EXPONENTIAL APPROXIMATION OF CONSUMPTION RATE OF CARBON SOURCE IN NUMERICAL SIMULATION OF MICROBIAL DEPOLYMERIZATION PROCESS

[1]MASAJI WATANABE, [2]FUSAKO KAWAI

[1]Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan
[2]Professor Emeritus, Okayama University, Okayama, Japan
E-mail: watan-m@okayama-u.ac.jp

Abstract - This study demonstrates the validity of assumption that a part of microbial consumption is converted directly to cells in microbial depolymerization processes. The exponential consumption of residual polymer in a culture medium is incorporated in a numerical simulation of a microbial depolymerization process. Weight distributions obtained experimentally were incorporated into inverse problems for a time factor and a molecular factor of a degradation rate. Once those inverse problems were solved, a microbial depolymerization process was simulated. A mathematical model is described, numerical techniques are described, and numerical results are introduced.

Keywords - Biodegradation, Polymer, Mathematical model, Numerical simulation, Inverse problem.

I. INTRODUCTION

The major industrial production of petroleum-based polymers started in the early part of the twentieth century. Althogh those macromolecules had been nonexistent before they were artificially produced, some of those synthesized materials are utilizable by microorganisms as carbon sources. Molecules liberate monomer units from their terminals to reduce from high-molecular-weight molecules to low-molecular-weight molecules in exogenous type microbial depolymerization processes. Polyethylene (PE) and polyethylene glycol (PEG) are polymers depolymerizable in exogenous type microbial depolymerization processes. Utilization of PEG with average molecular weight 20000 by Psedonomas aeruginosa was confirmed [1]. The degradation of PEG 20000 by anaerobic bacteria isolated from sludge of a municipal anaerobic digester was documented [2]. Efficient biodegradation of PEG by Pseudomonas stutzeri was reported [3]. Exogenous type microbial depolymerization processes have also been studied numerically with experimental outcomes integrated into analysis. A mathematical model was proposed for a simulation of biodegradation of PE [4]. Numerical techniques were reapplied to a biodegradation process of PEG [5].

Unlike exogenous type microbial depolymerization processes, molecules break down randomly in endogenous type depolymerization processes. Endogenously depolymerizable polymers include polyvinyl alcohol (PVA) and polylactic acid (PLA) A mathematical model was proposed and numerical techniques were developed for an enzymatic degradation process of PVA [6]. The numerical techniques were reapplied to an enzymatic hydrolysis of polylactic acid (PLA) [7]. Techniques originally developed for endogenous type microbial depolymerization processes were replied to exogenous type microbial depolymerization processes of PE and PEG [8]. This study revisited an exogenous type microbial depolymerization process of PEG. Weight distribution before and after cultivation of microbial consortium E-1 in culture media for two days, four days, and seven days were incorporated into a computational study. Inverse problems for a molecular factor and a time factor of a degradation rate were solved numerically. In particular, the Newton's method and the bisection method were applied to equations for three parameters associated with the time factor which corresponds to the microbial population, Once those inverse problems were solved, the biodegradation process of PEG was simulated.

II. DESCRIPTION OF MATHEMATICAL MODEL AND NUMERICAL FORMULATION

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 mass of polymer molecules with molecular weight M satisfying the condition $A \le M \le B$ at time t. The total weight v(t) is expressed in terms of the integral

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

The total weight v(t) of the residual polymer at time t is expressed in terms of the integral

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

Integral (1) is a reasonable approximation of the integral (2) with appropriate values of A and B. In this study, an integral with the lower limit 0 was replaced with an integral with the lower limit $A=10^{3.1}$, and an

integral with the upper limit ∞ was replaced with an integral with upper limit B=$10^{4.2}$. Note that $-v'(t)$ is the decreasing rate of the total weight. In an exogenous type depolymerization process, this is a consumption rate of monomer units liberated from molecules.

