

Numerical study on microbial depolymerization process of exogenous type

M. Watanabe* F Kawai†

Abstract

A microbial depolymerization process of exogenous type is studied with experimental data introduced into analysis. A mathematical model for exogenous depolymerization processes is introduced together with numerical techniques to analyze it. Given an initial weight distribution of polyethylene glycol with respect to the molecular weight and its final weight distribution obtained after cultivation of a microbial consortium E1, an inverse problem of an initial value problem was solved to determine an oxidation rate. Given the oxidation rate, the initial value problem was solved to simulate the transition of weight distribution. A numerical simulation shows that the mathematical method in analysis of an exogenous depolymerization process is practically acceptable. Numerical techniques illustrated here are applicable to analyses of other exogenous depolymerization processes.

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*Faculty of Environmental Science and Technology, Okayama University, 1-1, Naka 3-chome, Tsushima, Okayama 700-8530, JAPAN. This work is supported by JSPS Grant-in-Aid for Scientific Research (C) (16540106). <mailto:watanabe@ems.okayama-u.ac.jp>

†Research Institute for Bioresources, Okayama University

1 Introduction

In general, microbial depolymerization process falls into one of two categories: exogenous type or endogenous type. In an exogenous depolymerization process, polymers are depolymerized by separation of monomer units at their terminals. Examples include biodegradation processes of polyethylene (PE). PE is structurally a long-chain alkane (basically normal-type). The initial step of the oxidation of n -alkanes is hydroxylation to provide the corresponding primary (or secondary) alcohol, which is oxidized further to an aldehyde (or ketone) and then to an acid. Carboxylated n -alkanes are structurally analogous to fatty acids, and should be subject to β -oxidation processes to produce depolymerized alkanolic acids by two-carbon units (acetic acid). A mathematical model has been proposed and analyzed to study the PE biodegradation which involves the β -oxidation. [3, 4, 12, 10, 11]. Unlike exogenous depolymerization processes, where oxidation takes a substantial part, hydrolysis is often involved in a depolymerization process of endogenous type, in which internal cleavages take place. The enzymatic degradation of polyvinyl alcohol (PVA) is an example of endogenous depolymerization. PVA is a carbon-chain polymer with a hydroxyl group attached to every other carbon unit, which are known to be degraded by random oxidation of hydroxyl groups and hydrolysis of mono/diketones. A mathematical model was also proposed and analyzed to study the endogenous depolymerization of PVA [9, 10, 13].

In this paper, the analytical and numerical study of exogenous depolymerization is continued to cover the biodegradation of polyethylene glycol (PEG). PEG is one of polyethers that are utilized for constituents in a number of products including lubricants, antifreeze agents, inks, and cosmetics, and also for raw materials to synthesize detergents or polyurethanes. Polyethers are either water soluble or oily liquid, and eventually discharged into the environment through sewage [7]. Since they can not be incinerated or recycled, their biodegradability is an important issue as far as the carbon cycle is concerned [6]. PEG is produced in the largest quantity among polyethers, and its major part is consumed in production of nonionic surfactants. Besides PEG, polyethers include polypropylene glycol (PPG), polytetramethylene glycol (PTMG), polybutylene glycol (PBG), polyglycerin, polyglycidol, polyisobutylene oxide, polyglyoxylate, and so on. They are polymers that have chemical structures represented by the expression $\text{HO}(\text{R}-\text{O})_n\text{H}$, *e.g.*, PEG: $\text{R} = \text{CH}_2\text{CH}_2$, PPG: $\text{R} = \text{CH}_3\text{CHCH}_2$, PTMG: $\text{R} = (\text{CH}_2)_4$ [5].

In the following sections, the biodegradability of PEG is examined by analyzing its weight distribution obtained before and after a microbial consortium E-1. An inverse problem of an initial value problem was solved

numerically to determine an oxidation rate for which the solution of the initial value problem also satisfies a final condition. A result of a numerical simulation is presented to show that the mathematical model is appropriate for the PEG biodegradation. The significance of the outcome is discussed, and the dependence of PEG biodegradability on the molecular weight is examined.

