Numerical Techniques for Analysis of Microbial Population with Residual Polymer in Microbial Depolymerization Process

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Abstract**:** *This study shows that microbial consumption of carbon sources is a primary factor of microbial growth and decay in exogenous type microbial depolymerization processes. Values of residua polymer before and after cultivation of microorganisms were introduced into analysis for a microbial population. Once the microbial population was obtained, microbial depolymerization process was simulated. Those techniques were illustrated with analysis of an exogenous type depolymerization process of polyethylene glycol.*

Keywords: *biodegradation, xenobiotic polymer, mathematical model, inverse problem, numerical simulation*

1. Introduction

Major industrial production of petroleum based polymers started around the mid twentieth century. Although those xenobiotic macromolecular compounds had been nonexistent until they were invented, some of them can be utilized by microorganisms for carbon sources. Those petroleum based materials have been now accumulated on the surface of the earth to potential sources of carbon dioxide emission, and now mechanism of microbial depolymerization processes must be elucidated.

Microbial depolymerization processes are classified into exogenous type processes and endogenous type processes. The primary factor of an exogenous type depolymerization process is molecular reduction by terminal liberation. Polyethylene (PE) is depolymerizable in exogenous type depolymerization processes. A mathematical model was proposed and numerical techniques were developed in studies of PE biodegradation [1]. Polyethylene glycol (PEG) is also depolymerizable in an exogenous type depolymerization process [2]. Those mathematical techniques developed for PE biodegradation were applied to a microbial depolymerization process of PEG [3]. Time dependence of degradation rate was incorporated into formulation of a PEG depolymerization process [4].

Unlike exogenous type depolymerization processes, molecules are broken down arbitrarily in endogenous type depolymerization processes. A mathematical model was proposed for simulation of an enzymatic degradation process [5]. Mathematical techniques developed for the enzymatic degradation of PVA were applied to an enzymatic hydrolysis of PLA, and degradabilities of PVA and PLA were compared [6]. Time dependence of an degradation rate was incorporated into modeling of an enzymatic hydrolysis of PLA [7]. A mathematical model proposed for endogenous type depolymerization processes was applied to exogenous type depolymerization processes of PEG [8] and PE [9]. Mathematical techniques developed for the PE biodegradation were applied to a depolymerization process of PEG [10]. Time dependence of degradability was incorporated into formulation of a PEG depolymerization process [11].

This study revisited an exogenous type microbial depolymerization process of PEG. In previous studies, weight distributions before and after cultivation of microbial consortium E-1 were introduced into inverse analyses for a molecular factor and a time factor of a degradation rate. Inverse problemes for a molecular factor and a time factor of a deradation rate were solved numerically. An initial value problem was solved numerically, and a microbial depolymerization process was simulated. In this study, a set of residual PEG before and after cultivation was introduced into an inverse analysis for the microbial population. In the following sections, mathematical model is described, techniques to solve inverse problems for the micriobial population are

illustrated, and numerical results are presented. A numerical result for the microbial population and experimental outcomes for an optical density were compared.

2. Exogenous Type Microbial Depolymerization Model Propose in a Previous Study

Suppose that $w(t,M)$ [mg] is the weight distribution with respect to the molecular weight M at time t, and that $v(t)$ [mg] is the total weight of polymer molecules at time t. The total weight $v(t)$ of the entire residual polymer at time t is

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

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

$$\frac{\partial w}{\partial t}=\sigma(t)\left[-\lambda(M)w+c(M)\int_M^\infty \lambda(K)d(K)w(t,K)\,dK\right], \tag{1}$$

$$\frac{d\sigma}{dt}=k\left[1-h\frac{\sigma}{v(t)}\right]\sigma\,, \tag{2}$$

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

Parameter L is the molecular weight of a monomer unit, *e.g.* PE: $L=28$ $(\mathrm{CH_2CH_2})$, PEG: $L=44$ $(\mathrm{CH_2CH_2O})$. Function $\lambda(M)$ is the molecular factor of degradation rate, whereas the microbial population $\sigma(t)$ is the time factor of degradation rate.

