Study of Inverse Problem for Microbial Population in Biodegradation Process of Xenobiotic Polymer

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Abstract: This study shows validity of the bisection method in conjunction with the Newton-Raphson method in application to an inverse analysis of a three parameter model. Our techniques are demonstrated by application to microbial depolymerization process. Numerical results show applicability to a wide range of inverse problems.

Key words: *biodegradation, depolymerization, polyethylene glycol, inverse problem, numerical simulation*

INTRODUCTION

Petroleum based polymers has been produced industrially since around the mid twentieth century. Although those macromolecular compounds had been nonexistent until they were produced, some of those xenobiotic compounds are utilizable by microorganisms. Those petroleum based polymers have been accumulated on the surface of the earth, and they are now potential sources of carbon dioxide emission. It is an urgent task to elucidate mechanism of microbial depolymerization processes. Microbial depolymerization processes are categorized into exogenous type processes and endogenous type processes. In an exogenous type depolymerization process, molecules reduce in size through successive terminal liberation of monomer units. Unlike an exogenous type depolymerization process, molecules break up randomly in an endogenous type depolymerization process.

Polyethylene (PE) is an exogenously depolymerizable polymer. The weight reduction due to β-oxidation, and the direct consumption by cells are the primary factors in an exogenous type depolymerization process of PE. A PE molecule liberates a monomer unit (CH_2CH_2) in one cycle of β-oxidation. It reduces in size undergoing successive β-oxidation processes until it becomes small enough to be absorbed directly into a cell. According to this scenario, mathematical model was proposed for numerical simulation of PE biodegradation processes [1].

Polyethylene glycol (PEG) is another exogenously depolymerizable polymer. PEG is one of polyethers that are expressible with structural formula $HO(R-O)_nH$ (PEG: R = CH_2CH_2). A PEG molecule reduces in size liberating C_2 compounds (CH_2CH_2O) [2]. Numerical techniques that had been developed for PE biodegradation were applied to an exogenous type microbial depolymerization process of PEG [3]. Weight distributions with respect to the molecular weight before and after cultivation of microbial consortium E-1 were introduced into problems for a molecular factor of a degradation rate. Once a degradation rate was obtained, initial value problem was solved for simulation of a microbial depolymerization of PEG. The time dependence of degradation rates was also considered for formulation for a PEG biodegradation process [4].

Polyvinyl alcohol (PVA) and polylactic acid (PLA) are endogenously depolymerizable polymers. A mathematical model was proposed for an enzymatic degradation of PVA [5]. Mathematical techniques which had been developed for the enzymatic degradation of PVA were applied to an enzymatic hydrolysis of PLA, and degradabilities of PVA and PLA were compared [6]. The time dependence of a degradation rate was also considered for a PLA depolymerization process [7]. A mathematical model that had been proposed for endogenous type depolymerization processes was reformulated for exogenous type depolymerization processes of PEG [8] and PE [9]. Numerical techniques that had been developed for the PE biodegradation [9] were applied to an exogenous type depolymerization process of PEG [10]. The time dependence of degradability was

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considered in formulation of a PEG biodegradation process [11].

An exogenous type depolymerization process of PEG was revisited in this study. In the following sections, mathematical model for an exogenous type depolymerization process is described, and an inverse problem for the microbial population is illustrated. Numerical results show a practical applicability of the Newton's method in conjunction with the bisection method.

DESCRIPTION OF MATHEMATICAL MODEL FOR MICROBIAL DEPOLYMERIZATION PROCESS OF EXOGENOUS TYPE

Suppose that a polymer is the sole carbon source in a culture medium. Let $w(t, M)$ [mg] the weight distribution with respect to the molecular weight M at time t [day], and $v(t)$ [mg] be the total weight of polymer molecules with molecular weight between A and B at time t. The total weight $v(t)$ is the integral of $w(t, M)$ with respect to M over the interval $[A, B]$,

$$v(t) = \int_A^B w(t, M)\, dM \cdot$$
(1)

The total weight $v(t)$ of the entire residual polymer at time t is the integral of $w(t, M)$ with respect to M over the interval $[0, \infty)$,

$$v(t) = \int_0^\infty w(t, M)\, dM \cdot$$
(2)

Integral (1) is an appropriate approximation of the integral (2) for suitable values of A and B.

