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Bioprocess Modeling and Simulation

Aman KumarBT10B003

Kinetic model for polyhydroxybutyrate (PHB) production by *Hydrogenophaga pseudoflava* and verification of growth conditions

Mahmoudi *et al.*

Introduction

Biodegradable plastics are the necessity of today's world. Polyhydroxybutyrate (PHB) is a biopolymer belonging to the class of esters that can be used as a biodegradable thermoplastic material and can find several applications for waste management strategies and biocompatibility in medical devices.

The growth kinetics were first fitted with Monod's equation and thereafter a logistic model was also proposed to provide a correct description for PHA synthesis using experimental data.

The biokinetic coefficients were calculated from Lineweaver-Burk plot.

Data Obtained:

The following data were obtained using the software called **PlotDizitizer.**

Glucose Data					
Time	X (g/l)	S (g/l)	P(g/l)	X/X0	ln(X/X0)
0	0.459813	40	0	1	0
12.2873	0.8926	37.9962	0.0172	1.941224	0.663319
24.268	2.705	37.9389	0.2122	5.882826	1.772037
36.213	9.3904	31.9847	1.91	20.42222	3.016623
48.1645	15.1871	22.9962	2.604	33.02886	3.497382
60.1672	14.0261	20.0191	2.3059	30.50392	3.417855
72.025	12.16	17.9583	2.20841	26.44553	3.275087
84.0228	11.6119	18.0153	1.1529	25.25353	3.228966
96.0213	11.0026	14.9809	1.00956	23.92842	3.175067

Table 1

Fructose Data					
Time	X(g/l)	S(g/l)	P(g/l)	X/X0	ln(X/X0)
0	0.328	40	0	1	0
12.2873	0.4106	37.902	0	1.251829	0.224606
24.268	0.821355	37.963	0	2.504131	0.917942
36.213	2.505	36.0494	0.507	7.637195	2.03303
48.1645	5.094	31.0494	0.8037	15.53049	2.742805
60.1672	10.9651	28.9505	1.1037	33.43018	3.509459
72.025	15.1591	19.8765	1.25556	46.21677	3.833343
84.0228	18.152	17.963	1.6	55.34146	4.013522
96.0213	13.0815	15	1.4037	39.88262	3.685941

Table 2

Glucose Data	
1/S (l/g)	1/ μ (h)
0.0215	12.06
0.0286	16.27
0.033	18.47
0.0335	18.93
0.034	19.27
0.036	19.93
0.0378	20.4

Table 3

Fructose Data	
1/S (l/g)	1/ μ (h)
0.0238	11.33
0.0233	12.267
0.025	12.73
0.0328	14.73
0.0436	17.4
0.0478	21
0.0514	24.667

Table 4

Results (Proposed model, Assumptions and Simulation):

Monod's Kinetics:

The monod kinetic model was used to describe the production of PHB as follows:

$$\mu = \frac{\mu m\ S}{Ks+S}$$

where, μ is the specific growth rate, S is the substrate concentration, Ks and μm are defined as monod's constants.

Taking the inverse of the above equation, we get

$$\frac{1}{\mu} = \frac{Ks}{\mu m\ S} + \frac{1}{\mu m}$$

Plotting a graph between 1/μ and 1/S gives the value of Ks and μ_m. Matlab R2012b was used to plot the graphs and the graph is plotted using data from table 3 & 4 as below:

The graph below is Lineweaver plot for glucose consumption. From this graph, we find that the value of slope is 459.48 units and the value of intercept is 3.3401 units with a R^2 value of 0.9738.