System of equations (3), (4) is associated with initial conditions

$$w(0,M)=f_0(M), \tag{3}$$

$$\sigma(0)=\sigma_0\,, \tag{4}$$

where $f_0(M)$ and σ_0 are an initial weight distribution and an initial microbial population, respectively. The initial value problem (1), (2), (3), (4) is solvable for $w(t,M)$ and $\sigma(t)$ provided the function $\lambda(M)$ and values of parameters σ_0, k, and h are prescribed. In order to specify the function $\lambda(M)$ and values of parameters σ_0, k, and h, consider change of variables from t to τ defined by

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

Denote functions $w(t,M)$, $\sigma(t)$, and $v(t)$ by $W(\tau,M)$, $S(\tau)$, and $V(\tau)$, respectively, for which the relation (5) between t and τ holds. Equations

$$\frac{\partial W}{\partial \tau}=-\lambda(M)W+c(M)\int_M^\infty \lambda(K)d(K)W(\tau,K)\,dK\,, \tag{6}$$

$$\frac{dS}{d\tau}=k\left[1-h\frac{S}{V(\tau)}\right], \tag{7}$$

are derived from the equations (3) and (4), respectively. Note that $W(\tau,M)$ is the sole unknown variable of equation (9), whereas equation (4) involves two unknowns variables $w(t,M)$ and $\sigma(t)$. Note also that

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

holds. Suppose that $F_1(M)$ is the weight distribution $W(\tau,M)$ for $\tau=T_1$, that is, $F_1(M)=W(T_1,M)$, and that $F_2(M)$ is the weight distribution $W(\tau,M)$ for $\tau=T_2\ (0\le T_1<T_2)$, that is, $F_2(M)=W(T_2,M)$. Given $F_1(M)$ and $F_2(M)$, equation (9), the initial condition

$$W(T_1,M)=F_1(M), \tag{8}$$

and the final condition

$$W(T_2,M)=F_2(M) \tag{9}$$

form an inverse problem for $\lambda(M)$, for which the solution of the initial value problem (6), (8) also satisfies the final condition (9). Numerical techniques for the inverse problem 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, and values of T_1 and T_2 were set, and the inverse problem (6), (8), (9) was solved numerically. Once the function $\lambda(M)$ was specified, equation (9) was solved for $W(\tau,M)$ with the initial condition

$$W(0,M)=f_0(M). \tag{10}$$

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 $V(\tau_i)=v(t_i)\quad(i=1,2,\ldots,m)$.

A solution of the equation is not only a function of τ, but also a function of parameters σ_0, k, and h. Suppose $S(\tau,\sigma_0,k,h)$ is the solution of the equation (10) with initial value σ_0. The change of variables (8) leads to $t=q(\tau,\sigma_0,k,h)$, where

$$q(\tau,\sigma_0,k,h)=\int_0^{\tau}\frac{dr}{S(r,\sigma_0,k,h)} \tag{11}$$

Given m pairs of values of t and τ, $(t_i,\tau_i)\quad(i=1,2,\ldots,m)$, define functions $g_i(\sigma_0,k,h)\quad(i=1,2,\ldots,m)$ 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,

$$g_i(\sigma_0,k,h)=0\quad(i=1,2,\ldots,m). \tag{12}$$

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].

3. Microbial Population in Exogenous Type Microbial Depolymerization Process of PEG

Previous study shows that the function $V(\tau)$ is well approximated with an exponential function $V(\tau)=v(0)e^{-\mu\tau}$. In this study, system of equations (12) was analyzed. The results from cultivation of the symbiotic mixed culture E-1 on PEG 6000 was reported [16]. Let $t_0=0$, $t_1=1$, $t_2=3$, $t_3=4$, $t_4=5$, $t_5=7$, and $t_6=11$. The residual PEG $v_0,v_1,\ldots,v_6$ at time $t_0,t_1,\ldots,t_6$, 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 value of the parameter $\mu=2$ was set, and the Gauss-Newton method [19] was applied to the nonlinear least square problem for a minimizer of the expression. Table 1 sows the convergence process of a sequence of parameter values generated by the Gauss-Newton method. For those values of the parameters in the last row of the table 1, $t=q(\tau,\sigma_0,k,h)$ was generated numerically.

Figure 1 shows the residual PEG.

