Computational study of bacterial depolymerization process of xenobiotic polymer

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Abstract. This study shows an efficient applicability of computational techniques to analyses of microbial depolymerisation process. Microorganisms were cultivated in a culture media in which a polymer was a sole carbon source, and weight distributions before and after cultivation were introduced into inverse analysis for a molecular factor and a time factor of a degradation rate. An inverse problem for two parameter values associated with the time factor was solved numerically. Once the molecular factor and the time factor were given, microbial depolymerization process was simulated.

1 Introduction

In a biodegradation process of Polyethylene (PE), molecules liberate monomer units from their terminals. Such a depolymerization process is called an exogenous type depolymerization process. Polyethylen glycol (PEG) is another exogenously biodegradable polymer. Utilization of PEG of average molecular weight 20000 by Psedonomas aeruginosa was documented [1]. Degradation of PEG 20000 by anaerobic bacteria isolated from sludge of a municipal anaerobic digester was reported [2]. Efficient biodegradation of PEG by Pseudomonas stutzeri was observed [3]. A mathematical model was formulated and numerical techniques were applied to biodegradation of PE [4]. Those techniques were reapplied to a biodegradation process of PEG [5].

Unlike exogenous type processes, random scission is an essential mechanism of endogenous type processes. Polyvinyl alcohol (PVA) and polylactic acid (PLA) are distinctive endogenously depolymerizable polymers. A mathematical model was formulated and numerical techniques were applied to an enzymatic degradation process of PVA [6]. Techniques applied to the enzymatic degradation of PVA was reapplied to an enzymatic hydrolysis of polylactic acid (PLA) [7]. Techniques originally applied to endogenous type processes were replied to exogenous type processes [8].

This study revisited a biodegradation process of PEG. Experimental outcomes before and after cultivation of bacteria in culture media were incorporated into a computational analysis. Numerical solutions of inverse problems for a molecular factor and a time factor of a degradation rate were obtained. Once the inverse problems were solved, biodegradation process was simulated.

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2 Computational model for exogenous type microbial depolymerization process

Suppose that $w(t,M)$ [mg] is the weight distribution of a polymer with respect to the molecular weight M at time t, and that $v(t)$ [mg] is the total weight of polymer molecules with molecular weight between A and B at time t. The total weight $v(t)$ over the interval $[A,B]$ is expressible in terms of the integral of weight distribution with respect to the molecular weight M from A to B, as equation (1) shows,

$$v(t)=\int_A^B w(t,M)\,dM\,. \tag{1}$$

In particular, total weight $v(t)$ of the entire residual polymer at time t is the integral of weight distribution with respect to the molecular weight M from 0 to ∞, as equation (2) shows,

$$v(t)=\int_0^\infty w(t,M)\,dM\,. \tag{2}$$

Integral (1) becomes an appropriate approximation for the integral (2) with suitable values of A and B. In this study, integral over the infinite interval $[0,\infty)$ was approximated with an integral over a finite interval $[A,B]$, where $A=10^{3.2}$ and $B=10^{4.2}$. Similarly, integral with the lower limit 0 was approximated with an integral with the lower limit A, and an integral with the upper limit ∞ was approximated with an integral with upper limit B.

Suppose that $\sigma(t)$ denotes the microbial population at time t. System of equations (3), (4) for the weight distribution $w(t,M)$ and the microbial population $\sigma(t)$ was proposed in previous studies [8 - 12].

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

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

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

Here, parameter L is the molecular weight of a monomer unit, *e.g.* PE: $L=28$ (CH_2CH_2), PEG: $L=44$ (CH_2CH_2O). Note that function $\lambda(M)$ is the molecular factor of degradation rate, and that the microbial population $\sigma(t)$ is the time factor of degradation rate. Note also that equations (2) and (3) lead to equation (5)

$$v'(t)=\int_0^\infty \frac{\partial w}{\partial t}\,dM=\sigma(t)\int_0^M\left[-\lambda(M)w+c(M)\int_M^\infty \lambda(K)d(K)w(t,K)\,dK\right]dM \tag{5}$$

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

$$w(0,M)=f_0(M), \tag{6}$$

$$\sigma(0)=\sigma_0\,, \tag{7}$$

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

3 Numerical solutions of inverse problems for molecular factor and time factor of degradation rate

The initial value problem equations (3), (4), (6), (7) is tractable to numerical computation for approximations of $w(t,M)$ and $\sigma(t)$ provided molecular factor $\lambda(M)$ and values of parameters σ_0, k, and h are specified. So the function $\lambda(M)$ and values of parameters σ_0, k, and h must be obtained before the initial value problem is tackled.

