEG. The solution $x = w(t,M)$ of (2) is associated with the initial condition:

$$w(0,M) = f(M), \tag{3}$$

where $f(M)$ is some prescribed function that represents the initial weight distribution. Given the the degradation rate $\beta(t,M)$, the equation (2) and the initial condition (3) form an initial value problem to find the unknown function $w(t,M)$.

A time factor of the degradation rate such as microbial population, dissolved oxygen, or temperature should affect molecules regardless of their sizes. Then the dependence of the degradation rate on those time factors must be uniform over all the molecular weight classes, and the degradation rate should be a product of a time dependent part $\sigma(t)$ that represents

the magnitude of degradability, and a molecular dependent part $\lambda(M)$ that represents the molecular dependence of degradability:

$$\beta(t,M) = \sigma(t)\lambda(M). \tag{4}$$

Let

$$\tau = \int_0^t \sigma(s)\, ds, \tag{5}$$

and

$$\begin{aligned} W(\tau,M) &= w(t,M), \\ X &= W(\tau,M), \\ Y &= W(\tau,M+L). \end{aligned}$$

Then

$$\frac{dX}{d\tau} = \frac{dx}{dt}\frac{dt}{d\tau} = \frac{1}{\sigma(t)}\frac{dx}{dt}.$$

It follows that

$$\frac{dX}{d\tau} = -\lambda(M)X + \lambda(M+L)\frac{M}{M+L}Y. \tag{6}$$

This equation governs the transition of weight distribution $W(t,M)$ which changes with the time independent or time averaged degradation rate $\lambda(M)$. Given the initial weight distribution $f(M)$, The solution of the initial value problem is the solution of the equation (6) subject to the initial condition

$$W(0,M) = f(M). \tag{7}$$

The solution of the inverse problem is the degradation rate $\lambda(M)$ for which the solution of the initial value problem (6), (7) also satisfies the final condition

$$W(\mathcal{T},M) = g(M). \tag{8}$$

When the solution $W(\tau,M)$ of the initial value problem (6), (7) satisfies this condition, the solution $w(t,M)$ of the intiail value problem (2), (3) satisfies the condition

$$w(T,M) = g(M), \tag{9}$$

where

$$\mathcal{T} = \int_0^T \sigma(s)\, ds \tag{10}$$

The inverse problem consisting of the equation (6) and the conditions (7) and (8) was solved numerically with techniques developed in previous studies. Figure 3 shows the graph of the function $\lambda(M)$ based on the weight distribution before and after cultivation for three days (Watanabe and Kawai, 2004).

3 TIME FACTOR OF DEGRADATION RATE

A microbial population grows exponentially in a developing stage. Since the increase of biodegradability results from increase of microbial population, it is appropriate to assume that the time factor of the degradation rate $\sigma(t)$ is an exponential function of time:

$$\sigma(t) = e^{at+b}. \tag{11}$$

Then in view of the equation (5)

$$\tau = \int_0^t \sigma(s)\, ds = \int_0^t e^{as+b}\, ds = \frac{e^b}{a}\left(e^{at}-1\right).$$

Suppose that the weight distribution is given at $t = T_1$ and $t = T_2$, where $0 < T_1 < T_2$, and let

$$\mathcal{T}_1 = \int_0^{T_1} \sigma(s)\, ds, \tag{12}$$

$$\mathcal{T}_2 = \int_0^{T_2} \sigma(s)\, ds \tag{13}$$

It follows that

$$\sigma(t) = e^b e^{at} = \frac{a\mathcal{T}_1 e^{at}}{e^{aT_1}-1} \tag{14}$$

and

$$\tau = \mathcal{T}_1 \frac{e^{at}-1}{e^{aT_1}-1} \tag{15}$$

Now the equation (13) leads to

$$\mathcal{T}_2 = \mathcal{T}_1 \frac{e^{aT_2}-1}{e^{aT_1}-1},$$

which is equivalent to the equation

$$h(a) = 0, \tag{16}$$

where

$$h(a) = \frac{e^{aT_2}-1}{e^{aT_1}-1} - \frac{\mathcal{T}_2}{\mathcal{T}_1}.$$

Since

$$h'(a) = \frac{T_2 e^{aT_2}\left(e^{aT_1}-1\right) - T_1 e^{aT_1}\left(e^{aT_2}-1\right)}{\left(e^{aT_2}-1\right)^2},$$