System of equations (3), (4) for the weight distribution $w(t, M)$ and the microbial population $\sigma(t)$ was proposed in previous studies [10, 11, 12, 13, 14, 15, 20].

$$\frac{\partial w}{\partial t} = \sigma(t)\left[-\lambda(M)w + c(M)\int_M^\infty \lambda(K)d(K)w(t, K)\, dK \right],$$
(3)

$$\frac{d\sigma}{dt} = k\left(1 - h\frac{\sigma}{(-v'(t))} \right)\sigma,$$
(4)

where

$$c(M) = Me^{\rho M}, \quad d(M) = \frac{e^{-\rho M}}{M(1 - e^{-\rho M})}, \quad \rho = \frac{\log 2}{L}.$$

Parameter L is the molecular weight of a monomer unit, PE: $L = 28$ (CH_2CH_2), PEG: $L = 44$ (CH_2CH_2O). Function $\lambda(M)$ is the molecular factor of the degradation rate, while $\sigma(t)$ is the time factor that corresponds to the microbial population. Note that $-v'(t)$ is the total weight reduction of the polymer in the culture medium per unit time. In an exogenous type process, weight reduction is due to liberation of monomer units which are consumed by cells, and the weight reduction $-v'(t)$ is converted to the increase of cells per unit time. The function $-v'(t)/\sigma$ is the conversion of weight reduction to population increase per unit population.

System of equations (3), (4) forms an initial value problem with initial conditions

$$w(0, M) = f_0(M),$$
(5)

$$\sigma(0) = \sigma_0,$$
t (6)

where $f_0(M)$ and σ_0 are an initial weight distribution and an initial microbial population, respectively.

SOLUTION OF INVERSE PROBLEMS FOR MOLECULAR FACTOR AND TIME FACTOR OF DEGRADATION RATE

The initial value problem (3), (4), (5), (6) is solvable for unknown functions $w(t, M)$ and $\sigma(t)$ provided the function $\lambda(M)$ and values of parameters σ_0, k, and h are given. In order to specify the function $\lambda(M)$ and values of parameters σ_0, k, and h, change of variables from t to τ given by

$$\tau = \int_0^t \sigma(s)\, ds.$$
(7)

was applied to the equations (3) and (4). Denote by $W(\tau, M)$, $S(\tau)$, and $V(\tau)$ the functions $w(t, M)$, $\sigma(t)$, and $v(t)$ respectively, where the relation (7) between t and τ holds.

Note that equations

$$\frac{d\tau}{dt} = \sigma(t), \quad \frac{dt}{d\tau} = \frac{1}{\frac{d\tau}{dt}} = \frac{1}{S(\tau)}$$

hold. Note also that equations

$$\frac{\partial W}{\partial \tau} = \frac{\partial w}{\partial t}\frac{dt}{d\tau} = \frac{1}{S(\tau)}\frac{\partial w}{\partial t}, \qquad \frac{\partial S}{\partial \tau} = \frac{\partial \sigma}{\partial t}\frac{dt}{d\tau} = \frac{1}{S(\tau)}\frac{d\sigma}{dt}, \qquad \frac{\partial V}{\partial \tau} = \frac{dv}{dt}\frac{dt}{d\tau} = \frac{1}{S(\tau)}\frac{dv}{dt}$$

hold. The system of equations (3), (4) leads to

$$\frac{\partial W}{\partial \tau} = -\lambda(M)W + c(M)\int_M^\infty \lambda(K)d(K)W(\tau,K)\,dK,$$
(8)

$$\frac{dS}{d\tau} = k\left(1 - \frac{h}{(-V'(\tau))}\right).$$
(9)

Note that equations (8) involves $W(\tau,M)$ only, while equation (3) involves two unknown functions $w(t,M)$ and $\sigma(t)$. Note also that

$$V(\tau) = \int_0^\infty W(\tau,M)\,dM$$

holds.