TABLE I: Convergence process of a sequence generated by the Gauss Newton method. The initial values of σ_0, k, and h were 0.1, 0.2, and 400.0, respectively, and the residual at the n^{th} step $\sqrt{\left(\sigma_0^{(n)}-\sigma_0^{(n-1)}\right)^2+\left(k^{(n)}-k^{(n-1)}\right)^2+\left(h^{(n)}-h^{(n-1)}\right)^2}$ reduced to a value less than 10^{-12} after twenty three steps. Here $\sigma_0^{(n)}$, $k^{(n)}$, and $h^{(n)}$ are approximate values of σ_0, k, and h at the n^{th} step, respectively.

n	$\sigma_0^{(n)}$	$k^{(n)}$	$h^{(n)}$	Residual
0	0.1000000000000000	0.2000000000000000	400.0000000000000000	
1	0.0994712200790175	0.2647019893267910	453.5594521318570000	53.5594912157667000
2	0.0976723354256255	0.2698380666711560	431.3419505965090000	22.2175022018332000
3	0.0990035526379217	0.2668439034076890	429.8402255085150000	1.5017286629299200
4	0.0987054665615930	0.2683211108119130	430.4765200611240000	0.6362963371556590
5	0.0987741465360885	0.2679302821343420	430.2985094747810000	0.1780110286311540
6	0.0987589117356565	0.2680228992252650	430.3396351261650000	0.0411257584946348
7	0.0987623045164953	0.2680019590888500	430.3301874992620000	0.0094476507183294
8	0.0987615556325857	0.2680066364757630	430.3322933644540000	0.0021058705197855
9	0.0987617208562342	0.2680056005562030	430.3318262622940000	0.0004671033380734
10	0.0987616844459683	0.2680058293231320	430.3319294229440000	0.0001031609106899
11	0.0987616924673173	0.2680057788810690	430.3319066724800000	0.0000227505216587
12	0.0987616907005094	0.2680057899961860	430.3319116858780000	0.0000050134110688
13	0.0987616910896440	0.2680057875476640	430.3319105814590000	0.0000011044226628
14	0.0987616910039412	0.2680057880869710	430.3319108247190000	0.0000002432613418
15	0.0987616910228162	0.2680057879681910	430.3319107711420000	0.0000000535778426
16	0.0987616910186591	0.2680057879943510	430.3319107829410000	0.0000000117997003
17	0.0987616910195750	0.2680057879885870	430.3319107803410000	0.0000000025999677
18	0.0987616910193727	0.2680057879898600	430.3319107809150000	0.0000000005732105
19	0.0987616910194175	0.2680057879895810	430.3319107807910000	0.0000000001233505
20	0.0987616910194076	0.2680057879896410	430.3319107808160000	0.0000000000248975
21	0.0987616910194099	0.2680057879896270	430.3319107808090000	0.0000000000073328
22	0.0987616910194097	0.2680057879896290	430.3319107808110000	0.0000000000026148
23	0.0987616910194097	0.2680057879896280	430.3319107808110000	0.0000000000005116

Values of optical density $OD_{610,}$ O_0, O_1, O_2, O_3, O_4, O_5, and O_6 were recorded at $t_0=0$, $t_1=1$, $t_2=3$, $t_3=4$, $t_4=5$, $t_5=7$, and $t_6=11$ [16]. Figure 3 shows points $(O_0,\sigma(t_0))$, $(O_1,\sigma(t_1))$, $(O_2,\sigma(t_2))$, $(O_3,\sigma(t_3))$, $(O_4,\sigma(t_4))$, $(O_5,\sigma(t_5))$, and $(O_6,\sigma(t_6))$. A linear relation $\sigma=aO+b$ between the microbial population σ and the optical density O was assumed. Figure 2 also shows a result of the least square approximation for a and b ; $a\approx 0.023357$, $b\approx 0.117776$ based on $(O_0,\sigma(t_0))$, $(O_1,\sigma(t_1))$, $(O_2,\sigma(t_2))$, $(O_3,\sigma(t_3))$, $(O_4,\sigma(t_4))$, and $(O_5,\sigma(t_5))$. Figure 4 shows the curve $(t,\sigma(t))$. It also shows conversions of O_0, O_1, O_2, O_3, O_4, O_5, and O_6 according to the least square approximation.

4. Conclusion

In previous studies [10 - 15, 17, 18], weight distributions before and after cultivation of microorganisms in culture media were introduced into inverse analysis of the molecular factor and the time factor of a degradation rate. Those include the inverse problems for the molecular factor (6), (8), (9) and the time factor (12). This study demonstrates that an exogenous type microbial depolymerization process is simulated with a set of residual polymer before after cultivation of microorganisms.

