

## **Mathematical modeling of the evolution of a simple biological system**

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### **Abstract**

*As a part of teaching of physics it is common to record data on evolution of a physical system and then construct a mathematical model of the system. For example, movement of a simple pendulum can be recorded, and then a mathematical model can be constructed to simulate the observed movement. The comparison between the observed data and the predictions based on the model then tell us how satisfactory the model is. In physical systems the model is usually based on fundamental principles of physics such as Newton's Laws. This article will deal with the application of a mathematical model to a biological process. The process involves the complete reduction of 15um nitrate by a bacterial culture in the presence of 0.01% glucose in 46 hrs. The simple mathematical model captures the observed changes and a deterministic model measures the percentage of fit. Including light and phytoplankton in this mathematical model would elucidate the competition between bacteria and phytoplankton for nitrate.*

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### **Introduction**

In India, classroom education in biology does not generally include an exercise in which the data can be used to develop models. This has hampered exposure to quantitative tools in biology, much to the disadvantage of students. The purpose of this note is to report an exercise we carried out to expose traditional biologists educated in India to mathematical modelling of biological systems. The exercise consisted of a simple biological system

that was grown in a laboratory. Evolution of the system was recorded. A mathematical model was then constructed. In constructing the model it was pointed out that unlike in case of a physical system, where a model usually can be developed from well established fundamental principles, in case of biological systems such principles are not always available offering an opportunity for innovation. The success of the innovation, of course, is measured by how well it reproduces the observations. The next section describes the experiment. Section 3 discusses development of a suitable model to simulate the

experimental observations. Success of the model and possible future studies on the biological system are discussed in Section 4.

### **Nitrate reduction by *Halomonas cupida* under heterotrophic conditions: a deterministic model**

The bacterial strain *Halomonas cupida* has the unique property of carrying out both phases of nitrate reduction (nitrate to nitrite and nitrite to  $N_2$ ) processes. Reduction of nitrate to nitrite has been reported in some members of *Halomonadaceae* family (Dobson and Franzmann, 1996), (Kaye and Baross, 2000) such as in *H. compisalis* (Mormile et al., 1999) and *Halomonas halodenitrificans* (Dobson and Franzmann, 1996). The present study demonstrates the property of complete nitrate reduction ( $15\mu M$ ) by the culture under heterotrophic conditions (0.01% glucose) in 46 hrs. This work predicts the cell numbers using a deterministic model and measures the percentage of fit.

Definitions:

$N_0, N_1, N_n$  = the number of cells at time zero ( $t_0$ ),  $t_1$  and  $t_n$  respectively

Delta N ( $\Delta N$ ) = the change in cell numbers

Delta t ( $\Delta t$ ) = the change in time

$dN/dt$  = rate of change in cell number with respect to time

$A_0, A_1, A_n$  = nitrate concentration at time zero ( $t_0$ ),  $t_1$  and  $t_n$  respectively

Delta A ( $\Delta A$ ) = change in the nitrate concentration

$dA/dt$  = rate of change in the nitrate concentration with respect to time

$G_0, G_1, G_n$  = glucose concentration at time zero ( $t_0$ ),  $t_1$  and  $t_n$  respectively

Delta G ( $\Delta G$ ) = change in glucose concentration

$dG/dt$  = rate of change in glucose concentration with respect to time

$K_a$  = change in nitrate due to bacteria

$K_g$  = change in glucose due to bacteria

$K_n$  = change in bacterial numbers due to death/growth

$K_{na}$  = change in bacteria due to nitrate

$K_{ng}$  = change in bacteria due to glucose

Where  $K$  is a rate constant for a particular change in parameter due to another parameter and is always positive.

I. Change in nitrate concentration at a given time is dependent on the number of bacteria at time zero.

$$\Delta A = (K_{na}N) \Delta t \quad \text{where } K_{na} = 1.5 \times 10^{-8} \text{ (trial and error) equation 1}$$

II. Change in glucose concentration at a given time is dependent on the number of bacteria at time zero.

$$\Delta G = (K_{ng}N) \Delta t \quad \text{where } K_{ng} = 9.9 \times 10^{-8} \text{ (trial and error) equation 2}$$

III. Change in bacterial number  $\Delta N$  at a given time  $t$  is dependent on the initial nitrate concentration  $A_0$ , initial glucose concentration  $G_0$  and the initial bacterial number  $N_0$ .

$$\Delta N = (K_a A + K_g G) \Delta t \quad \text{equation 3}$$

## Material and methods

### *Experimental Design*

Kinetics of nitrate reduction by *H. cupida* was monitored for 46 hours at  $28(\pm 1)^\circ\text{C}$  and 1 atmospheric pressure. The isolate was enriched in aged offshore seawater amended with 0.01% glucose for 48 hours. The cells were spun at 8000 rpm at  $4^\circ\text{C}$  and washed with physiological saline. This was repeated thrice and cells suspended in saline formed the inoculum for nitrate reduction experiments. Nitrate reduction experiments were done in the presence of 0.01% glucose. Fall in level of nitrate, appearance of nitrite (Bendschneider and Robinson, 1952) and the remaining nitrate (Wood et al., 1967) were monitored along with the growth of the culture in presence and absence of nitrate. Cell counts were made using a cell counting chamber. Uninoculated controls were incorporated wherever required.