Suppose that the weight distribution for $\tau = T_1$, $F_1(M)$, is given, that is, $F_1(M) = W(T_1,M)$ holds, and that the weight distribution for $\tau = T_2$ $(0 \leq T_1 < T_2)$, $F_2(M)$, is given ,that is, $F_2(M) = W(T_2,M)$ holds. Equation (8), the initial condition

$$W(T_1,M) = F_1(M),$$
(10)

and the final condition
$$W(T_2,M) = F_2(M)$$
(11)

form an inverse problem for $\lambda(M)$, for which the solution of the initial value problem (8), (10) also satisfies the final condition (11).

Numerical techniques for the inverse problem (8), (10), (11) were developed in previous studies. Weight distributions after before cultivation of the microbial consortium E1 were assigned to the functions $F_1(M)$ and $F_2(M)$, respectively, values of T_1 and T_2 were set, and the inverse problem (8), (10), (11) was solved numerically. Once the function $\lambda(M)$ was obtained, equation (8) was solved for $W(\tau,M)$ with the initial condition

$$W(0,M) = f_0(M).$$
(12)

A previous study shows that $V(\tau)$ is well approximated by an exponential function $v_0 e^{-\mu\tau}$, which indicates that $V(\tau)$ is approximated by the solution of the initial value problem

$$\frac{dV}{d\tau} = -\mu V, \quad V(0) = v_0,$$
(13)

and that $v(t)$ is approximated by the solution of the initial value problem

$$\frac{dv}{dt} = -\mu\sigma v, \quad v(0) = v_0,$$
(14)

where

$$v(0) = \int_0^\infty w(0,M)\,dM = \int_0^\infty f_0(M)\,dM.$$

Suppose that $V(\tau) = v_0 e^{-\mu\tau}$ is the solution of the initial value problem (13) with given values of v_0 and μ. A solution of the equation (9) with the initial value σ_0 is not only a function of τ, but also a function of parameters σ_0, k, and h, and we denote it by $S(\tau,\sigma_0,k,h)$. The change of variables (8) leads to $t = q(\tau,\sigma_0,k,h)$, where

$$q(\tau,\sigma_0,k,h) = \int_0^\tau \frac{dr}{S(r,\sigma_0,k,h)}$$
(13)

Given $m+1$ pairs of values of t and $v(t)$, $(t_0,v_0),(t_1,v_1),\ldots,(t_m,v_m)$, corresponding the values of τ, $\tau_0,\tau_1,\ldots,\tau_m$ are obtained by solving $V(\tau_i) = v(t_i)$ $(i = 1, 2,\ldots, m)$. Given m pairs of values of t and τ, (t_i,τ_i) $(i = 1, 2,\ldots, m)$, define functions $g_i(\sigma_0,k,h)$ $(i = 1, 2,\ldots, m)$ by

$$g_i(\sigma_0,k,h) = q(\tau_i,\sigma_0,k,h) - t_i,$$

and consider the equations for σ_0, k, and h,

$$g_i(\sigma_0,k,h) = 0 \quad (i = 1, 2,\ldots, m).$$
(14)

Various methods were applied to systems of equations such as system (14). Those are the Newton-Raphson method in conjunction with the bisection method, the Newton-Raphson method in conjunction with the Newton's method, the Newton-Raphson method, and the Gauss-Newton method [12 – 15, 17, 18, 20].