$h'(a) > 0$ if and only if

$$\frac{T_1 e^{aT_1}}{e^{aT_2}-1} < \frac{T_2 e^{aT_2}}{e^{aT_2}-1}$$

For $x > 0$

$$q(x) = \frac{xe^{ax}}{e^{ax}-1} = \frac{x}{1-e^{-ax}}$$

Then

$$\begin{aligned} q'(x) &= \frac{1-e^{-ax}-axe^{-ax}}{(1-e^{-ax})^2} \\ &= \frac{e^{-ax}(e^{ax}-1-ax)}{(1-e^{-ax})^2} > 0. \end{aligned}$$

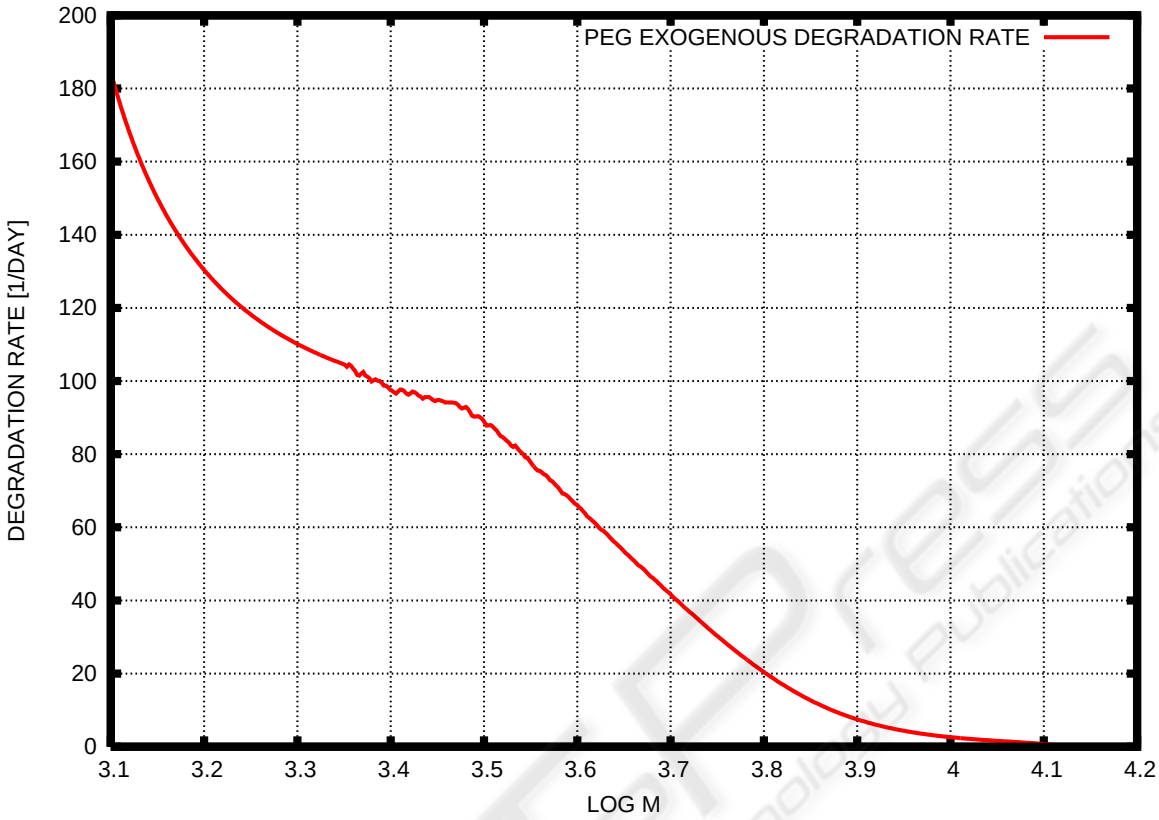

Figure 3: Degradation rate based on the weight distribution of PEG before and after cultivation of a microbial consortium E1 for three days.

It follows that the $q(x)$ is a strictly increasing function, and it follows that $h'(a) > 0$.