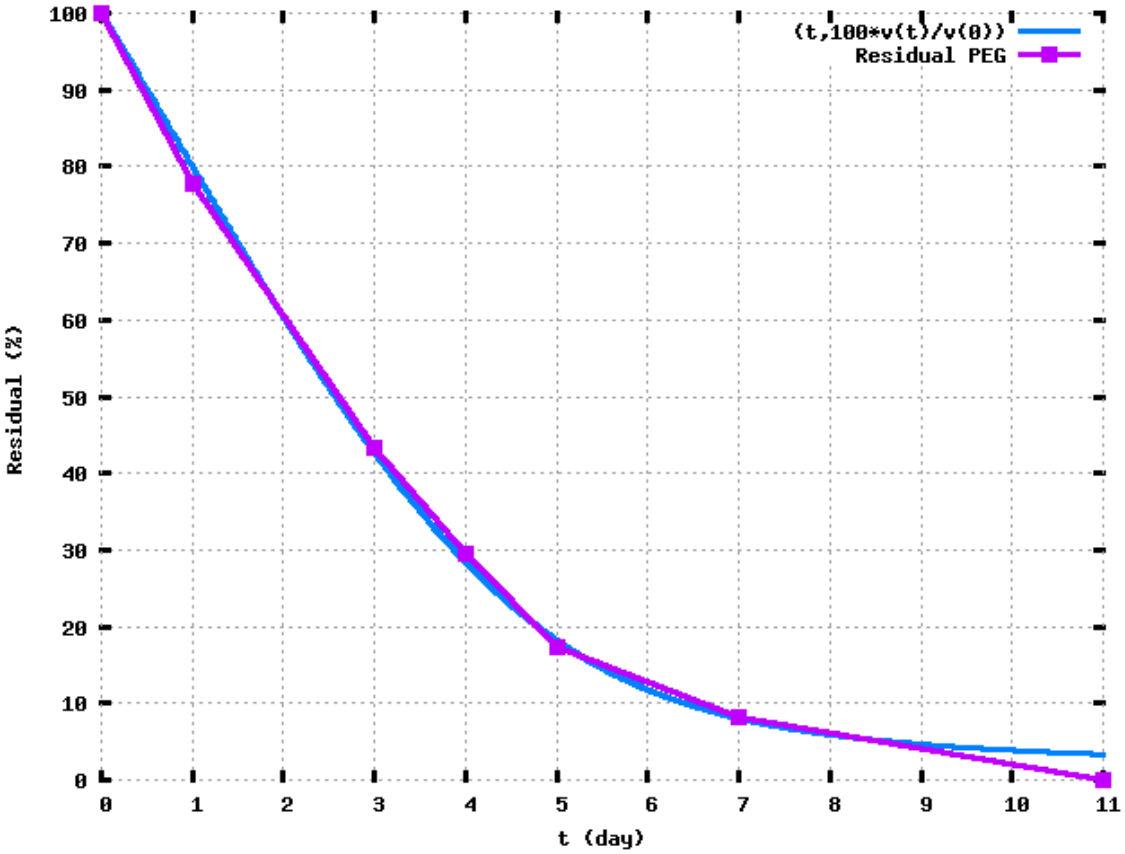

Fig. 1: Residual PEG. Curve $(t, y) = (q(\tau, \sigma_0, k, h), 100.0 \times V(\tau)/v(0))$. The figure also shows Residual PEG at $t_0 = 0$, $t_1 = 1$, $t_2 = 3$, $t_3 = 4$, $t_4 = 5$, $t_5 = 7$, and $t_6 = 11$.

Figure 3 shows the transition of viable cell density whereas the optical density of culture media was an outcome from a mixture of viable cells and inviable cells. The symbiotic mixed culture E-1 consists of *S. terrae* and *Rhizobium* sp. The results concerning the residual PEG are outcomes from a microbial depolymerization process where *S. terrae* was a primary PEG utilizing bacteria. Figure 4 shows the transition of viable *S. terrae* whereas *Rhizobium* sp. as well as inviable cells were incorporated in the outcomes of the OD_{610}. Viable cell density of *S. terrae* and *Rhizobium* sp. were measured by colony counting on nutrient agar plates in another experiment [16]. A further study is required to validate the numerical results by comparison with the experimental results.