2 Exogenous depolymerization model

Metabolism of polyethers has been well documented with regards to PEG. PEG is depolymerized exogenously by liberating C₂ compounds, either aerobically or anaerobically [7], and the PEG biodegradation can be presumed to follow the exogenous depolymerization model, which is originally developed for the PE biodegradation. It is based on the assumption that a molecule loses its weight by a fixed amount per unit time, that some low molecules are directly consumed by microorganisms, and that the consumption rate depends on the sizes of molecules. Those assumptions led to the following mathematical model of PE biodegradation [3, 4, 11].

$$\frac{dx}{dt} = -\alpha(M)x + \beta(M+L)\frac{M}{M+L}y, \quad (1)$$

where $\alpha(M) = \rho(M) + \beta(M)$. Here t and M represent the cultivation time and the molecular weight respectively, and $x = w(t, M)$ denotes the total weight of M -molecules (the PE molecules with molecular weight M) present at time t . The parameter L represents the amount of the weight loss due to the terminal separation. The variable y is given by $y = w(t, M+L)$, *i.e.*, the total weight of $(M+L)$ -molecules present at time t . The function $\rho(M)$ represents the direct consumption rate per unit time, and the function $\beta(M)$ represents the weight conversion rate per unit time from the class of M -molecules to the class of $(M-L)$ -molecules.

The mathematical model (1), which was originally developed for the PE biodegradation, can be viewed as a general biodegradation model involving exogenous depolymerization processes. A PEG molecule is first oxidized at its terminal, and the an ether bond is split. This process correspond to β -oxidation in the PE biodegradation. We call this process oxidation, because oxidation is involved throughout its depolymerization process [7]. Note that $L = 44$ (CH₂CH₂O) in the exogenous depolymerization of PEG.

The solution $x = w(t, M)$ of (1) is associated with the initial condition

$$w(0, M) = f(M). \quad (2)$$

Here, $f(M)$ is some prescribed function that represents the initial weight distribution. Given the total consumption rate and the oxidation rate, the equation (1) and the initial condition (2) form an initial value problem to find the unknown function $w(t, M)$. On the other hand, given the initial condition (2) and an additional final condition at $t = T > 0$:

$$w(T, M) = g(M), \quad (3)$$

an inverse problem is formulated to determine the degradation rates $\alpha(M)$ and $\beta(M)$ for which the solution $w(t, M)$ of the initial value problem (1) and (2) also satisfies the final condition (3).

It can be shown that the condition

$$0 < g(M) < f(M) + \frac{M\beta(M+L)}{M+L} \int_0^T w(s, M+L) ds. \quad (4)$$

is a sufficient condition for a unique positive total degradation rate $\alpha(M)$ to exist, given the β -oxidation rate $\beta(M+L)$ and the weight distribution $w(t, M+L)$ [11]:

Note that molecules must penetrate through the membranes to enter into the cells in order to become subject to the direct consumption. On the other hand, the rate of the membrane transport decreases, as the molecular size increases. Moreover, there should be a value of the molecular weight, which we call M_ρ , such that $\rho(M) = 0$ for $M \geq M_\rho$. Since $\alpha(M) = \rho(M) + \beta(M)$,

$$\alpha(M) = \beta(M) \quad (5)$$

for $M \geq M_\rho$. In the present case, we deal with the case where the molecular weight is so large that the equation (5) holds.

3 Computational analysis of inverse problem

Here a numerical scheme developed in [12] to solve the inverse problem is introduced, and a numerical result is presented. In order to solve the inverse problem, cover the interval $[a, b]$ with the intervals of length L : $[a + iL, a + (i + 1)L]$, $i = 0, \dots, l - 1$, extending the definition of $f(M)$ and $g(M)$ if necessary. Here the positive integer l satisfies the condition

$$\frac{b-a}{L} \leq l < \frac{b-a}{L} + 1,$$

Choose a positive integer m , and set

$$M_{i,j} = a + iL + j\delta M, \quad i = 0, 1, \dots, l-1, \quad j = 0, 1, \dots, m \quad \left(\delta M = \frac{L}{m} \right).$$

Denote by $\alpha_{i,j}$ and $\beta_{i,j}$ approximate values of $\alpha(M_{i,j})$ and $\beta(M_{i,j})$, respectively. Note that $\alpha_{i,j} = \beta_{i,j}$, $0 \leq i \leq l$, $0 \leq j \leq m$, in view of the equation (5). Let $f_{i,j} = f(M_{i,j})$, $g_{i,j} = g(M_{i,j})$. Choose a positive integer n , and set

$$t_k = k\delta t, \quad k = 0, 1, 2, \dots, n \quad \left(\delta t = \frac{T}{n} \right)$$