It is easily seen that

$$\lim_{a\to\infty} h(a) = \infty$$

Suppose that

$$\frac{T_2}{T_1} < \frac{\mathcal{T}_2}{\mathcal{T}_1} \tag{17}$$

Then by L'Hospital's rule

$$\begin{aligned} \lim_{a\to 0+} h(a) &= \lim_{a\to 0+} \left\{ \frac{e^{aT_2}-1}{e^{aT_1}-1} - \frac{\mathcal{T}_2}{\mathcal{T}_1} \right\} \\ &= \lim_{a\to 0+} \frac{e^{aT_2}-1}{e^{aT_1}-1} - \frac{\mathcal{T}_2}{\mathcal{T}_1} \\ &= \lim_{a\to 0+} \frac{T_2 e^{aT_2}}{T_1 e^{aT_1}} - \frac{\mathcal{T}_2}{\mathcal{T}_1} \\ &= \frac{T_2}{T_1} - \frac{\mathcal{T}_2}{\mathcal{T}_1} < 0 \end{aligned} \tag{18}$$

It follows that the condition (17) is a necessary and sufficient condition for the equation (16) to have a unique positive solution.

In order to determine a and b, the values of T_1, T_2, $\mathcal{T}_1$, and $\mathcal{T}_2$ must be set. Let $T_1 = \mathcal{T}_1 = 3$. The initial value problem (6), (7) was solved numerically with the degradation whose graph is shown in Figure 3 to find the weight distribution at $\tau = 30$ (Figure 4). Figure 4 also shows the weight distribution after cultivation for five days.

Figure 4 shows that it is appropriate to set $T_2 = 5$ and $\mathcal{T}_2 = 30$. Figure 5 shows the graph of $h(a)$ with those values of parameters.

Figure 5 shows that there is a unique solution of the equation (16). It was solved numerically with the Newton's method, and a numerical solution, which was approximately equal to 1.136176 was found.