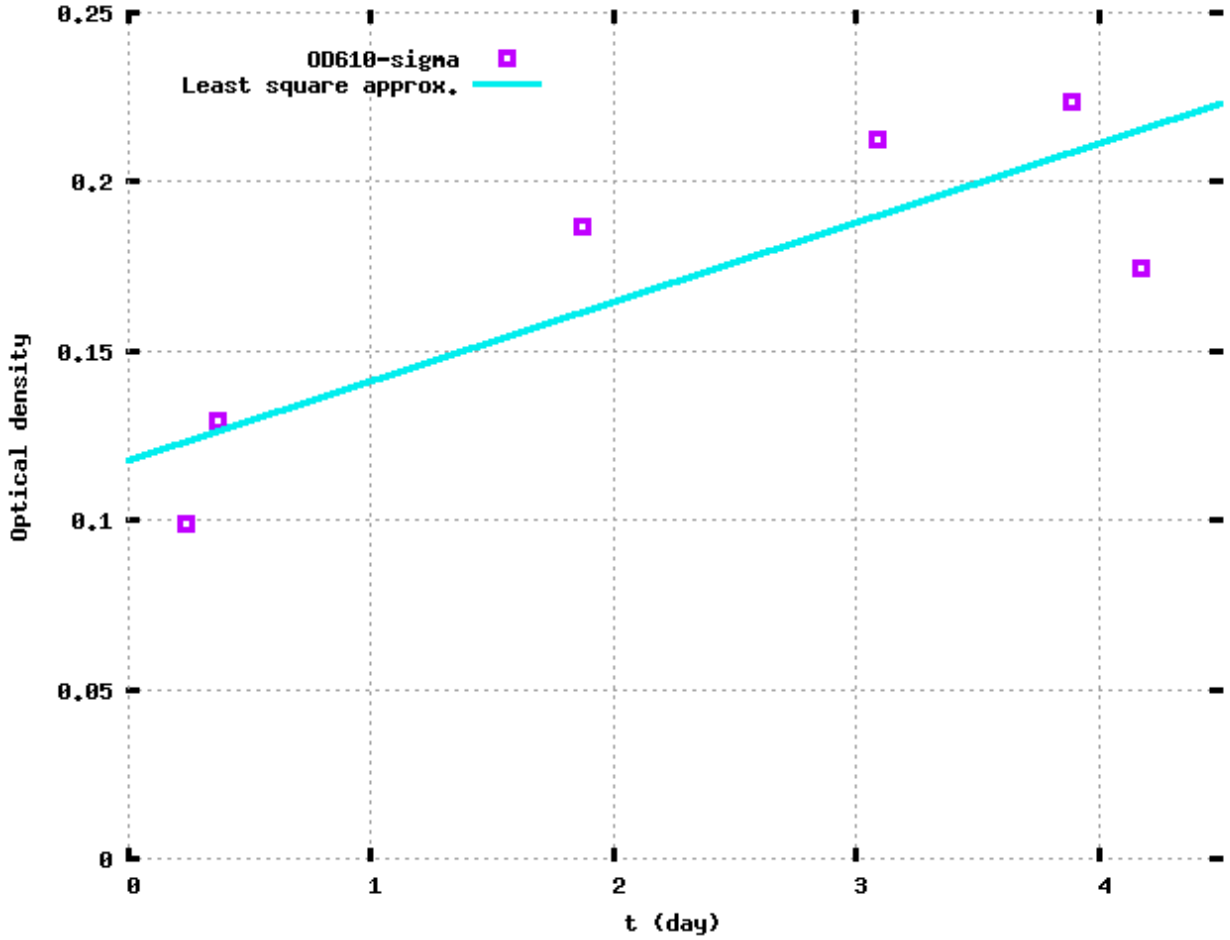

Fig. 2: $(O_0, \sigma(t_0))$, $(O_1, \sigma(t_1))$, $(O_2, \sigma(t_2))$, $(O_3, \sigma(t_3))$, $(O_4, \sigma(t_4))$, and $(O_5, \sigma(t_5))$. A least square approximation $\sigma = aO + b$ is also shown ($a \approx 0.023357$, $b \approx 0.117776$).