NUMERICAL RESULTS FOR MICROBIAL POPULATION IN EXOGENOUS TYPE MICROBIAL DEPOLYMERIZATION PROCESS OF PEG

The results from cultivation of the symbiotic mixed culture E-1 on PEG 6000 was reported [16]. Let $t_i = i$ $(i = 0, 1, \ldots, 6)$, $t_7 = 8$ and $t_8 = 10$. The residual PEG $v_0, v_1, \ldots, v_8$ at time $t_0, t_1, \ldots, t_8$, respectively, are given. Recall that function $S(\tau, \sigma_0, k, h)$ is the solution of the equation (7) with the

initial value σ_0. The values of the parameters $\mu = 2$ and $\sigma_0 = 0.1$ were set, and the Newton's method was applied to the equation

$$g_2(\sigma_0, k, h) = 0$$
(15)

for the function $k = \psi(h)$ which satisfies $g_2(\sigma_0, \psi(h), h) = 0$. Fig. 1 (a) shows a numerically generated graph of $k = \psi(h)$ for $120.0 \le h \le 160.0$. Fig. 1 (b) shows the curve $(h, g_4(\sigma_0, \psi(h), h))$ for $120.0 \le h \le 160.0$. Fig. 1 (b) shows that there is a solution of

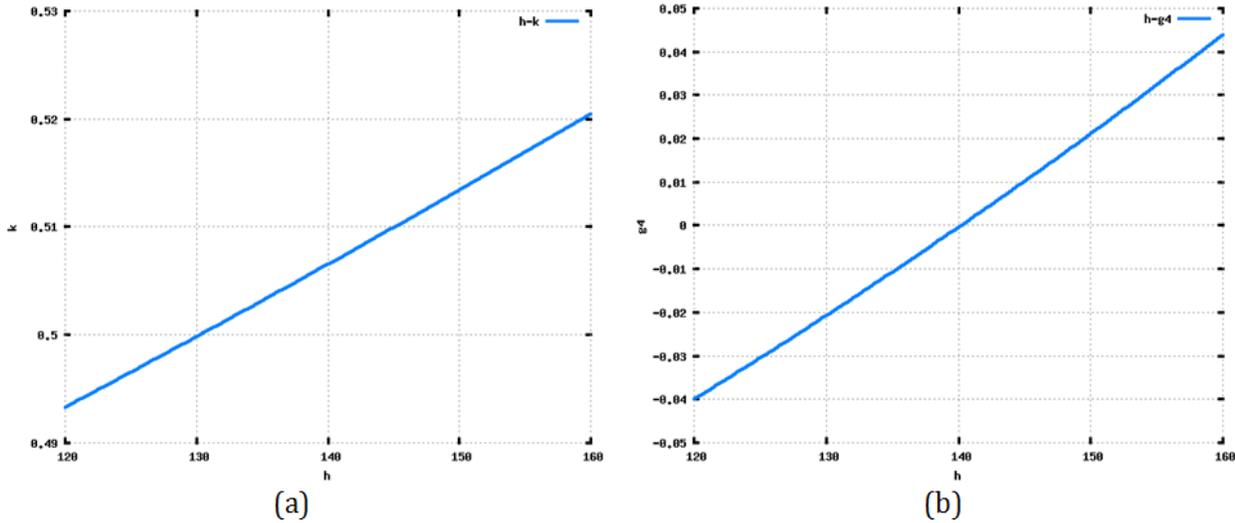$$g_4(\sigma_0, \psi(h), h) = 0$$
(16)

in the interval $[120.0, 160.0]$.

Fig. 1: (a) Graph of $k = \psi(h)$. Equation (15) was solved numerically by the Newton's method for values of h that satisfy $120.0 \le h \le 160.0$. (b) curve $(h, g_4(\sigma_0, \psi(h), h))$..

The bisection method was applied to the equation (16). Table 1 shows the iteration process of bisection method. It shows that it took thirty four steps of iterations for $|g_4(\sigma_0, \psi(h), h)|$ to reduce to a value less

than 10^{-12}. Fig. 2 (a) shows a numerical result for the residual PEG in the culture medium. Fig. 2 (b) shows a numerical result for the microbial population $\sigma(t)$.