Consider the change of variables from t to τ defined by equation

$$\tau = \int_0^t \sigma(s)\, ds. \tag{8}$$

Suppose that functions $W(\tau,M)$, $S(\tau)$, and $V(\tau)$ correspond to functions $w(t,M)$, $\sigma(t)$, and $v(t)$, respectively, where the relation (8) between t and τ holds. Note that equation (9)

$$v'(t) = \frac{dv}{dt} = \frac{dV}{d\tau}\frac{d\tau}{dt} = V'(\tau)\sigma(t), \tag{9}$$

holds, and that equations (10) and (11)

$$\frac{\partial W}{\partial \tau} = -\lambda(M)W + c(M)\int_M^\infty \lambda(K)d(K)W(\tau,K)\, dK \tag{10}$$

$$\frac{dS}{d\tau} = -kV'(\tau) - h \tag{11}$$

hold in view of equations (3) and (4). Let $F_1(M)$ be the weight distribution $W(\tau,M)$ for $\tau = T_1$ so that equation (12)

$$W(\tau_1,M) = F_1(M) \tag{12}$$

holds, and let $F_2(M)$ be the weight distribution $W(\tau,M)$ for $\tau = T_2$ $(0 \le T_1 < T_2)$ so that equation (13)

$$W(\tau_2,M) = F_2(M) \tag{13}$$

holds. Equation (10), the initial condition (12), and the final condition (13) form an inverse problem for $\lambda(M)$, for which the solution of the initial value problem of equations (10), (12) also satisfies the final condition as equation (13) shows.

Numerical techniques developed in previous studies were applied to the inverse problem. In particular, weight distributions of PEG before and after cultivation of microbial consortium E-1 for two days, four days, and seven days were introduced into the inverse analysis. Denote the weight distribution before cultivation by $f_0(M)$, and denote the weight distributions after cultivation for two days, four days, and seven days by $f_1(M)$, $f_2(M)$ and $f_3(M)$, respectively, that is $w(t_0,M) = f_0(M)$, $w(t_1,M) = f_1(M)$, $w(t_2,M) = f_2(M)$, $w(t_3,M) = f_3(M)$, ($t_0 = 0$, $t_1 = 2$, $t_2 = 4$, $t_3 = 7$).

Weight distributions after cultivation of the microbial consortium E-1 for two days $f_1(M)$ and four days $f_2(M)$ were set for functions $F_1(M)$ and $F_2(M)$, respectively, values of and the inverse problem (10), (11), (12) was solved numerically for $T_1 = 0$ and $T_2 = 2$.

Once the function $\lambda(M)$ was obtained, initial value problem of equation (10) was solved for $W(\tau, M)$ subject to the initial condition,

$$W(\tau_0, M) = f_0(M), \tag{14}$$

where $\tau_0 = 0$. Once the initial value problem (10), (14) was solved, equations $V(\tau_i) = v(t_i)$ $(i = 1, 2, 3)$ were solved numerically. A previous study shows that function $V(\tau)$ is well approximated by an exponential function $V(\tau) = v_0 e^{-\mu\tau}$ $\left(v_0 = \int_0^\infty f_0(M)\,dM\right)$. Note that $V'(\tau) = -\mu v_0 e^{-\mu\tau}$ for this approximation. In this study, $V(\tau)$ was approximated by the exponential function with $\mu \approx 0.511$, and approximate values $\tau_1 \approx 0.632$, $\tau_2 \approx 2.593$, and $\tau_3 \approx 7.764$ were obtained.