Then the time interval $[0, T]$ is divided into n intervals: $[t_{k-1}, t_k]$, $k = 1, 2, \dots, n$. Let $w_{i,j,k}$ denote an approximate value of $w(t_k, M_{i,j})$. Given $w_{i+1,j,k}$, $j = m-1, m-2, \dots, 0$, $k = 0, 1, \dots, n$, and $\beta_{i+1,j}$, $j = m-1, m-2, \dots, 0$, $\eta = \alpha_{i,j}$ is a solution of the equation

$$\Phi_{i,j}(\eta) = 0, \tag{6}$$

where

$$\Phi_{i,j}(\eta) = e^{-\eta T} \left[f_{i,j} + \frac{\beta_{i+1,j} M_{i,j}}{M_{i+1,j}} \times \frac{\delta t}{2} \sum_{k=1}^n (e^{\eta t_{k-1}} w_{i+1,j,k-1} + e^{\eta t_k} w_{i+1,j,k}) \right] - g_{i,j},$$

Start the numerical process at $i = l-1$ setting appropriate values of $w_{i,j,k}$, $j = 0, 1, \dots, m-1$, $k = 0, 1, \dots, n$, and march backward: $i = l-1, l-2, \dots, 0$. At each step, evaluate a numerical solution $\eta = \alpha_{i,j}$ of (6) by the Newton's method for $j = m-1, m-2, \dots, 0$, examining the condition

$$0 < g_{i,j} < f_{i,j} + \frac{\beta_{i+1,j} M_{i,j}}{M_{i+1,j}} \times \frac{\delta t}{2} \sum_{k=1}^n (w_{i+1,j,k-1} + w_{i+1,j,k}),$$

which is a sufficient condition for the equation (6) to have a unique positive solution $\eta = \alpha_{i,j}$. Once an approximate solution $\eta = \alpha_{i,j}$ of the equation (6) is found, approximate values $w_{i,j,k}$ of $w(t_k, M_{i,j})$ are given by

$$w_{i,j,k} = e^{-\eta t_k} f_{i,j} + \frac{\beta_{i+1,j} M_{i,j}}{M_{i+1,j}} e^{-\eta t_k} \frac{\delta t}{2} \sum_{p=1}^k \{ e^{\eta t_{p-1}} w_{i+1,j,p-1} + e^{\eta t_p} w_{i+1,j,p} \}$$

with $\eta = \alpha_{i,j}$ for $k = 0, 1, \dots, n$.

The microbial consortium E-1 was cultivated on a culture medium containing PEG as its sole carbon source. Gel permeation chromatography (GPC) patterns were analyzed to obtain the initial weight distribution of PEG with respect to the molecular weight, and its weight distribution obtained after the cultivation for three days, and they are shown in Figure 2. The inverse problem was to solved numerically to find the exogenous degradation rate $\beta(M)$ based on the initial weight distribution and the final weight distribution shown in Figure 1 with the values of the parameters: $L = 44$, $T = 6$, $n = 2400$, $\Delta t = 0.0025$, $m = 10$. The values of the parameters $a \approx 4466.835922$, $b \approx 15848.931925$, and $l = 259$ were also set. Figure 1 shows the graph of the exogenous degradation rate generated numerically.