Denote by $\sigma(t)$ the total population of viable cells at time t. System of equations

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

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

where

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

was proposed in previous studies [10 - 15]. Here, the constant L denotes the molecular weight of a monomer unit, e.g. PE: L=28 (CH_2CH_2), PEG: L=44 (CH_2CH_2O), and k and h are positive parameters. The function $\lambda(M)$ is the molecular factor of the degradation rate, and microbial population $\sigma(t)$ is the time factor of degradation rate. Note that Eq. 2 and Eq.3 lead to

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

Let $f_0(M)$ and σ_0 the initial weight distribution and the initial microbial population, respectively. Eq. 3and Eq. 4 are associated with the initial conditions

$$w(0,M) = f_0(M), \quad (6)$$

$$\sigma(0) = \sigma_0 \quad (7)$$

The initial value problem Eq. 3, Eq. 4, Eq. 6, Eq. 7 is solvable when the molecular factor $\lambda(M)$ of the degradatin rate and values of the parameters σ_0, k, and h are prescribed. Numerical techniques to solve inverse problems for the function $\lambda(M)$ and the parameters σ_0, k, and h, were developed in previous studies. The change of variables from t to τ

$$\tau = \int_0^t \sigma(s)\,ds. \quad (8)$$

converts Eq. 3 and Eq. 4 to equations suitable for the an inverse analysis. Let $W(\tau,M)$, $S(\tau)$, and $V(\tau)$ be the functions corresponding to w(t,M), $\sigma(t)$, and v(t) , respectively, according to the change of variables Eq. 8. Note

$$\frac{\partial}{\partial\tau} = \frac{\partial}{\partial t}\frac{\partial t}{\partial\tau} = \frac{1}{\sigma(t)}\frac{\partial t}{\partial\tau},$$

and Eq. 3 and Eq. 4 are converted to

$$\frac{\partial W}{\partial\tau} = -\lambda(M)W + c(M)\int_M^\infty \lambda(K)d(K)W(\tau,K)\,dK, \quad (9)$$

$$\frac{dS}{d\tau} = kV'(\tau) - h, \quad (10)$$

respectively. Note that

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

and that

$$V'(\tau) = \int_0^\infty\left[-\lambda(M)W(\tau,M) + c(M)\int_M^\infty \lambda(K)d(K)W(\tau,K)\,dK\right]dM. \quad (11)$$

Suppose that the function $F_1(M)$ is the weight distribution for $\tau = T_1$, that is,

$$W(T_1,M) = F_1(M), \quad (12)$$

and that the function $F_2(M)$ be the weight distribution for $\tau = T_2$, that is,