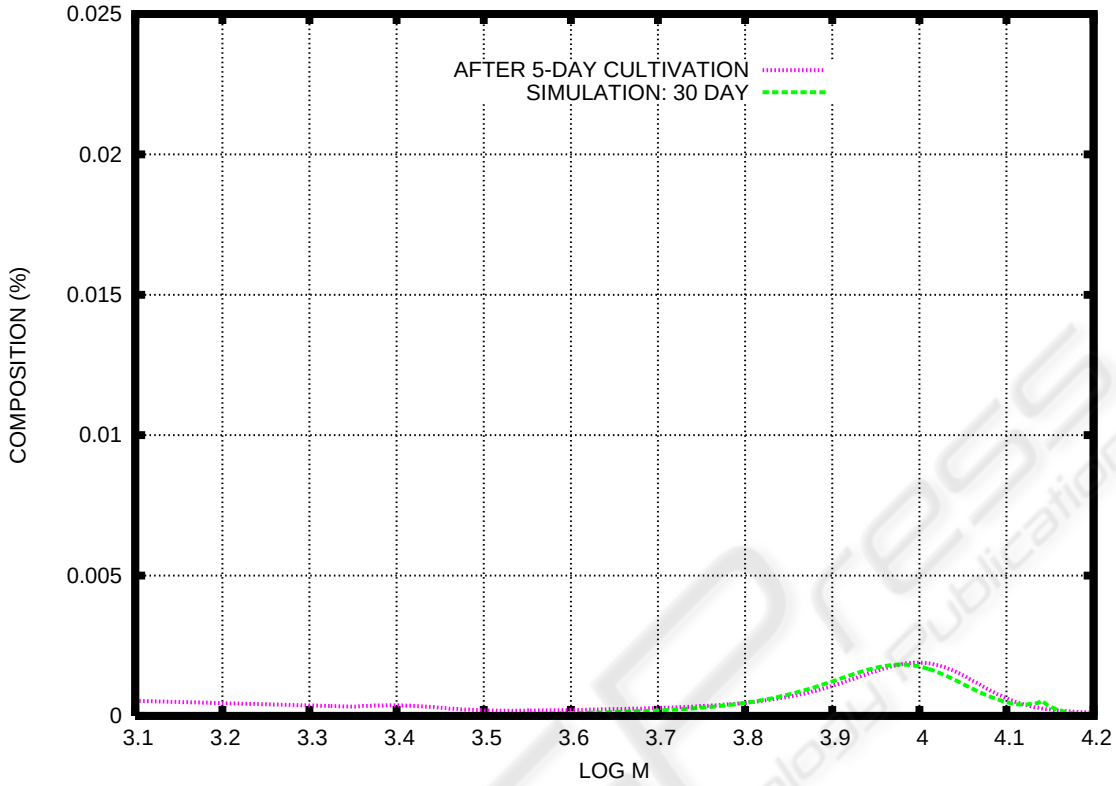

Figure 4: Weight distribution of PEG after cultivation for 30 days according to the time independent model based on the initial value problem (6), (7), and the degradation rate shown in Figure 3. The experimental result obtained after cultivation for 5 days is also shown.

4 SIMULATION WITH TIME DEPENDENT DEGRADATION RATE

Once the degradation rate $\sigma(t)\lambda(M)$ are given, the initial value problem (2) and (3) can be solved directly to see how the numerical results and the experimental results agree. Here the initial value problem was solved numerically with techniques base on previous results (Watanabe et al., 2003; Kawai et al., 2004; Watanabe et al., 2004).

Choose a positive integer N and set

$$\Delta M = \frac{b-a}{N}$$

$$M_i = a + i\Delta M, \quad i = 0,1,2,\cdots,N.$$

An approximate solution of the differential equation (1) at $M = M_i$ is denoted by $w_i = w_i(t)$ $(i = 0,1,2,\cdots,N)$. There is a non-negative integer K and a constant R such that $L = K\Delta M + R$, $0 \le R < \Delta M$, and that the inequalities

$$M_{i+K} \le M_i + L < M_{i+K+1}$$

hold. Then approximate values of $w(t, M_i + L)$ and $\beta(M_i + L)$ can be obtained by using the approximations

$$\begin{aligned} w(t, M_i + L) &\approx \left(1 - \frac{R}{\Delta M}\right) w(t, M_{i+K}) \\ &\quad + \frac{R}{\Delta M} w(t, M_{i+K+1}), \\ \lambda(M_i + L) &\approx \left(1 - \frac{R}{\Delta M}\right) \lambda(M_{i+K}) \\ &\quad + \frac{R}{\Delta M} \lambda(M_{i+K+1}). \end{aligned}$$

Substituting these expressions in the differential equation (2) and setting $M = M_i$, we obtain the linear