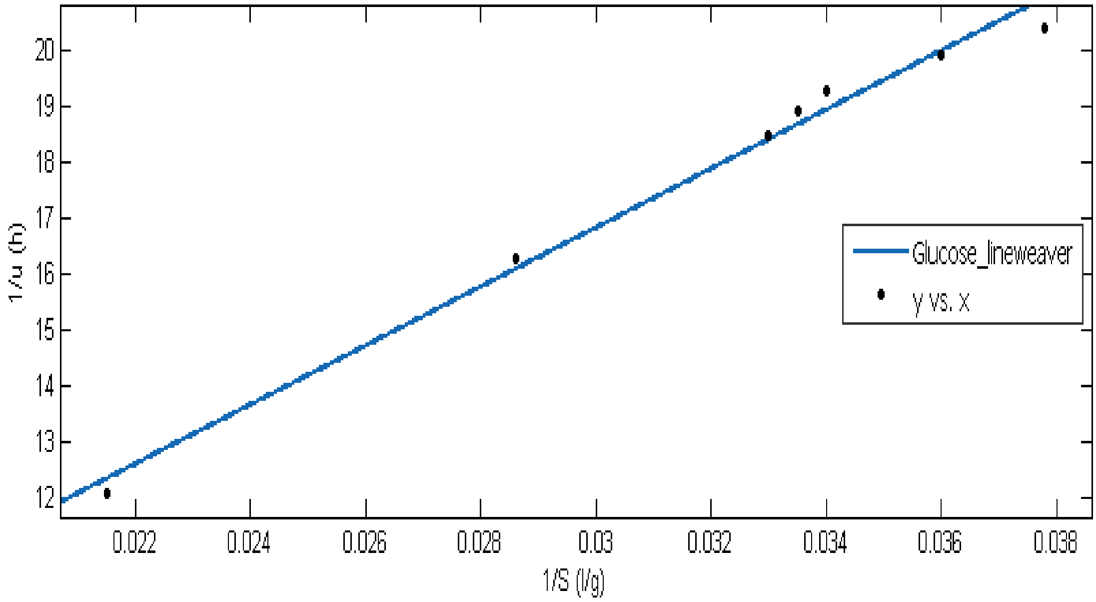

Figure 1

We find similarity in the readings with the paper taken, and we find that the value of Ks as 137.56 g/l while that of μ_m as 0.299 h^{-1} as compared to the value of Ks as 106 g/l and μ_m as 0.36 h^{-1} in the research paper.

When the organism uses fructose as the carbon source, we find that the value of slope as 335.68 units while that of intercept as 3.9273 units with a R^2 value of 0.9524.

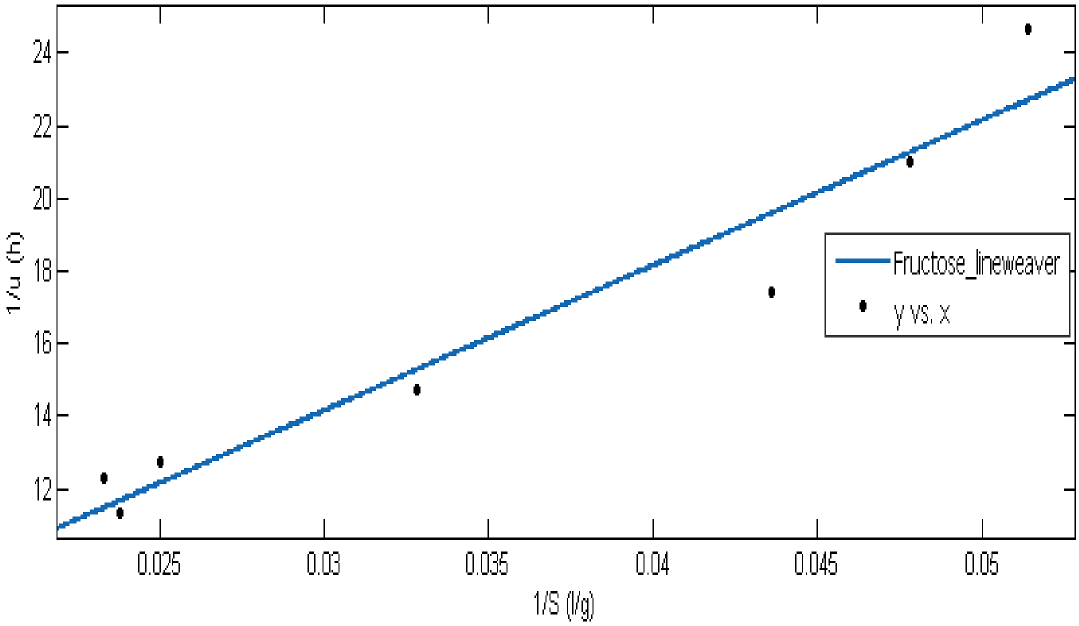

Figure 2

We find that the value of Ks as 85.47 g/l while that of μ_m as 0.2546 h^{-1} as compared to the value of Ks as 80 g/l and μ_m as 0.24 h^{-1} in the research paper.