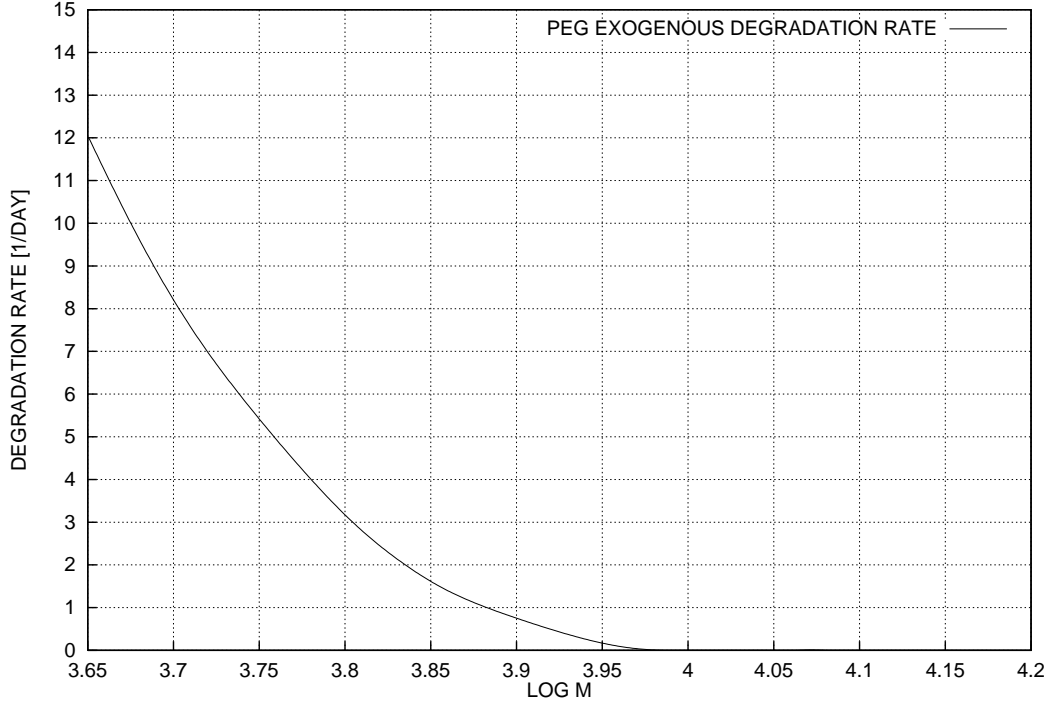


Figure 1: The exogenous degradation rate $\beta(M)$ based on GPC profiles obtained before and after cultivation of the microbial consortium E-1 (Figure 2).

4 Simulation of PEG biodegradation

In the process of evaluating the exogenous degradation rate, the approximate values $w_{i,j,k}$ of $w(t, M)$ at $t = t_k$ and $M = M_{i,j}$ are also evaluated. On the other hand, once the approximate values of the total consumption rates and the β oxidation rates are given, we can solve the initial value problem (1) and (2) directly to see how appropriately the numerical results and the experimental results agree. Here the initial value problem was solved numerically with techniques developed previously [4, 11, 12].

Choose a positive integer N and set

$$M_i = a + i\Delta M, \quad i = 0, 1, 2, \dots, N \quad \left(\Delta M = \frac{b-a}{N} \right).$$

We denote an approximate solution of the differential equation (1) at $M = M_i$ by $w_i = w_i(t)$ ($i = 0, 1, 2, \dots, N$). There are a non-negative integer K and a constant R such that $L = K\Delta M + R$, $0 \leq R < \Delta M$, and that the

inequalities

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

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

$$w(t, M_i + L) \approx \left(1 - \frac{R}{\Delta M}\right) w(t, M_{i+K}) + \frac{R}{\Delta M} w(t, M_{i+K+1}),$$

$$\beta(M_i + L) \approx \left(1 - \frac{R}{\Delta M}\right) \beta(M_{i+K}) + \frac{R}{\Delta M} \beta(M_{i+K+1}).$$

Substituting these expressions in the differential equation (1) and setting $M = M_i$, we obtain the linear system:

$$\frac{dw_i}{dt} = -\alpha_i w_i + \beta_i w_{i+K} + \gamma_i w_{i+K+1}, \quad i = 0, 1, 2, \dots, N. \quad (7)$$

Here the coefficients α_i , β_i , and γ_i are defined by

$$\alpha_i = \alpha(M_i), \quad \beta_i = \sigma_i \frac{M_i}{M_i + L} \left(1 - \frac{R}{\Delta M}\right), \quad \gamma_i = \sigma_i \frac{M_i}{M_i + L} \cdot \frac{R}{\Delta M},$$

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

Approximate values of the degradation rates $\alpha(M_i)$ and $\beta(M_i)$ can be linear approximations in terms of the approximate values $\alpha_{j,k}$ and $\beta_{j,k}$ obtained by solving the inverse problem.

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 (7) are absent when $i+K$ exceeds N , so that the system (7) becomes a closed system to be solved for unknown functions $w_i = w_i(t)$, $i = 0, 1, 2, \dots, N$. In view of the condition (2), these functions are subject to the initial condition

$$w_i(0) = f_i = f(M_i). \quad (8)$$

Given the initial weight distribution shown in Figure 2 and the corresponding degradation rates shown in Figure 1, the initial value problem (7) and (8) was solved numerically implementing the forth-order Adams-Bashforth-Moulton predictor-corrector in PECE mode (P: predictor, E: evaluation, C: corrector) in conjunction with the Runge-Kutta method to generate approximate