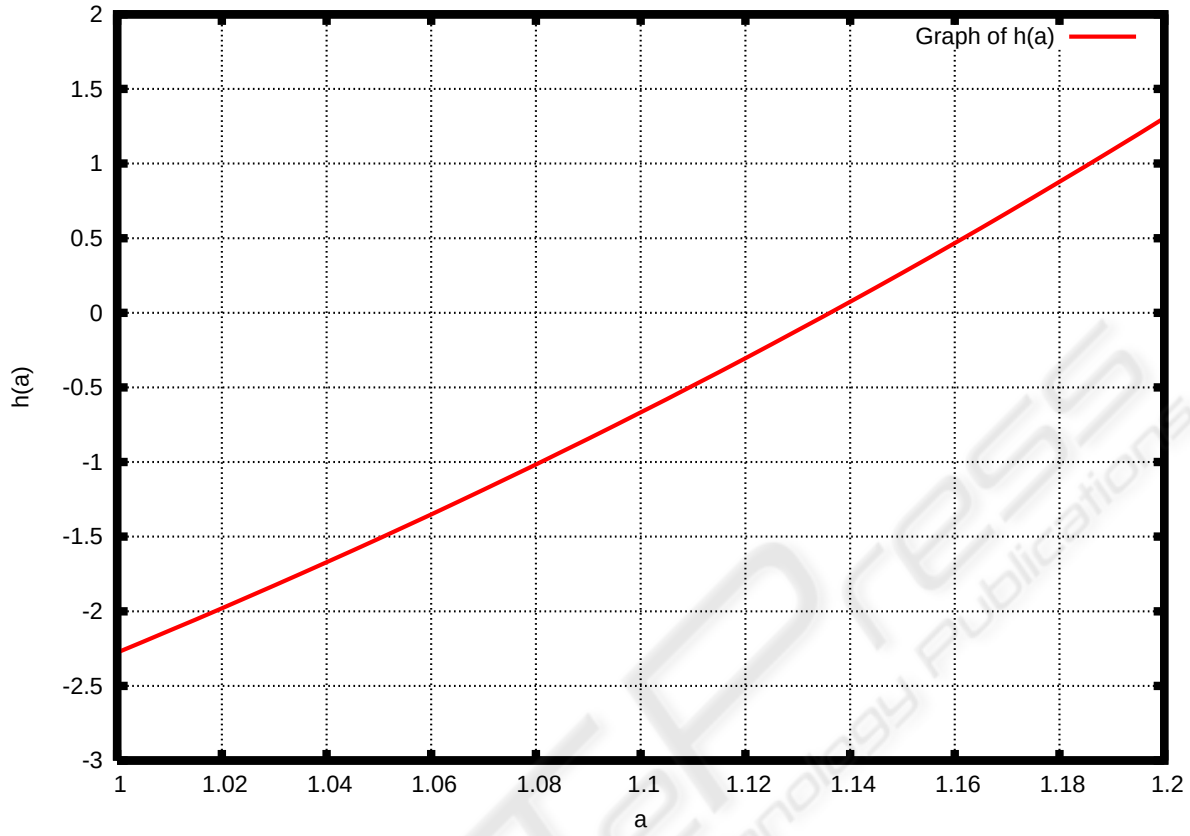

Figure 5: Graph of $h(a)$ with $T_1 = \tau_1 = 3$, $T_2 = 5$ and $\tau_2 = 30$.

system:

$$\frac{dw_i}{dt} = \sigma(t)\left(-\alpha_i w_i + \beta_i w_{i+K} + \gamma_i w_{i+K+1}\right), \quad i = 0,1,2,\cdots,N. \tag{19}$$

The coefficients α_i, β_i, and γ_i are given by

$$\alpha_i = \lambda(M_i),$$

$$\beta_i = \phi_i \frac{M_i}{M_i + L}\left(1 - \frac{R}{\Delta M}\right),$$

$$\gamma_i = \phi_i \frac{M_i}{M_i + L} \cdot \frac{R}{\Delta M},$$

$$\phi_i = \left(1 - \frac{R}{\Delta M}\right)\lambda(M_{i+K}) + \frac{R}{\Delta M}\lambda(M_{i+K+1}).$$

Approximate values of the degradation rates $\lambda(M_i)$ can be obtained from the numerical solution of the inverse problem by the linear approximation.

For all sufficiently large M, the oxidation rate becomes 0. In particular, we may assume that the last two terms on the right-hand side of the equation (19) are absent when $i+K$ exceeds N, so that the system (19) becomes a closed system to be solved for unknown functions $w_i = w_i(t)$, $i = 0,1,2,\ldots,N$. In view of the condition (3), these functions are subject to the initial condition

$$w_i(0) = f_i = f(M_i). \tag{20}$$

Given the initial weight distribution shown in Figure 2, the degradation rate $\lambda(M)$ shown in Figure 3, and the function $\sigma(t)$ given by the equation (14) with the value of a obtained numerically, the initial value problem (19) and (20) was solved numerically implementing the forth-order Adams-Bashforth-Moulton predictor-corrector in PECE mode in conjunction with the Runge-Kutta method to generate approximate solutions in the first three steps (Lambert, 1973) by using $N = 10000$, and a time interval $\Delta t = 5/24000$. Figure 6 shows the transition of the weight distribution during cultivation of the microbial consortium E-1 for five days.

Figure 7 shows the numerical result and the experimental results for the weight distribution after one day cultivation of the microbial consortium E1.