Malthus model:

The Malthus model is given as:

$$\mu = \frac{1dX}{Xdt}$$

Where, X is cell mass concentration and t is time.

Solving the above equation, we get in form of logarithm as foolows:

$$\ln\frac{X}{X0} = \mu t$$

Plotting $\ln(X/X_0)$ vs t gives the value of μ for the organism growing on different substrate viz. glucose and fructose.

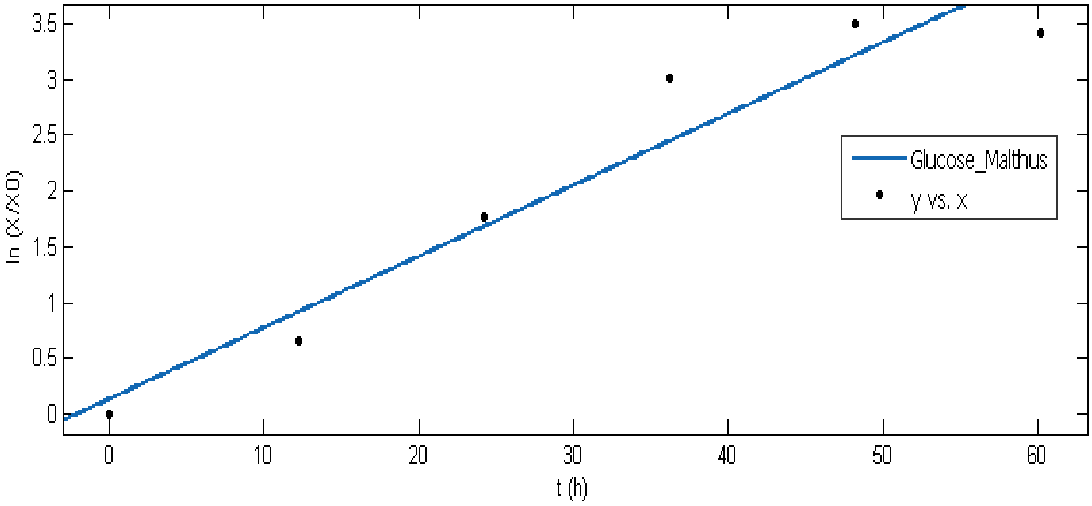

Figure 3

The value of μ was obtained as 0.0752 h^{-1} with a R^2 value of 0.9806 on glucose as a substrate.

The same experiment was repeated for fructose as a substrate and the plot was obtained as;

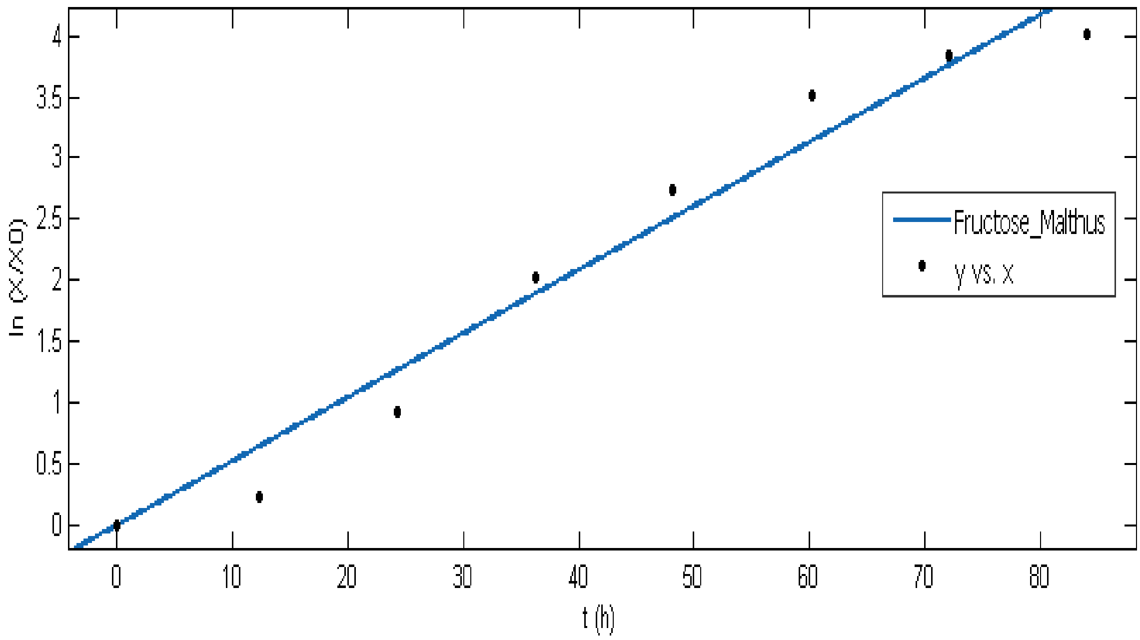

Figure 4

The value of μ was obtained as 0.0557 h^{-1} with a R^2 value of 0.9664 on fructose as a substrate.