5. Acknowledgements

The authors thank K. Yamamoto for his technical support. This work was supported by JSPS KAKENHI Grant Number 16K05276.

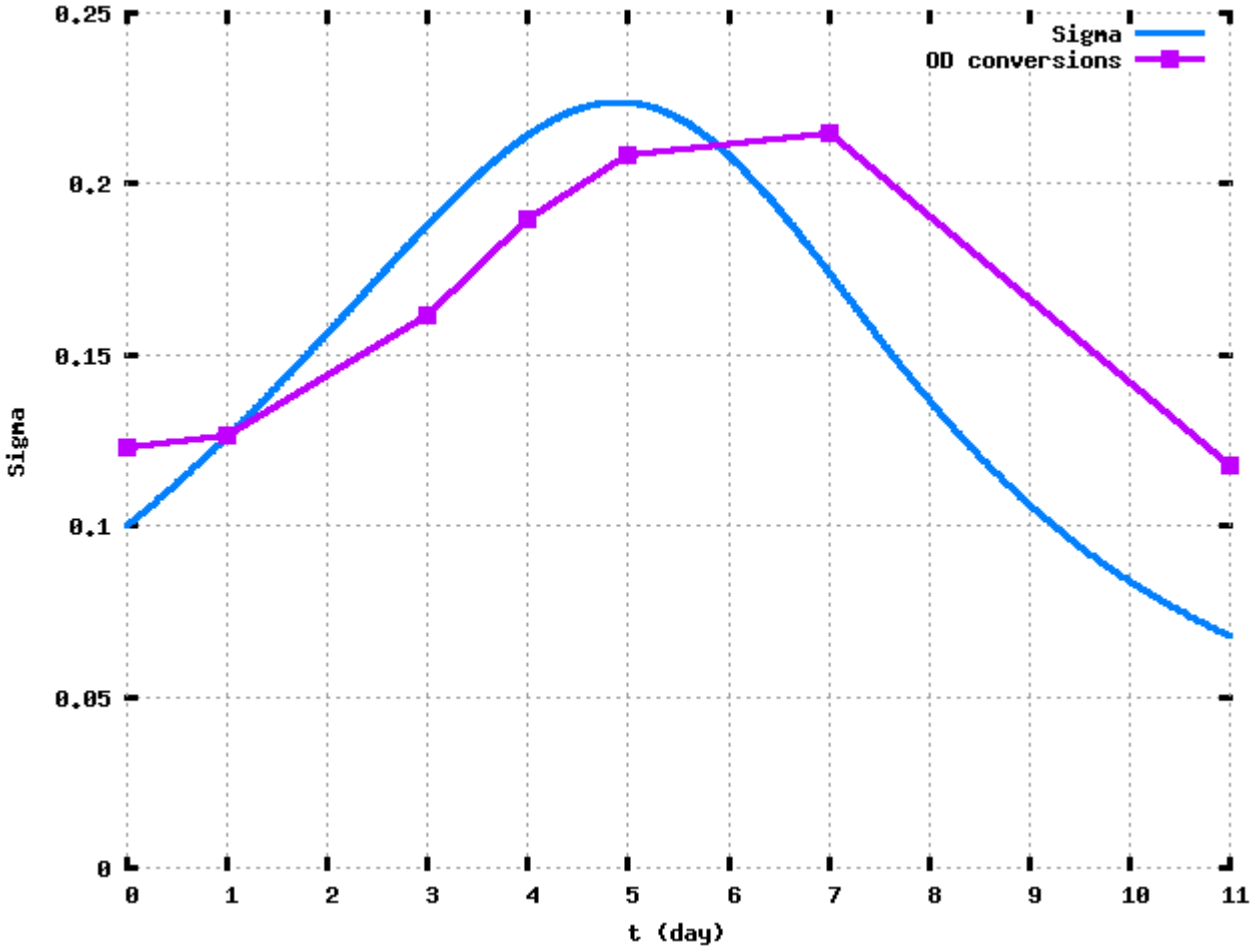

Fig. 3: Curve $(t,\sigma(t))=(q(\tau,\sigma_0,k,h),S(\tau,\sigma_0,k,h))$ and conversions of OD_{610}. Values of OD_{610} O_i $(i=0,1,\ldots,6)$ were converted according to the least square approximation (Fig. 3).

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