Table 1. Iteration process of the bisection method. Entries in the first column n are the iteration counts of the bisection method, and the entries in the third column m are the number of iterations of the Newton's method for residual between successive approximations to reduce to a value less than 10^{-12}.

n	h	m	σ_0	$k = \psi(h)$	$g_4(\sigma_0, \psi(h), h)$
0	120.0000000000000000	7	0.1000000000000000	0.4933183148733690	0.0400006931561174
0	160.0000000000000000	7	0.1000000000000000	0.5204876672523610	0.0439858757038953
1	140.0000000000000000	7	0.1000000000000000	0.5065355165547730	0.0004042817499919
5	141.2500000000000000	6	0.1000000000000000	0.5073853686307030	0.0022205404845517
10	140.1953125000000000	6	0.1000000000000000	0.5066681164666870	0.0000046117595556
15	140.1940917968750000	5	0.1000000000000000	0.5066672874996230	0.0000020547593582
20	140.1931381225580000	4	0.1000000000000000	0.5066666398710060	0.0000000571147627
25	140.1931107044210000	3	0.1000000000000000	0.5066666212517080	0.0000000003177143
30	140.1931108906860000	2	0.1000000000000000	0.5066666213782060	0.0000000000746851
34	140.1931108557610000	2	0.1000000000000000	0.5066666213544840	0.0000000000001164

Viable cell density $[10^9/\text{ml}]$ of *S. terrae* s_i at $t_i (i = 0,1,\ldots,8)$ were reported [16]. A linear relation $s = a\sigma + b$ between the cell density s and microbial population σ was assumed and the cell density was assumed. The least square approximation based on $(\sigma(t_i), s_i)$ $(i = 0,1,2,3)$ led to the values of a and b,

$a \approx 45.400549072956856$, $b \approx -4.7609816633566258$ (Fig 3 (a)). Figure 3 (b) shows the conversion of the curve $(t, \sigma(t)) = (q(\tau, \sigma_0, k, h), S(\tau, \sigma_0, k, h))$ according to the linear function.
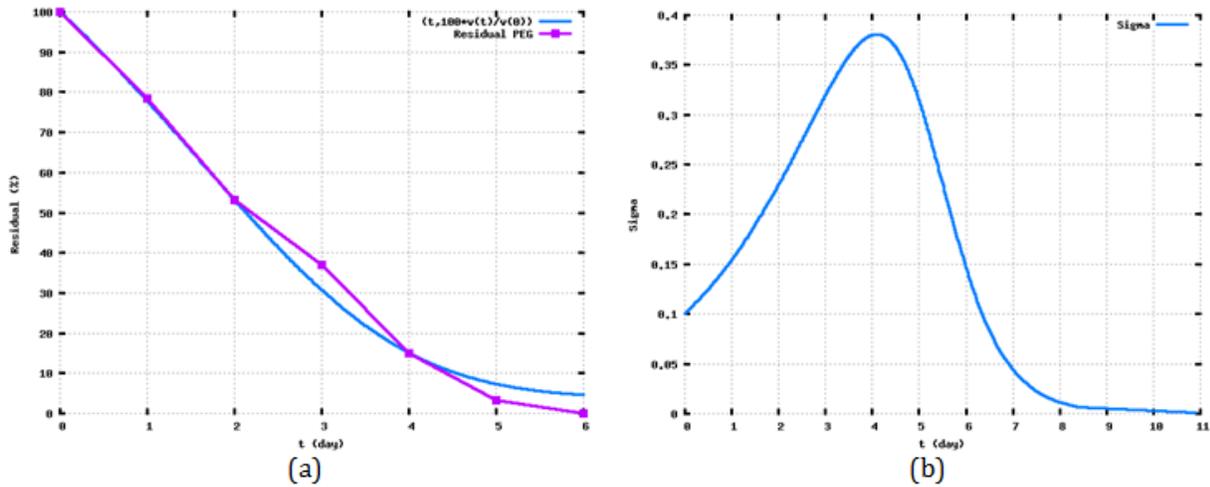