A solution of equation (11) with some initial value σ_0 depends not only on τ but also on σ_0, k, and h. So let the solution of the equation (11) with initial value σ_0 be denoted by $S(\tau, \sigma_0, k, h)$. In view of the expression (8), $t = q(\tau, \sigma_0, k, h)$, where equation (15) holds.

$$q(\tau, \sigma_0, k, h) = \int_0^\tau \frac{dr}{S(r, \sigma_0, k, h)} \tag{15}$$

Consider functions $g_i(\sigma_0, k, h) = q(\tau_i, \sigma_0, k, h) - t_i$, and consider the equations for σ_0, k, and h,

$$g_1(\sigma_0, k, h) = 0, \quad g_2(\sigma_0, k, h) = 0. \tag{16}$$

In this study, σ_0 was fixed $(\sigma_0 = 0.1)$, and the Newton's method in conjunction with the bisection method was applied to system (16). Specifically, equation $g_1(\sigma_0, k, h) = 0$ was solved numerically with the Newton's method for function $k = \psi(h)$ such that $g_1(\sigma_0, \psi(h), h) = 0$, and equation $g_2(\sigma_0, \psi(h), h) = 0$ was solved for h with the bisection method. At each step of the bisection method, the Newton's method was applied to the equation $g_1(\sigma_0, k, h) = 0$, and iterations were repeated until errors between successive approximations to reduced to a value less than or equal to 10^{-12}. It took thirty seven iterations for $|g_2(\sigma_0, \psi(h), h)|$ to reduce to a value less than or equal to 10^{-10}. Final vales of *k* and *h* are approximately equal to 0.00623 and 0.489, respectively. Figure 1 shows values of optical density OD 630, O_0, O_1, O_2, and O_3, before and after cultivation of the microbial consortium E-1 for two days, four days, and seven days. It also shows the OD conversion of the microbial population, that is, where $y(t) = \sigma(t) / S_a * O_a$. Here $\sigma(t) = S(\tau, \sigma_0, k, h)$ for $t = q(\tau, \sigma_0, k, h)$, and S_a and O_a are averages of the microbial population and the optical density, respectively, that is, $S_a = \frac{1}{t_3 - t_0}\int_{t_0}^{t_3} \sigma(t)\,dt$, $O_a = \frac{O_0 + O_1 + O_2 + O_3}{4}$.

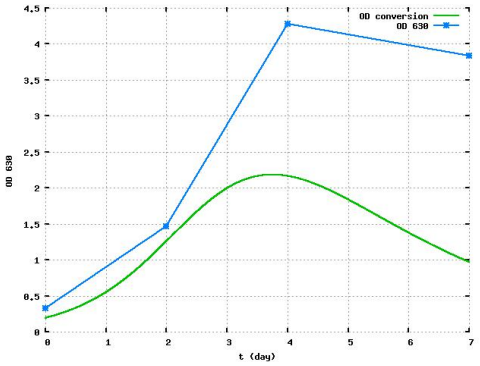
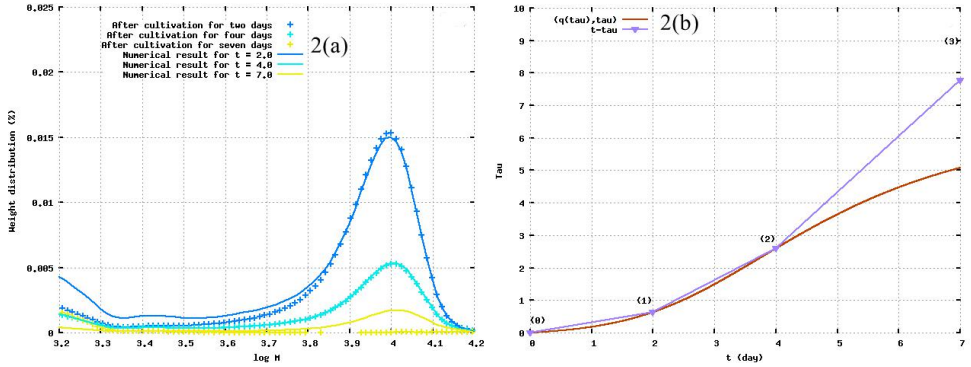


Fig. 1. OD 630 and OD 630 conversion of microbial population. The figure shows OD 630, O_0, O_1, O_2, and O_3, before and after cultivation of the microbial consortium E-1 for two days, four days, and seven days, and the OD conversion of the microbial population $y(t)=\sigma(t)/S_a * O_a$ $\left(S_a=\frac{1}{t_3-t_0}\int_{t_0}^{t_3}\sigma(t)\,dt,\quad O_a=\frac{O_0+O_1+O_2+O_3}{4}\right)$.