$$W(T_2,M) = F_2(M). \quad (13)$$

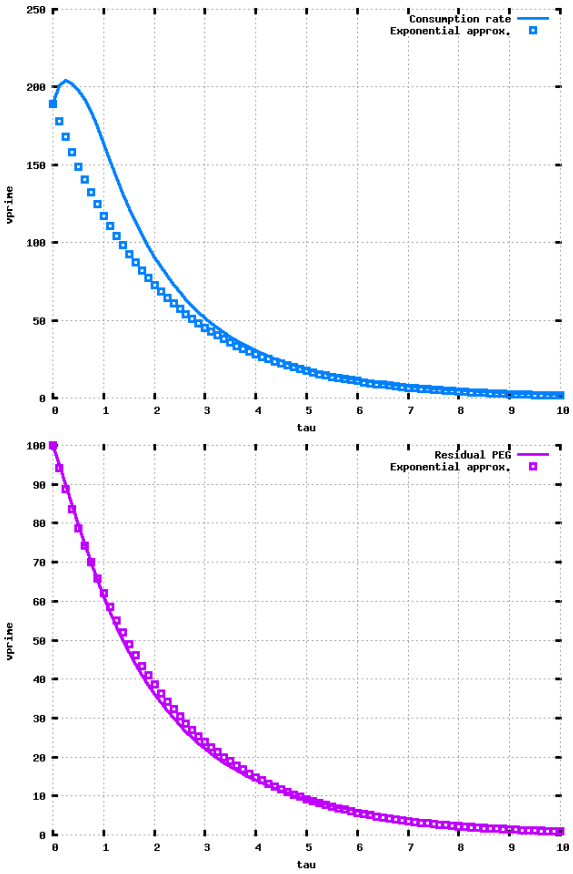
Eq. 9, the condition Eq. 12, and the condition Eq. 13 form an inverse problem for $\lambda(M)$, for which the solution of the initial value problem Eq. 9, Eq. 12 satisfies the final condition Eq. 13. In previous studies, weight distributions of PEG before after cultivation of microbial consortium E-1 for two days, four days, and seven days, $f_0(M)$, $f_1(M)$, $f_2(M)$, and $f_3(M)$, were incorporated into analysis. Note that $w(t_i,M) = f_i(M)$ (i = 0, 1, 2, 3) for $t_0 = 0$, $t_1 = 2$, $t_2 = 4$, and $t_3 = 7$, respectively. Functions $f_1(M)$ and $f_2(M)$ were assigned to $F_1(M)$ and $F_2(M)$, respectively, and the inverse problem for $\lambda(M)$ was solved numerically for $T_1 = 0$ and $T_2 = 2$.

Once the inverse problem for $\lambda(M)$ was solved, Eq. 9 was solved for $W(\tau,M)$ with the initial condition $W(\tau_0,M) = f_0(M)$, where $\tau_0 = 0$, A previous study shows that function $V(\tau)$ is well approximated with an exponential function

$$V(\tau) = v_0e^{-\mu\tau} \quad \left(v_0 = \int_0^\infty f_0(M)\,dM\right) \quad (14)$$

Note that $V'(\tau) = -\mu v_0e^{-\mu\tau}$. In a previous study, function $V(\tau)$ was approximated with the exponential function Eq. 14 with $\mu \approx 0.506$ [16]. Conversely, the value of μ was determined with Eq. 11 as the least squrare approximation in this study, and $\mu \approx 0.47654$ was obtained. Figure 1 (a) shows the graphs of the consumption rate Eq. 11 and the exponential approximation $V'(\tau) = -\mu v_0e^{-\mu\tau}$.

Figure 1 (b) shows the graphs of residual PEG $V(\tau)$ and the exponential approxmation Eq. 14.



(a)(b)

Figure 1: (a) Curves $(\tau, V'(\tau))$ and $(\tau, -\mu v_0 e^{-\mu\tau})$, (b) Curves $(\tau, 100V(\tau)/V(0))$ and $(\tau, 100e^{-\mu\tau})$, $\mu \approx 0.47654$.

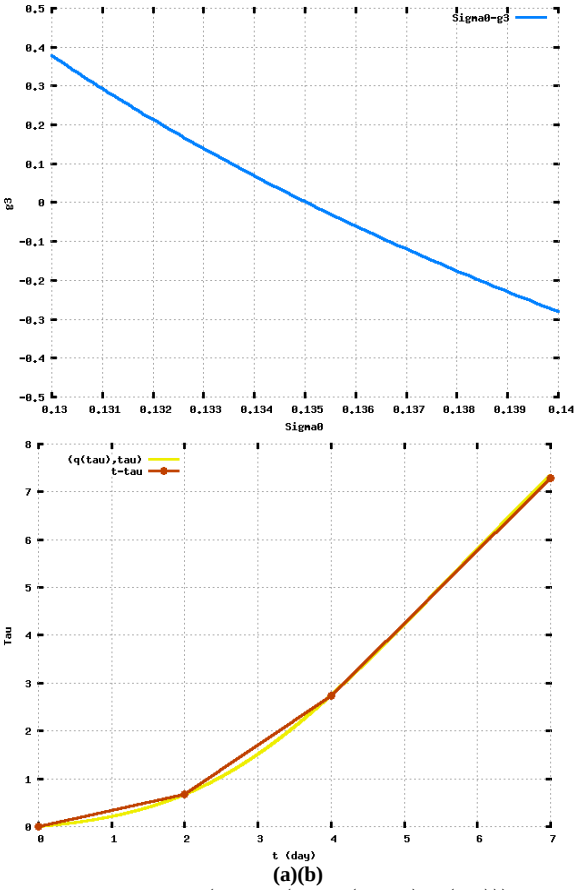
Once $V(\tau)$ was obtained, equations $V(\tau_i) = v(t_i)$ $(i = 1, 2, 3)$ were solved numerically, and the values $\tau_1 \approx 0.67392$, $\tau_2 \approx 2.74105$, and $\tau_3 \approx 7.29052$ were obtained. Suppose that $S(\tau, \sigma_0, k, h)$ is the solution of Eq. 11 with initial value σ_0. According to the change of variables Eq. 8, the expression $t = q(\tau, \sigma_0, k, h)$, where