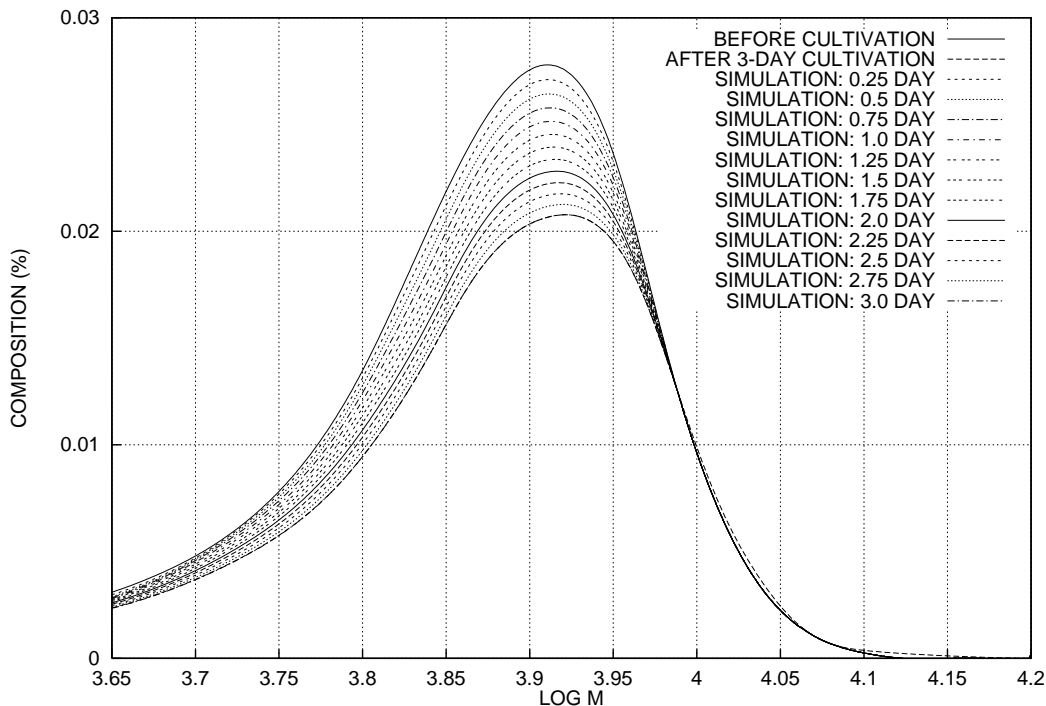


Figure 2: Transition of the Weight distribution of PEG during 3-day cultivation of the microbial consortium E-1.

solutions in the first three steps [8] by setting $N = 10000$, and $\Delta t = 0.0025$. Here Δt is the steplength in time. Figure 2 also shows the simulated transition of the weight distribution during cultivation of the microbial consortium E-1. Note that the experimental result obtained after cultivation of the microbial consortium E-1 and the corresponding numerical result are hardly distinguishable.

5 Discussion

Recall that the oxidation rate $\beta(M)$ is the ratio of the weight of M -molecules degraded per unit time. It also represents the ratio of the number of M -molecules that undergo exogenous depolymerization processes. Since the reciprocal of $\beta(M)$ can be regarded as the average time it takes for one cycle of exogenous depolymerization process to be completed, the oxidation rate $\beta(M)$ might be presumed independent of the molecular size M , for the exogenous depolymerization processes take place only at the terminals of

the molecules. In practice, however, this is the case only for molecules of moderate sizes.

As the metabolic enzymes are located in cell membranes, the exogenous depolymerization processes take place inside a cell, and at least one end of a molecule must penetrate through its outer membrane to become subject to an exogenous depolymerization process in the periplasm. When a molecule makes contact with a microorganism, a part of a fixed length should be taken into a cell in the fixed period. If that part contains one of the terminals, the enzyme takes effect and the exogenous biodegradation process starts. The possibility for a part of a fixed length to contain a terminal becomes less when the molecule becomes large. The result shown in Figure 1 clearly indicates the dependence of the rate of membrane transport with respect to the molecular weight, and our mathematical analysis has revealed the role of membrane transport in exogenous depolymerization processes.

The exogenous depolymerization model originally developed for PE biodegradation has been applied successfully to PEG biodegradation. The inverse problem has been solved numerically to find the oxidation rate. The validity of the numerical result has been confirmed by the numerical simulation. Different GPC patterns obtained before and after cultivation of a microbial consortium E-1 were taken into mathematical analysis of PEG biodegradation [14]. However, the weight distributions shown in Figure 2 were based on GPC patterns recently obtained, and this is the first time they are presented.

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