Logistic Model:

The logistic model is given as follows:

$$X = \frac{X0\ \exp(\mu m\ t)}{1-\left(\frac{X0}{Xm}\right)(1-\exp(\mu m\ t)}$$

This is a suitable kinetic model for prediction of growth rate.

The plot is obtained as follows with glucose as the substrate:

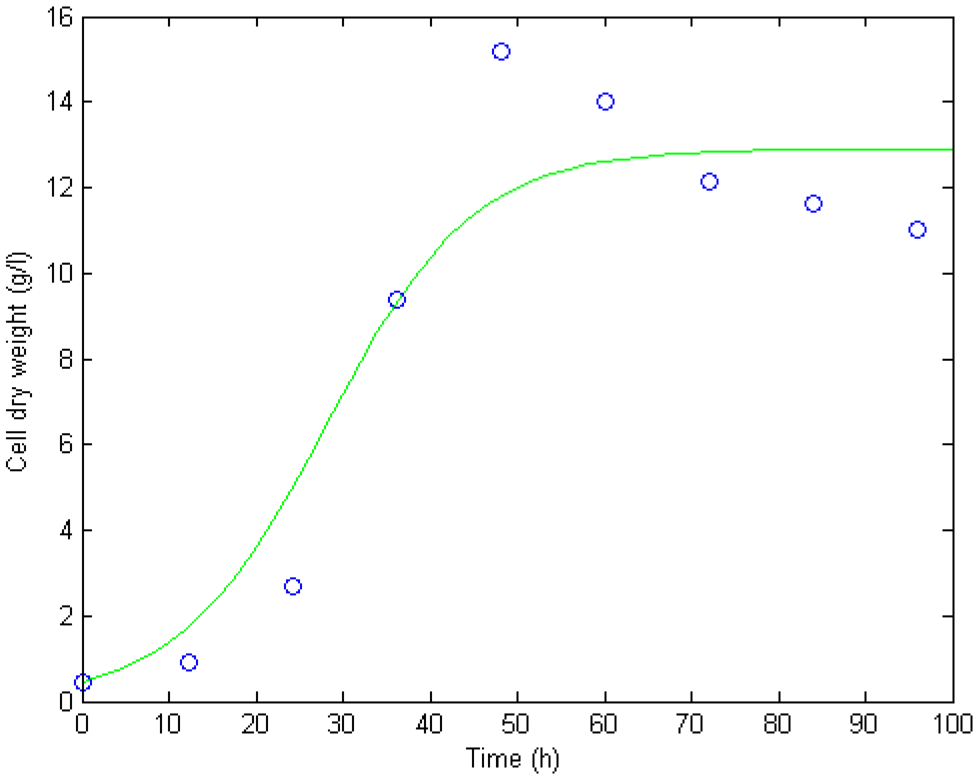

Figure 5

The value of parameters X_m was obtained to be 12.9105g/l and μ_m was obtained as $0.1175h^{-1}$ with R^2 value of 0.909. The value that was obtained in original paper was $\mu_m = 0.18h^{-1}$. The values are almost same while discrepancy may arise because the researchers have not used all the data points rather they have chosen points which gives best R^2 values.

