1.Summary

Lac repressor is a quaternary protein with four identical subunits each has an inducer bonding site. When galactose is absent, lac repressor will bind with the operator gene O to repress the lac operator and block the combination of RNA polymerase with the P gene sequence in order to repress the initiation of transcription. However, when inducer combine with the the repressor, the structure of the repressor will be altered, and it will dissociate from the operator gene O. Therefore, RNA polymerase is no longer blocked, and transcription begins. In fact, galactose itself is not the inducer in our operation system. As soon as lactose enter the cell, it will undergo a catalytic reaction by β-galactosidase to form galactose--the psychological inducer. While in our experiment, we use isopropyl-β-d-thiogalactopyranoside (IPTG) as the inducer. IPTG is a strong and stable inducer that cannot be metabolized by bacteria.

However, IPTG is not an innocuous inducer; instead, it exacerbates the toxicity of haloalkane substrate and causes appreciable damage to the E. coli BL21(DE3) host, which is already bearing a metabolic burden due to its content of plasmids carrying the genes of the synthetic metabolic pathway.[1]

2.Introduction

In our experiment, the expression of TodD is bottomed so that the protein concentration is particularly low. This added difficulties to our future experiments. Thus, we hope through the modeling we can extrapolate the optimal concentration of IPTG. i.e. How to reach the highest expression under certain concentration of IPTG in a fixed period of time.

3.Theoretical basis:

We considered two things through modeling:

First, IPTG can influence the expression of E.coli and augment the expression as it significantly weakens the effect of repressor, increases the total expression of the gene.

Second, IPTG will influence the growth of E.coli, reduce number of E.coli, and lower the total expression of the gene.

4.Model

K	Control parameter of growth rate of E.coli
M	Control parameter of growth rate of E.coli
t	times
n	Control parameter of concentration of IPTG
C	Control parameter of concentration of IPTG
k_x	dissociation constant for IPTG to Lac
k_d	dissociation constant for Lac repressor

Due to the theoretical basis and the background knowledge we learn, there are two factors that contribute to the growth of the E.coli, times and the concentration of the IPTG. So the model that illustrates the pattern of E.coli growth is two-dimensional. Basically, the growth of E.coli would follow logistic growth:

$\frac{\partial[E]}{dt}$=k*[E](M-[E])

Solving the partial derivative, we get the parameter function(M,k are parameters):

E[t]=$\frac{M*e^{M*k*t}}{e^{M*k*t}-1}$

As we take account for the effect of IPTG, it need to be clear that instead of changing the whole pattern in which E.coli growth, concentration of IPTG would partially affect the growth of E.coli. So we can improve the logistic growth equation in this way(nw+1 would make sure that the model could also illustrate the growth when the concentration of IPTG equals to 0):

E[t,w]=$\frac{M}{ce^{-\frac{kMt}{nw+1}}+1}$

As we get more than three thousand sets of data from the experiment of IPTG inducing, we are able to use those data to fit with the equation we just wrote.

The most fitted parameters are attained by least square method.

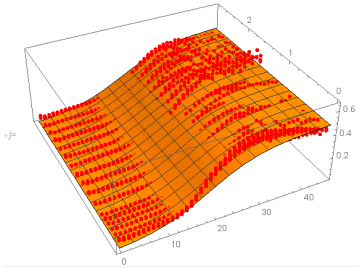
k=0.368188

c=28.2155

M=0.481054

n=0.0406467

Graph visually embody the how well the data have been fitted:



Since the RNA polymerase starts to transcript when IPTG binds the promotor, the rate of transcription is direct proportional to the possibility of the combination between IPTG molecule and the promotor. The function illustration of IPTG binding is shown by MM function below (information related to MM function is in the appendix) :

P= $\frac{X^*}{X^* + Kd}$

Since every two IPTG molecules bind with one repressor during IPTG induction, we have the following function, which is based on Hill equation.([EIPTG]means the concentration of the E.coli that is bond to IPTG):

X^*=[EIPTG]=$\frac{X_T W^2}{K_x^2 + W^2}$

$X^T$is the total concentration at the binding site, which is the concentration of E.coli.