(a) (b)

Fig. 2. (a) The weight distributions obtained experimentally and numerically. The figure shows experimental results and numerical results for the weight distribution after cultivation of microbial consortium E-1 for two days, four days, and seven days. (b) Curve $(t,\tau)=(q(\tau,\sigma_0,k,h),\tau)$ and points (t_i,τ_i) $(i=1,2,3)$. Values of $q(\tau_1,\sigma_0,k,h)$ and $q(\tau_2,\sigma_0,k,h)$ well agree with t_1 and t_2. However the value of $q(\tau_3,\sigma_0,k,h)$ disagrees with t_3.

Once the molecular factor $\lambda(M)$ and the time factor $\sigma(t)$ of the degradation rate were obtained, initial value problem (3), (6) was solved numerically for simulation of the microbial depolymerization process. Figure 2(a) shows experimental results and numerical results for the weight distribution before and after cultivation of the microbial consortium E-1 for two days, four days, and seven days. Figure 2(b) shows the curve $(t,\tau)=(q(\tau,\sigma_0,k,h),\tau)$. Note that values of $q(\tau_1,\sigma_0,k,h)$ and $q(\tau_2,\sigma_0,k,h)$ well agree with t_1 and t_2, respectively. However the values of wa disagrees with t_3. Figure 2(b) shows why the numerical result and the experimental result for the weight distribution after cultivation for seven days disagree. The numerical result for the weight distribution after cultivation for seven days should be appropriate for the one after cultivation for five days.

4 Discussion and conclusion

Equation (4) was derived from the fact that the consumption of monomer unit by microorganisms is converted to the increase in the microbial population. Note that $-v'(t)$ is the total amount of monomer units consumed by the microorganisms per unit time. Equation (4) asserts that this amount is converted to the increase of the microbial population. Our numerical results show that the conversion rate per mg and unit population was approximately 0.5.

In a previous study [12], equation (4) was applied to a set of residual PEG values. However in this study, it was applied to the set of weight distributions. The Newton's method in conjunction with the bisection method was applied to the system of equations (16) for two parameters of microbial population. The residual PEG was approximated by an exponential function $V(\tau)=v_0 e^{-\mu\tau}$, where the value of μ was obtained from a numerical result of initial value problem (10), (14).

Results of this study are summarized in Figures 1 and 2. Figure 1 shows the curve $(t,\sigma(t))=(q(\tau,\sigma_0,k,h),S(\tau,\sigma_0,k,h))$ and values of OD 630. Values of optical density involve viable cells as well as inviable cells, whereas the microbial population $\sigma(t)$ is the population of viable cells. Another factor concerning the optical density was involved in the PEG degradation process. The symbiotic mixed culture E-1 consists of *S. Terrae* and *Rhizomium* sp. A study [13] shows that *S. terrae* is the main degrader in a PEG biodegradation process. It shows that *S. terrae* cells increase while sufficient carbon sources are available, and that they rapidly decrease after consumption of PEG. The OD 630 conversion of the microbial population (Figure 1) corresponds to viable *S. terrae* cells. The numerical result shows the viable *S. terrae* cells started decreasing after four days.

Figure 2(a) shows numerical results for the function $w(t_i,M)$ $(i=1,2,3)$, and the weight distributions after cultivation of the microbial consortium E-1 for two days, four days and seven days. Acceptable agreements between the numerical results and the experimental results for weight distributions after cultivation for two days and four days show that the model (3), (4) is applicable to inverse analysis with a set of weight distributions.

This study demonstrated efficient applicabilities of the Newton's method and the bisection method to inverse problems that arise in studies of microbial depolymerization processes. In particular, equation for two parameters were analyzed numerically with those numerical methods. Applicabilities of those numerical techniques to problems involving three parameters or more will be further investigated in our future study.