The plot below is obtained with fructose as the substrate as:

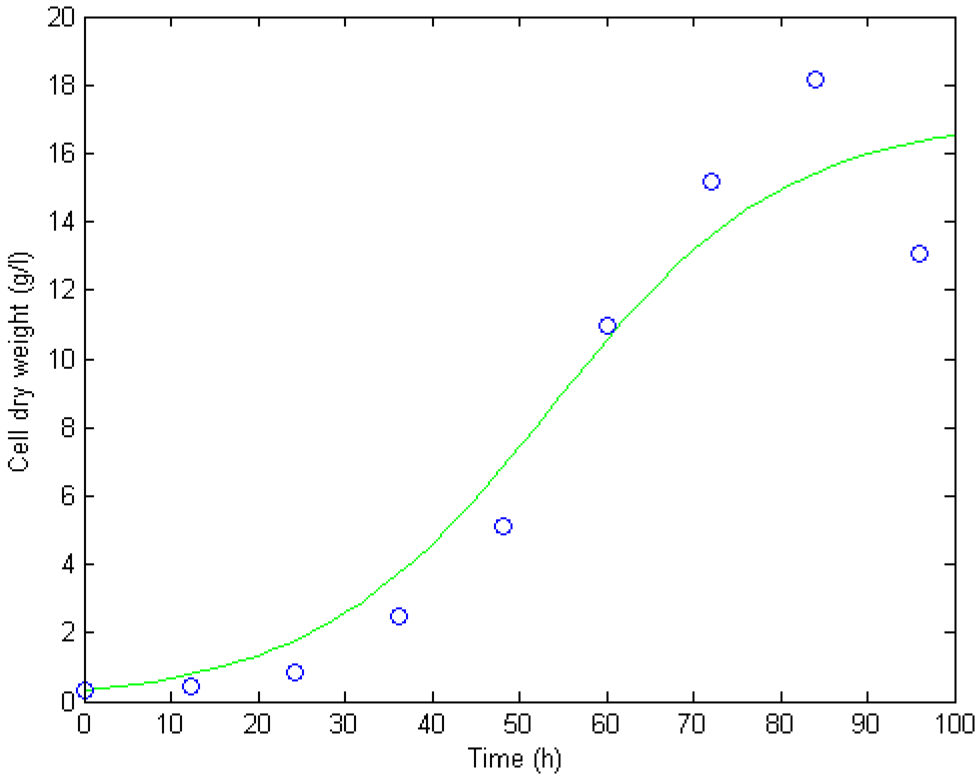

Figure 6

The value of parameters X_m was obtained to be 17.0276g/l and μ_m was obtained as $0.0735h^{-1}$ with R^2 value of 0.9374. The value that was obtained in original paper was $\mu_m = 0.14h^{-1}$.

Improvisation for the model:

In the last section, we calculated X_m and μ_m using the logistic model. There is another model which has given better results for various cases. The idea here is to incorporate that model and check for any possible positive outcomes.

Gompertz model: The equation for the model is given as given below

$$X = X_{max}.\exp\{-\exp[\frac{Rmax\ .e}{Xmax}(\lambda\text{-}t) + 1\ ]\}$$

Where X is the biomass formed per litre of reactor volume at fermentation time t

X_{max} is the potential maximum biomass formed per litre of reactor volume. It is like the carrying capacity X_m in the logistic model.

R_{max} is the maximum rate of product formed. It is like the apparent specific growth rate μ_m in the logistic model

λ_i is the lag time to exponential biomass formed.

Simulation was done for this model using glucose as a substrate (Please refer supplementary information for Matlab code).

The values of X_{max} was found to be 15.3506 g/l, R_{max} was obtained as 0.2052 and λ as 10.4055h.

Similarly, for fructose the values using the model were obtained as:

$X_{max} = 56.05$ g/l, $R_{max} = 0.3495$, and $\lambda = 45.6769$

The error obtained for the two cases are 0.8368units and 0.9379units.

These results have much lower error values than the logistic model. But, the general trend on reduction in specific growth rate was not observed with respect to substrate. Thus, no conclusion can be made from the results obtained from the model.

Discussions:

The model was simulated and the results almost complied with the results obtained by the authors. There were some discrepancy observed in the values which might be because of the listed reasons:

-While obtaining data with the help of PlotDizitizer, it is not possible to get the exact data which was obtained experimentally by the authors.

-The authors have removed the outliers on the basis of R^2 values to get the best fit model. Thus, incorporating those data points gives little different values of parameters. This analysis has been done with almost all the data points.

Furthermore, the model was improvised by introducing another model called Gompertz model. The model was simulated and the values of the unknown parameters were calculated.

Conclusions:

The paper was successfully repeated with some discrepancies. Some improvisation was also tried by introducing new model.

Acknowledgements:

I would like to thank you my classmates Miss Manali Naik, Mr. Akhil Sai Valluri and Mr. Eddy Hudson for helping me with my doubts in the project wherever required.