Therefore, the input function of promoter is:

f(W)=$\frac{\beta * \frac{[E]*W^2}{K_x^2 + W^2}}{\frac{[E]*W^2}{K_x^2 + W^2} + Kd}$

The objective function of the whole model that explains the net impact of IPTG to the biological system are expressed as the number of E.coli*the transcription rate of a single E.coli:

g(t)=$\frac{\beta * \frac{[E]*W^2}{K_x^2 + W^2}}{\frac{[E]*W^2}{K_x^2 + W^2} + Kd} * \frac{M}{c*e^{-M*t*(\frac{k}{n*W+1})} + 1}$

Value of k_x, k_d can be found in the wiki of Universe of Aberdeen 2009:

k_x=1*10^{-6} M

k_d=1.3 μM

Where k_x is the dissociation constant for IPTG to Lac and k_d is dissociation constant for Lac repressor.

Result:

```
In[18]:= g = 0.481054 / (28.2155 * E^(-0.481054 * t * 0.368188 / (0.0481054 * w + 1)) + 1)
                                  自然常数

Out[18]= 0.481054 / (1 + 28.2155 e^(-0.177118 t / (1 - 0.0481054 w)))

In[21]:= p = g * w^2 / (1 * 10^(-12) + w^2)

Out[21]= 0.481054 w^2 / ((1 + 28.2155 e^(-0.177118 t / (1 - 0.0481054 w))) (1 / 1000000000000 + w^2))

In[23]:= Maximize[{p / (p + 1.3 * 10^(-12)) * g, w > 0, t < 241}, {t, w}]
         最大点值

Out[23]= {0.481054, {t → 240.962, w → 0.764906}}
```

Input the value of k_x, k_d, k, c, M, n into g(t), finding the maximum value of g(t) is attained in what concentration of IPTG. The whole programming is done in Wolfram Mathematica.

The result of programming instructs us that when the concentration of IPTG is 0.764906mM, E.coli could produce the largest amount of todD.