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References

1. J. R. HAINES AND M. ALEXANDER, Microbial Degradation of Polyethylene Glycol, APPLIED MICROBIOLOGY, p. 621-25 (May 1975)
2. DARYL F. DWYER AND JAMES M. TIEDJE, Degradaton of Ethylene Glycol and Polyethylene Glycols by Methanogenic Consortia, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, p. 185-90 (July 1983)

3. NURIA OBRADORS AND JUAN AGUILAR, Efficient Biodgradation of High-Molecular-Weight Polyethylene Glycols by Pure Cultures of *Pseudomonas stutzeri*, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, p. 2383-2388 (Aug. 1991)
4. Masaji Watanabe, Fusako Kawai, Masaru Shibata, Shigeo Yokoyama, Yasuhiro Sudate, Shizue Hayashi, Analytical and computational techniques for exogenous depolymerization of xenobiotic polymers, Mathematical Biosciences, 19-37, 192 (2004)
5. M. Watanabe and F. Kawai, Effects of microbial population in degradation process of xenobiotic polymers, In P. Howlett, M. Nelson, and A. J. Roberts, editors, Proceedings of the 9th Biennial Engineering Mathematics and Applications Conference, EMAC-2009, vol **51** ANZIAM J, p. C682--C96, http://journal.austms.org.au/ojs/index.php/ANZIAMJ/article/view/2433 (September 2010)
6. Masaji Watanabe, Fusako Kawai, Mathematical modelling and computational analysis of enzymatic degradation of xenobiotic polymers, Applied Mathematical Modelling 30 1497-1514 (2006)
7. Masaji Watanabe, Fusako Kawai, Sadao Tsuboi, Shogo Nakatsu, Hitomi Ohara, Study on Enzymatic Hydrolysis of Polylactic Acid by Endogenous Depolymerization Model, Macromolecular Theory and Simulations 16, 619-26 (2007)
8. Masaji Watanabe, Fusako Kawai, Simulation for microbial depolymerization processes of polyethylene glycol, Proceedings of the 2013 International Conference on Advances in Intelligent Systems in Bioinformatics, Editors: Dr. Ford Lumban Gaol, Dr. Benfino Soewito, Prof. Dr. Mohamed Bououdina, Prof. Dr. Mu-Song Chen, intel-13, Advances in Intelligent Systems Research, Atlantis Press, 71 – 76. ISBN: 978-94-6252-000-4, ISSN: 1951-6851 http://www.atlantis-press.com/php/pub.php?publication=intel-13 (February 2014)
9. Masaji Watanabe, Fusako Kawai, Numerical Techniques for Inverse Problems from Modeling of Microbial Depolymerization Processes, International Journal of Applied Engineering Research ISSN 0973-4562 Vol. **11**, Number 8, p. 5461-5468. http://www.ripublication.com/ijaer16/ijaerv11n8_18.pdf (2016)
10. Masaji WATANABE and Fusako KAWAI, Simulation of Microbial Depolymerization Process with Exponential Consumption of Carbon Source, 2016 International Conference on Computer, Mechatronics and Electronic Engineering (CMEE 2016), DEStech Publications, Inc., Lancaster, Pennsylvania, U.S.A, 426-431, ISBN: 978-1-60595-406-6 (2016)
11. Masaji Watanabe, Fusako Kawai, Numerical Techniques for Simulation of Microbial Depolymerization Process with Time Factor of Degradation Rate, Submitted.
12. M Watanabe and F Kawai, Computational analysis for microbial depolymerization process of xenobiotic polymer, Proceedings of 2017 the 5th International Conference on Mechanical Engineering, Material Science and Civil Engineering (ICMEMSCE 2017), Paper ID: 34 (December, 2017)
13. FUSAKO KAWAI AND SHOGO ENOKIBARA, Symbiotic Degradation of Polyethylene Glycol (PEG) 20,000-Phthalate Polyester by Phthalate Ester Ester- and PEG 20,000-Utilizing Bacteria, JOURNAL OF FERMENTATION AND BIOENGINEERING, Vol. **82**, No.6, 575-579, (1996)