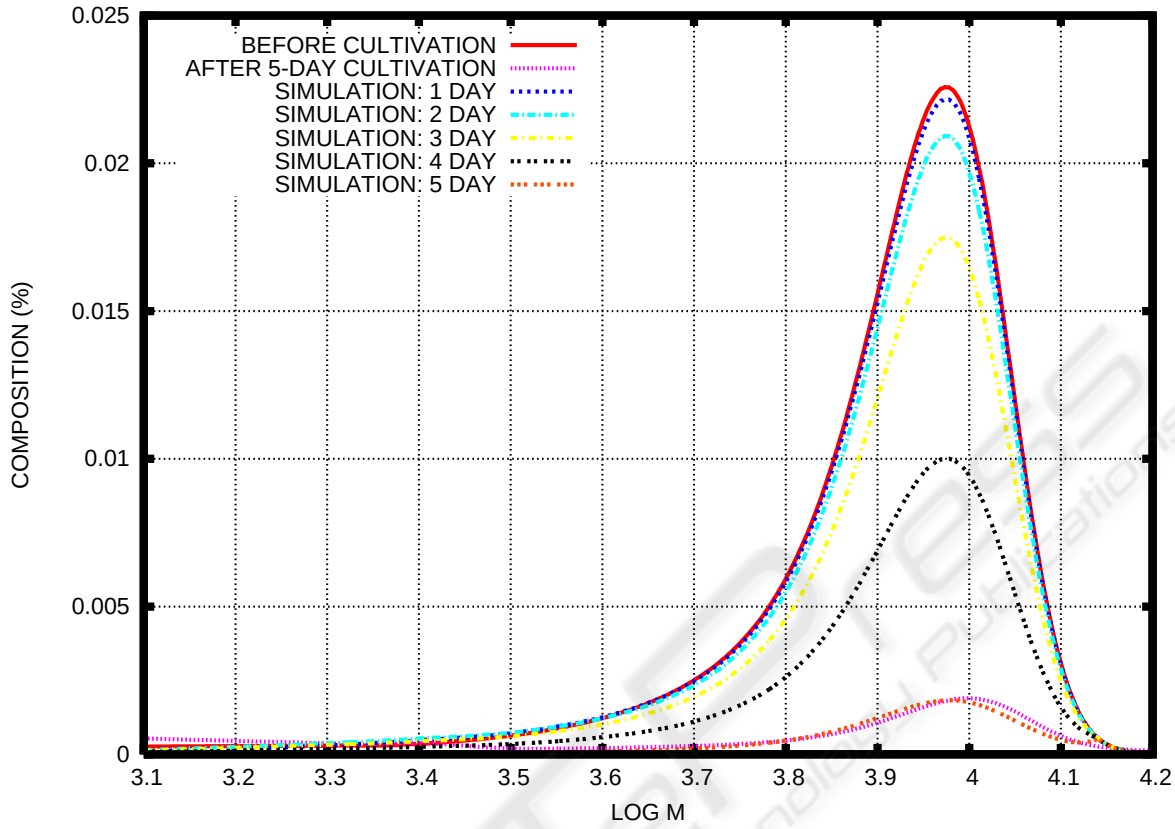

Figure 6: The weight distribution of PEG before and after 5-day cultivation, and the transition of the weight distribution based on the initial value problem (2), (3) with $\sigma(t) = e^{at+b}$, $a \approx 1.136176$, $b = \ln\{a\tau_1/(e^{aT_1}-1)\}$, $\tau_1 = T_1 = 3$.

5 DISCUSSION

Early studies of biodegradation of xenobiotic polymers are found in the second half of the 20th century. It was found that the linear paraffin molecules of molecular weight up to 500 were utilized by several microorganisms (Potts et al., 1972). Oxidation of *n*-alkanes up to tetratetracontane ($C_{44}H_{90}$, mass of 618) In 20 days was reported (Haines et al., 1974). Biodegradation of polyethylene was shown by measurement of $^{14}CO_2$ generation (Albertsson et al., 1987). The weight distribution of polyethylene before and after cultivation of the fungus Aspergillus sp. AK-3 for 3 weeks was introduced into analysis based on the time dependent exogenous depolymerization model. The transition of weight distribution for 5 weeks was simulated with the degradation rate based on the initial weight distribution and the weight distribution after 3 weeks of cultivation. The numerical result was found to be acceptable in comparison with an experimental result (Watanabe et al., 2004). The result shows that the microbial population was fully developed in 3 weeks, and that the biodegradation was with the constant rate.