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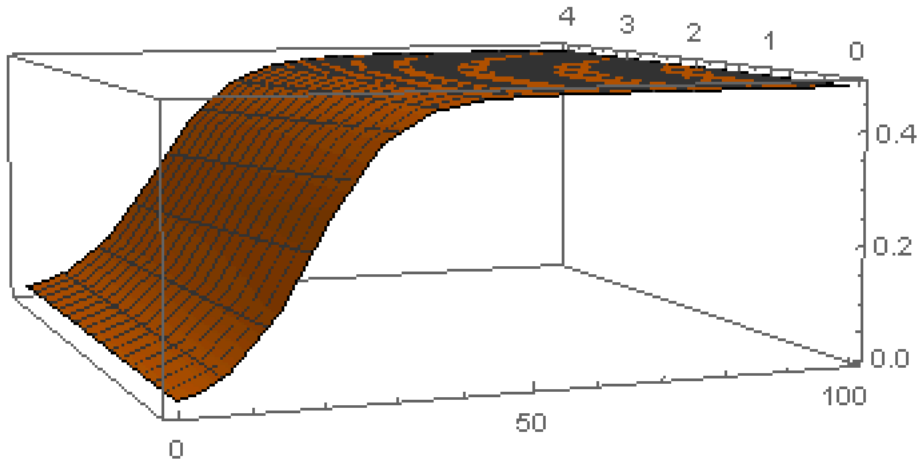
In[24]:= Plot3D[p / (p + 1.3 * 10^(-12)) * g, {t, 0, 100}, {w, 0, 4}]
         绘制三维图形
```

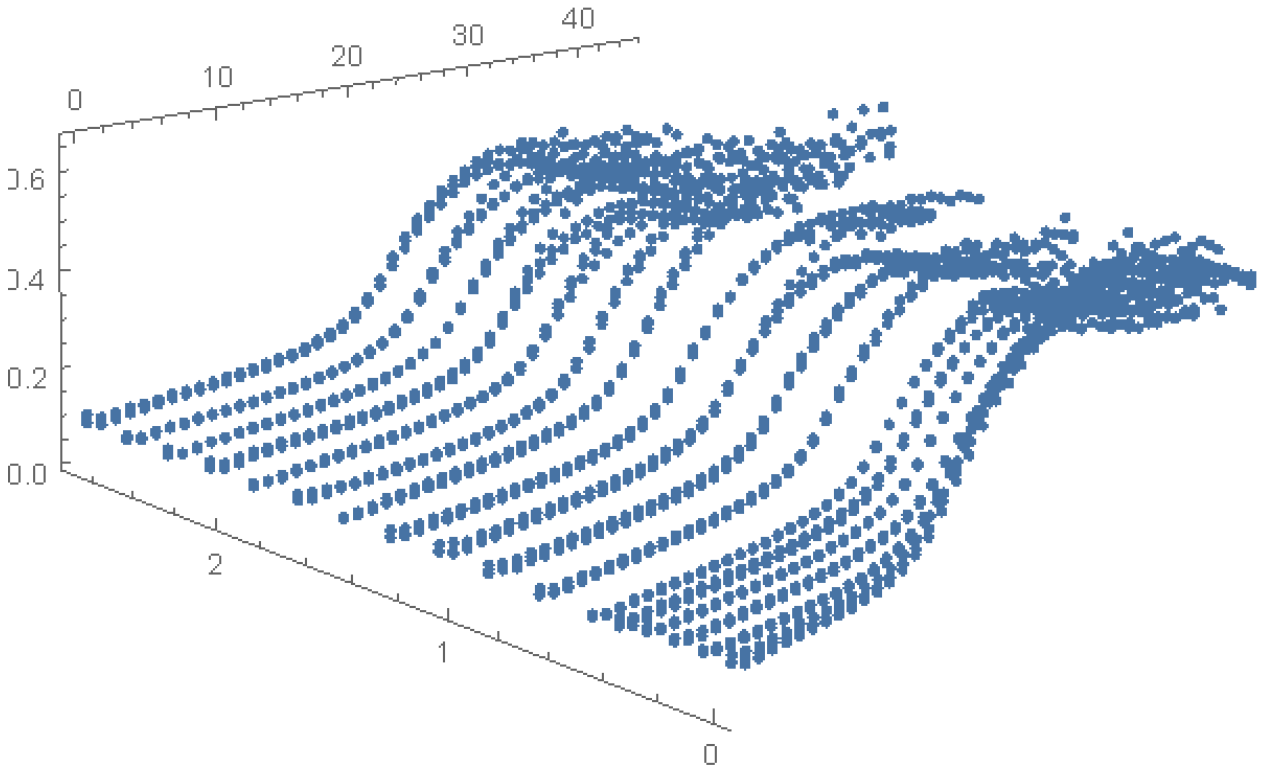
Out[24]=



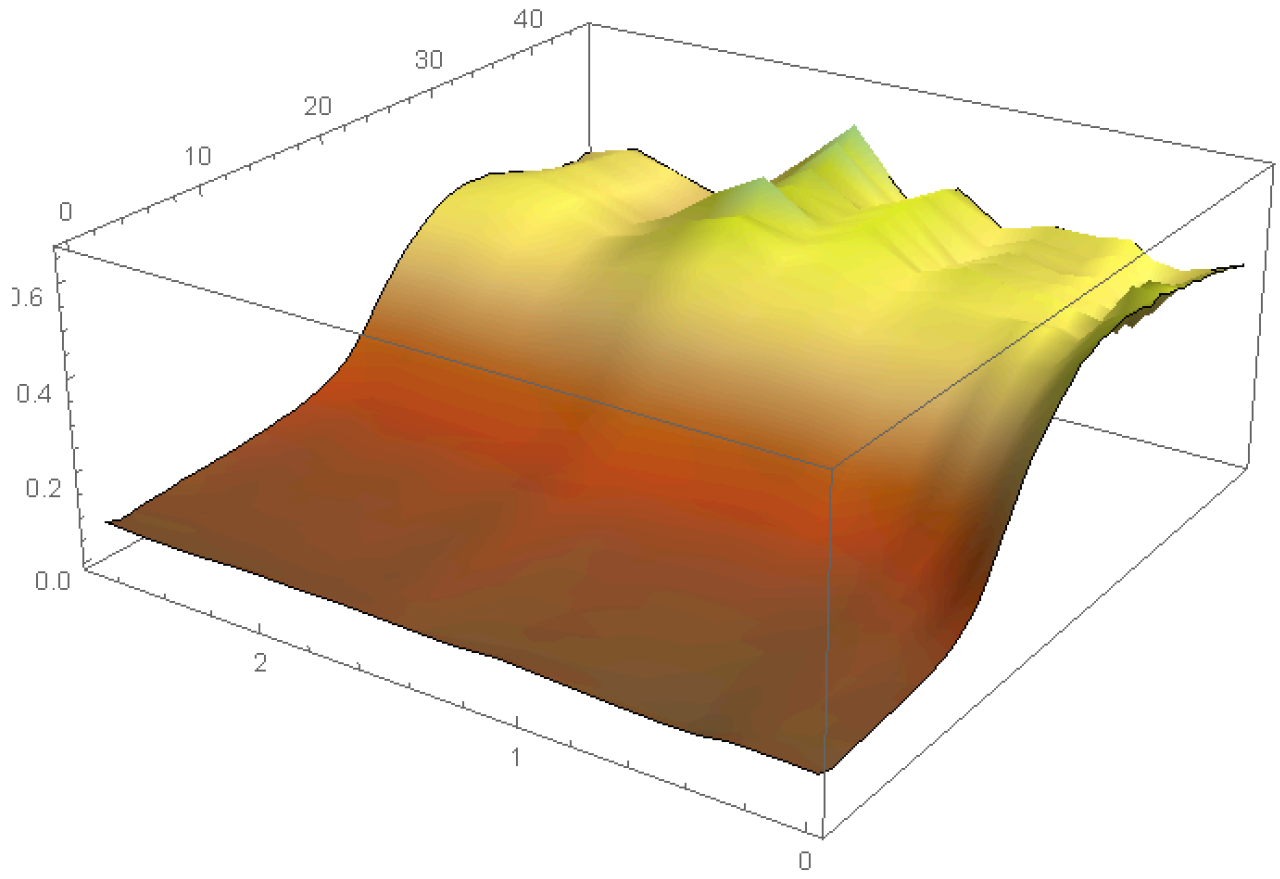
The graphics of objective function shows that the producing rate of todD would slowing down significantly when time value approaches 50, which is 12.5 hours in the real world. To increase the efficiency of the lab and to save time, 12.5 hours of IPTG induction is enough for the production of todD.

Appendix

Visualization of data collected from the experiment:



MM equations：



$$[XS_x]=\frac{X_T S_x}{S_x + K_x}$$

S_x are inducers
X are repressors
X_T=[XS_x]+X
K_x is dissociation constant

Reference

[1] Dvorak1, P., Lukas, Nikel3, P. I., Fedr2, R., Soucek2, K., Sedlackova5, M., … Damborsky1, J. (2015, December 21). Exacerbation of substrate toxicity by IPTG in Escherichia coli BL21(DE3) carrying a synthetic metabolic pathway. Retrieved October 14, 2019, from https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-015-0393-3.