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

is obtained. Given three pairs of values of t and τ, (t_1, τ_1), (t_2, τ_2), and (t_3, τ_3), equations for σ_0, k, and h,

$$g_1(\sigma_0, k, h) = 0, \quad g_2(\sigma_0, k, h) = 0, \quad g_3(\sigma_0, k, h) = 0, \quad (16)$$

where $g_i(\sigma_0, k, h) = q(\tau_i, \sigma_0, k, h) - t_i$, were set. For fixed values of σ_0 and h, the first equation is solved for $k = k(\sigma_0, h)$ with the Newton's method. The equation $g_2(\sigma_0, k(\sigma_0, h), h) = 0$ was solved for $h = h(\sigma_0)$ again with the Newton's method. Finally the third equation $g_3(\sigma_0, k(\sigma_0, h), h(\sigma_0)) = 0$ was solved with the bisection method. Figure 2, (a) shows the curve $(\sigma_0, g_3(\sigma_0, k(\sigma_0, h), h(\sigma_0)))$. Figure 2, (a) shows that there is a solution of $g_3(\sigma_0, k(\sigma_0, h), h(\sigma_0)) = 0$ in the interval $[0.13, 0.14]$. Applying those numerical method, the values of parameters $\sigma_0 \approx 0.135$, $k \approx 0.00419$, and $h \approx 0.07737$ were obtained. Figure 2, (b) shows the curve $(q(\tau, \sigma_0, k, h), \tau)$, and the points (t_1, τ_1), (t_2, τ_2), and (t_3, τ_3).



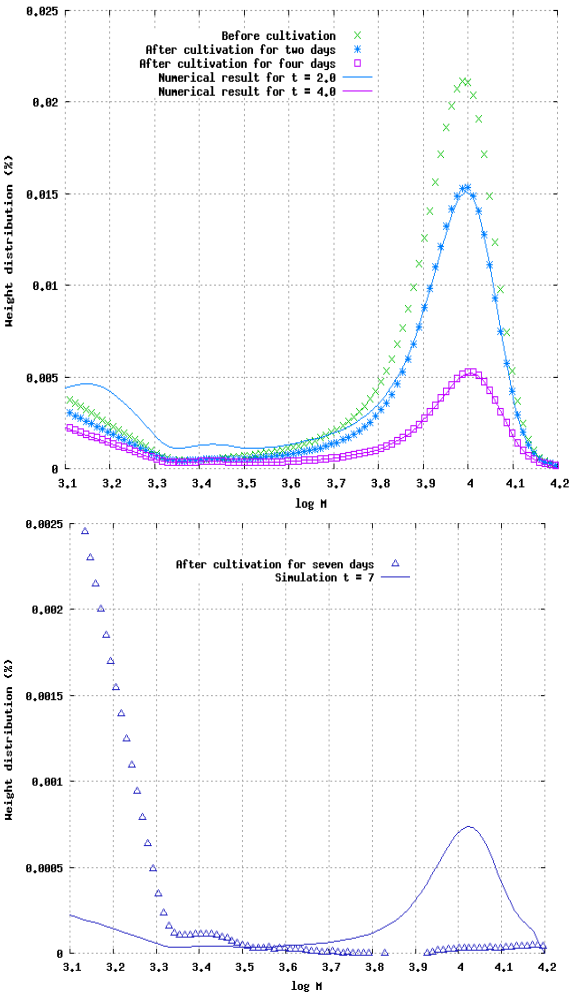
(a)(b)