The degradation rate changed over the cultivation period in the depolymerization processes of PEG. The development of microbial population accounts for the increase of degradability over the first five days of cultivation. In a depolymerization process where the microbial population becomes an essential factor, it is necessary to consider the dependence of the degradation rate on time. The numerical results based on the time dependent exogenous depolymerization model show reasonable agreement with the experimental results. Those results show that it is appropriate to assume that the degradation rate is a product of a time factor and a molecular factor. It has also been shown that the molecular factor can be determined by the weight distribution before and after cultivation experimentally. In the environment or sewer disposal, the time factor should also depends on other factors such as temperature or dissolved oxygen. Once those essentials are incorporated into the time dependent factor, the time dependent exogenous depolymerization

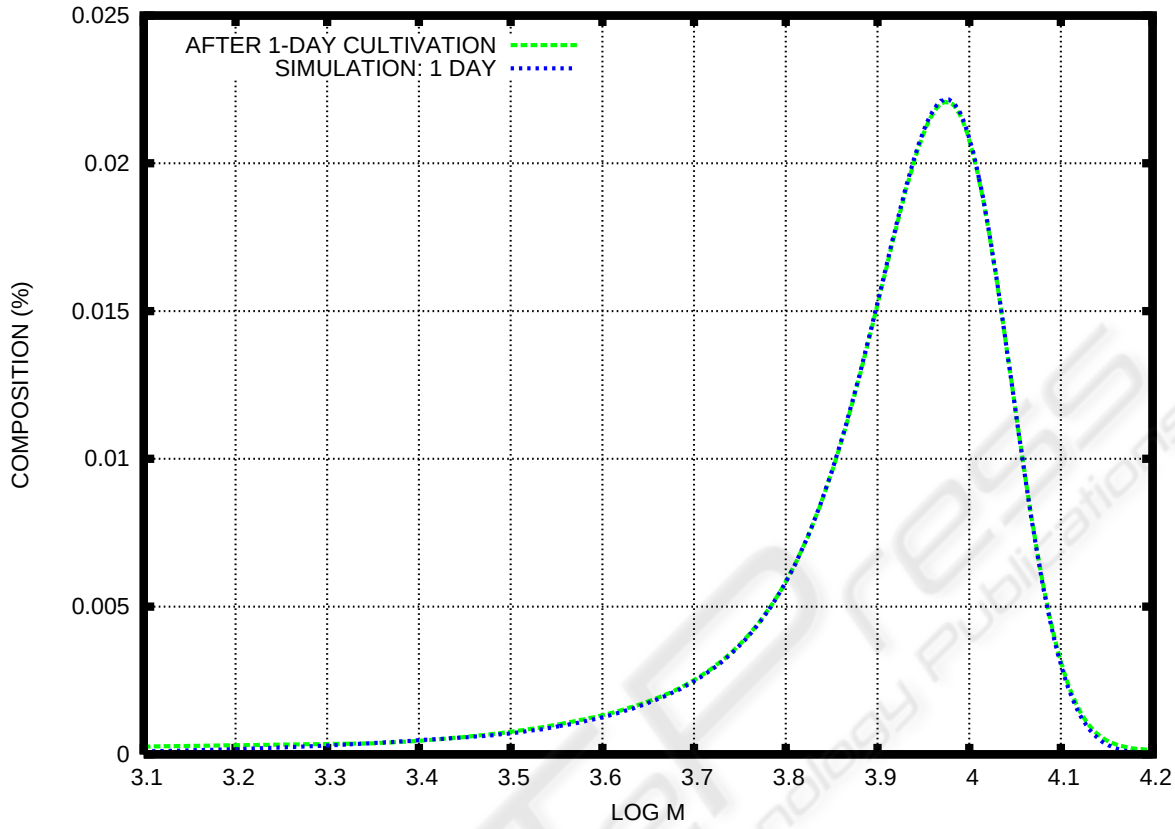

Figure 7: The weight distribution of PEG after 1-day cultivation, and the weight distribution based on the initial value problem (2), (3) with $\sigma(t) = e^{at+b}$, $a \approx 1.136176$, $b = \ln\{a\tau_1/(e^{aT_1}-1)\}$, $\tau_1 = T_1 = 3$, $t = 1$.

model and the techniques based on the model should be applicable to assess biodegradability of xenobiotic polymers.

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