Supplementary Information:

Matlab codes:

Code 1: Plotting the best curve fit of figure 1-4

Example code is given below: For getting best fit, best fir tool was used from Matlab whose code is given below:

```
function [fitresult, gof] = createFit(x, y)
%CREATEFIT(X,Y)
%  Create a fit.
%
%  Data for 'untitled fit 1' fit:
%      X Input : x
%      Y Output: y
%  Output:
%      fitresult : a fit object representing the fit.
%      gof : structure with goodness-of fit info.

%% Fit: 'untitled fit 1'.
[xData, yData] = prepareCurveData( x, y );

% Set up fittype and options.
ft = fittype( 'poly1' );
opts = fitoptions( ft );
opts.Lower = [-Inf -Inf];
opts.Upper = [Inf Inf];

% Fit model to data.
[fitresult, gof] = fit( xData, yData, ft, opts );

% Plot fit with data.
figure( 'Name', 'untitled fit 1' );
h = plot( fitresult, xData, yData );
legend( h, 'y vs. x', 'untitled fit 1', 'Location', 'NorthEast' );
% Label axes
xlabel( 'x' );
ylabel( 'y' );
grid on
```

Code 2: This was used to generate plot 5[1] and plot 6.

This code is for Figure 6.

```
%Trying to fit the data with all the points
global xCurveData;
t = [0, 12.2873 ,24.268 ,36.213 ,48.165 ,60.1872 ,72.025 ,84.0228
,96.0213]';
x = [0.328, 0.4106, 0.821355, 2.505, 5.094, 10.9651, 15.1591, 18.152,
13.0815]';
xCurveData = horzcat(x, t);
```

---

[1] Figure 5 was plotted using the same code just replacing the data points wherever required.

```
figure(1);

fprintf('Fitting the data to the curve...\n\n');
[params,error] = fminsearch(@calcErrorForOptFunc,[0,18])

x0 = 0.328;
xAxis = t;
x1CurveData = [];
for i = 1:length(xAxis)
    x1CurveData(i) = x0*exp(params(1)*xAxis(i)) / (1-(x0/params(2))*(1-
exp(params(1)*xAxis(i))));
end

[r,p] = corrcoef(x1CurveData,x)
rSq = r(1,2)^2

%Function to calculate error
function error = calcError(params,data)

k = params(1);
m = params(2);

error = 0;
x0 = 0.328;
indXFromEq = [];
endLimit = size(data);
endLimit = endLimit(1);
for i = 1:endLimit
    indXFromEq = x0*exp(k*data(i,2)) / (1-(x0/m)*(1-exp(k*data(i,2))));
    xExperimental = data(i,1);
    error = error + (xExperimental - indXFromEq)*(xExperimental -
indXFromEq);
end

clf
xAxis = 0:2:100;
for i = 1:length(xAxis)
    indXFromEq(i) = x0*exp(k*xAxis(i)) / (1-(x0/params(2))*(1-
exp(params(1)*xAxis(i))));
end
plot(xAxis,indXFromEq,'g','LineWidth',1.2)
hold on;
plot(data(:,2),data(:,1),'bo','LineWidth',1.2)
pause(0.01);

end

%function to calculate error for optimum function
function error = calcErrorForOptFunc(inputParams)
    global xCurveData;
    error = calcError(inputParams,xCurveData);
end
```

Code3 : This code is for simulating the Gompertz model. This is an example code to simulate using glucose as a substrate. Similar code can be written for fructose by just changing the data points.

```
t1 = [0
12.2873
24.268
36.213
48.1645
60.1672
72.025
84.0228
96.0213
];
>> x1 = [0.459813
0.8926
2.705
9.3904
15.1871
14.0261
12.16
11.6119
11.0026
];
>>  p_ini=[15 0.01 1];
>> [p_gompertz_growth gompertz_error]=fminsearch(@(p)
error_gompertz_growth(p,x1,t1),p_ini)

%function for GOmpertz error
function [ e3 ] = error_gompertz_growth( p,product,t2 )
e3=0;
for i=1:9
product_calc(i)=p(1)*2.718281828459045235336^(-
2.71828182845904523536^((p(2)*2.718281828459045235336/p(1))*(p(3)-
t2(i))+1));
end
for i=1:9
e3=e3+(((product_calc(i)-product(i))^2)/product(i)^2);
end
end
```