Figure 2: (a) Curve $(\sigma_0, g_3(\sigma_0, k(\sigma_0, h), h(\sigma_0)))$, (b) Curve $(q(\tau, \sigma_0, k, h), \tau)$, and the points (t_1, τ_1), (t_2, τ_2), and (t_3, τ_3).

Numerical Simulation of PEG microbial depolymerization process

Once the molecular factor of the degradation rate

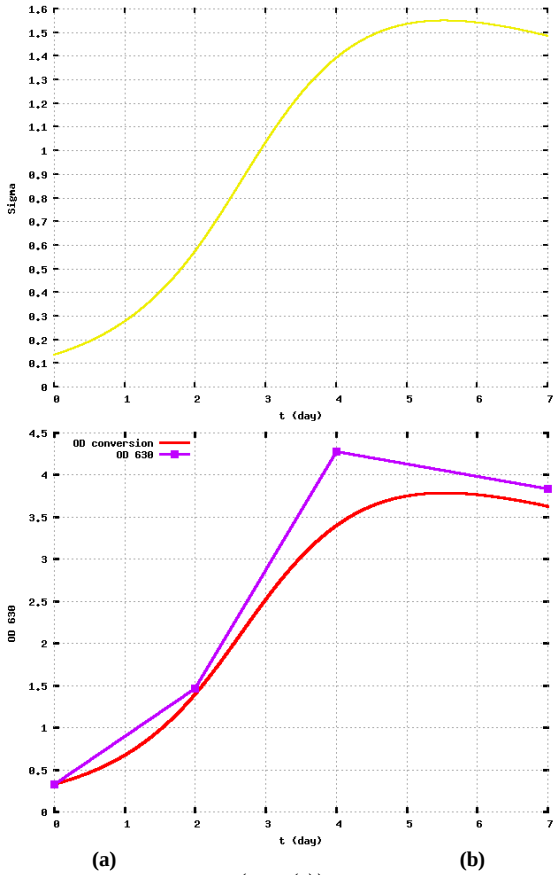
$\lambda(M)$ and the value of the parameters σ_0, k, and h were obtained, the initial value problem (3), (4), (6), (7) became solvable. Figures 3, (a) and 3, (b) show numerical results for weight distributions after cultivation of the microbial consortium E-1 for two days, four days, and seven days. The figures also show the corresponding experimental results Figure 4, (a) shows the numerical result for the microbial population $\sigma(t)$.



(a)(b)

Figure 3: (a) Weight distributions before and after cultivation of microbial consortium E-1 for two days and four days, (b) Weight distribution after cultivation of microbial consortium E-1 for seven days.

The optical density OD 630 was recorded before and after cultivation of the microbial consortium E-1. Figure 4, (b) shows values of optical density OD 630, O_0, O_1, O_2, and O_3 for $t_0 = 0$, $t_1 = 2$, $t_2 = 4$, and $t_3 = 7$, and the OD conversion of the microbial population.



(a) (b)

Figure 4: (a) Curve $(t,\sigma(t))$, (b) OD 630 and OD conversions of microbial population. OD 630, O_0, O_1, O_2, and O_3 for $t_0 = 0$, $t_1 = 2$, $t_2 = 4$, and $t_3 = 7$, and the OD conversion of the microbial population.