Fig. 2: (a) Curve $(t, y) = (q(\tau, \sigma_0, k, h), 100.0 \times V(\tau)/v(0))$. The figure also shows Residual PEG $v_0, v_1, \ldots, v_6$ at time $t_0, t_1, \ldots, t_6$. (b) Curve $(t, \sigma(t)) = (q(\tau, \sigma_0, k, h), S(\tau, \sigma_0, k, h))$.
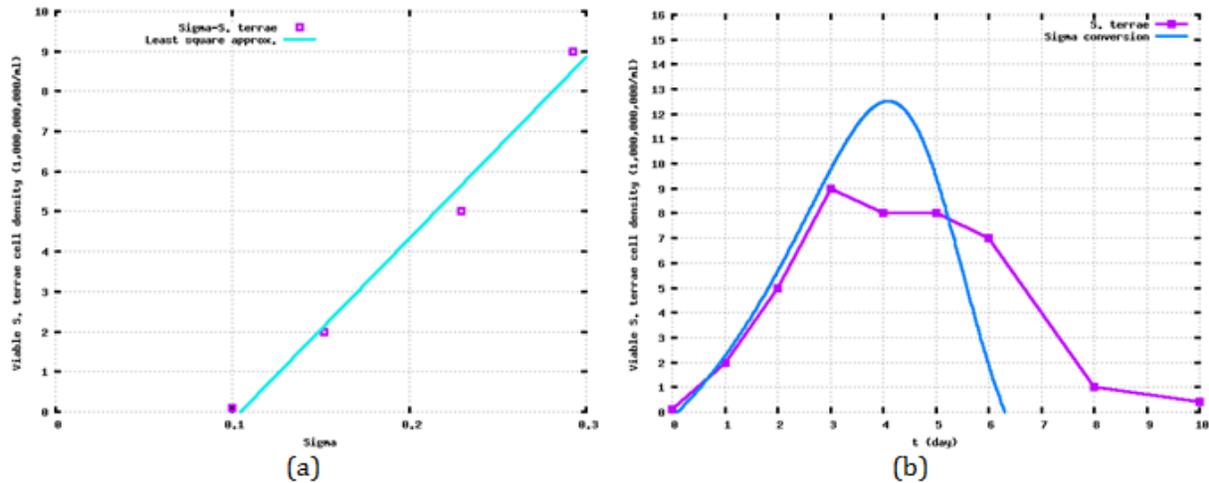
Fig. 3: (a) $\left(\sigma(t_i), s_i\right)$ $(i=0,1,2,3)$. A least square approximation $s = a\sigma + b$ is also shown $(a \approx 45.400549072956856,\ b \approx -4.7609816633566258)$. (b) Cell density *S. terrae* s_i at $t_i(i=0,1,\ldots,8)$ and the conversion of the curve $(t,\sigma(t)) = \left(q(\tau,\sigma_0,k,h), S(\tau,\sigma_0,k,h)\right)$ according to the linear function.

CONCLUSION

In previous studies [10 - 15, 17, 18], weight distributions before and after cultivation of microorganisms in culture media were introduced into inverse analysis for the molecular factor $\lambda(M)$ and the time factor $\sigma(t)$. A previous study [20] demonstrated that an exogenous type microbial depolymerization process is simulated with a set of residual polymer before after cultivation of microorganisms. The Gauss-Newton method [19] was applied to a nonlinear least square problem derived from the equation

$$\frac{d\sigma}{dt} = k\left(1 - h\frac{\sigma}{v(t)}\right)\sigma.$$
(17)

In this study, the Newton's method in conjunction with the bisection method was applied to the system of equations $g_2(\sigma_0,k,h)=0,\ g_4(\sigma_0,k,h)=0$.

The symbiotic mixed culture E-1 is an aggregate of *S. terrae* and *Rhizobium* sp. In the microbial depolymerization process, *S. terrae* was a primary PEG utilizing bacteria. Viable cell density of *S. terrae* was measured by colony counting on nutrient agar plates [16]. Figure 3 (b) shows the transition of viable cell density of *S. terrae* and a conversion of the numerical result for $\sigma(t)$. It shows an acceptable agreement between the experimental results and the numerical results for $t_1, t_2, t_3,$ and t_5, while it shows notable disagreements between the experimental results

and the numerical results for $t_4, t_6, t_7,$ and t_8. A further study is required for verification of our numerical techniques.

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