DISCUSSION

In this study, a value of μ was determined with Eq. 11 as the least squrare approximation. The initial value problem (3), (4), (6), (7) was solved after the inverse problems for $\lambda(M)$ and parameters σ_0, k, and h were solved. Figure 3, (a) and Figure 3, (b) show numerical results and experimental results for the weight distributions after cultivation of the microbial consortium E-1 for two days, four days, and seven days. Note that the scales of vertical axes are different, and the discrepancy between the experimental result and the numerical result that Figures 3, (b) shows is not so unsatisfactory as it may appear. Reasonable agreements between the numerical results and the experimental results for weight distributions after cultivation show that our techniques are applicable to simulation of exogenous type microbial depolymerization process with a set of experimental results. The optical density reflets inviable cells as well as viable cells, whereas the microbial population $\sigma(t)$ corresponds to viable cells. Figure 4, (b) shows that the OD 630 outcomes after cultivation for four

days and seven days are sum of conversions of microbial population and inviable cells.

ACKNOWLEDGEMENT

The authors thank Ms. Y. Shimizu for her technical support. This work was supported by JSPS KAKENHI Grant Number 16K05276.

REFERENCES

[1] J. R. HAINES AND M. ALEXANDER, Microbial Degradation of Polyethylene Glycol, APPLIED MICROBIOLOGY, May 1975, p. 621-625.

[2] DARYL F. DWYER AND JAMES M. TIEDJE, Degradaton of Ethylene Glycol and Polyethylene Glycols by Methanogenic Consortia, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1983. p. 185-190.

[3] NURIA OBRADORS AND JUAN AGUILAR, Efficient Biodgradation of High-Molecular-Weight Polyethylene Glycols by Pure Cultures of Pseudomonas stutzeri, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1991. p. 2383-2388.

[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 192 (2004) 19-37.

[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., pp C682--C96, September 2010 http://journal.austms.org.au/ojs/index.php/ANZIAMJ/article/view/2433

[6] Masaji Watanabe, Fusako Kawai, Mathematical modelling and computational analysis of enzymatic degradation of xenobiotic polymers, Applied Mathematical Modelling 30 (2006) 1497-1514

[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 (2007) 619-626.

[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, February 2014, 71 – 76. ISBN: 978-94-6252-000-4, ISSN: 1951-6851 http://www.atlantis-press.com/php/pub.php?publication=intel-13

[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 Volume 11, Number 8 (2016) pp 5461-5468. http://www.ripublication.com/ijaer16/ijaerv11n8_18.pdf

[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., 2016, 426-431, ISBN: 978-1-60595-406-6

[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 biodegradation of exogenously depolymerizable polymer, 2017 the 5th International Conference on Mechanical Engineering, Material Science and Civil Engineering, December, 2017, Kuala Lumpur, Malaysia, 012015, IOP Conference Series: Materials Science and Engineering, Published online: 6 April 2018. IOP Publishing http://iopscience.iop.org/issue/1757-899X/324/1

[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.

[14] MASAJI WATANABE, FUSAKO KAWAI, APPLICATION OF THE NEWTON'S METHOD AND BISECTION METHOD TO THREE PARAMETER PROBLEM FOR MICROBIAL DEPOLYMERIZATION PROCESS, Proceedings, ISER INTERNATIONAL CONFERENCE, 1st – 2nd April, 2018| Venue: Kuala Lumpur, Malaysia

[15] M Watanabe and F Kawai, Computational study of bacterial depolymerization process of xenobiotic polymer, Submitted.

[16] Masaji Watanabe and Fusako Kawai, Numerical Study of Exogenous Type Microbial Depolymerization Process, 12th PATTAYA International Conference on Chemical, Agricultural, Biological and Environmental Sciences (PCABES-18), Pattaya (Thailand) May 31-June 2, 2018. ISBN: 978-93-86878-17-5

★★★