## Results and discussion

Figures 1a–b shows the kinetics of nitrate reduction and appearance of nitrite. Within 28 hours, *H. cupida* reduced  $\sim 15\mu\text{M}$  of  $\text{NaNO}_3$  to non-detectable levels (Figure 1a). Nitrite values increased to  $1.04\mu\text{M}$  in 8 hours and decreased to non-detectable levels in 24 hours (Figure 1b). Glucose decreased from  $95\mu\text{g}\cdot\text{ml}^{-1}$  to non detectable values in 46 hrs (Figure 1c). Bacterial cell numbers increased from  $4.68 \times 10^7 \text{ ml}^{-1}$  to  $2.73 \times 10^8 \text{ ml}^{-1}$  in 28 hours and decreased to  $2.13 \times 10^8 \text{ ml}^{-1}$  by 46 hours (Figure 1d).

### ***Percentage Fit***

Figure 2 presents the deviation of the predicted values from the observed values using different constant varying from 0.001 to 0.05. With a  $K_n=0.05$  the percentage fit was 98% and with  $K_n=0.001$  it was 100%.

### ***Development of a model***

The bacterial numbers modeled were derived by using equation no. 3 which uses the following constants:

Constant for change of nitrate due to bacteria  $K_a = 1.5 \times 10^{-8}$

Constant for change in glucose due to bacteria  $K_g = 9.9 \times 10^{-8}$ .

The predicted bacterial growth curve which is sigmoidal in nature gave a 100% fit with the observed experimental values.

### ***Biological Description***

The growth curve is typical, showing lag, log and stationary phase. The bacteria inoculated into a medium grew after a lag phase of 16 hours. The exponential phase lasted for 8 hours before evolving into a stationary phase after 28 hours, when there is apparently no net increase or decrease in cell number for a long time. Comparison of growth rate in the presence of glucose and, glucose and nitrate showed that nitrate could improve growth rate by 5-38% at different phases (Table 1). However, the general trend shows that there could be 14% improvement in cell growth at the beginning which could increase to 20% towards the end of the experiment.

### ***Model Equations***

Rate of change of nitrate concentration and glucose concentration can be expressed by

$$dA/dt = -K_{na} * N \quad (1) \text{ and}$$

$$dG/dt = -K_{ng} * N \quad (2)$$

The growth rate of bacteria depends upon the nitrate and glucose concentrations, so the equation representing the change in bacterial concentration can be written as

$$dN/dt=K_a*A+K_g*G \quad (3)$$

After differentiating the equation (3) with respect to “t”, we get

$$d^2N/dt^2=K_a*dA/dt+K_g*dG/dt$$

Now substituting the value of dA/dt and dG/dt into above equation

$$d^2N/dt^2=-K_a*K_{na}*N-K_g*K_{ng}*N =-(K_a*K_{na}+K_g*K_{ng})*N$$

$$d^2N/dt^2+\alpha^2*N=0, \text{ where } \alpha^2=(K_a*K_{na}+K_g*K_{ng}) \quad (4)$$

Eq. (4) is a homogeneous second order linear differential equation and its general solution 5 is given by

$$N=A_1*\sin(\alpha*t)+A_2*\cos(\alpha*t) \quad (5)$$

where A1 and A2 are constants

They are easily derived from initial conditions when we take time to be zero. That is at t=0

$$N_{(t=0)}=A_1*\sin(\alpha*0)+A_2*\cos(\alpha*0)$$

$$N_0=A_1*0+A_2*1$$

$$A_2=N_0$$

Taking time derivative of (5) at t=0, we obtain

$$(dN/dt)_{(t=0)}=A_1*(d\sin(\alpha*t)/dt)_{(t=0)}+A_2*(d\cos(\alpha*t)/dt)_{(t=0)}$$

$$(dN/dt)_{(t=0)}=A_1*(\alpha*\cos(\alpha*t))_{(t=0)}+A_2*(-\alpha*\sin(\alpha*t))_{(t=0)}$$

$$(dN/dt)_{(t=0)}=A_1*(\alpha*\cos(\alpha*0))+A_2*(-\alpha*\sin(\alpha*0))$$

$$(dN/dt)_{(t=0)}=A_1*(\alpha*1)+A_2*(-\alpha*0) \quad (\text{since } \cos(0)=1 \text{ and } \sin(0)=0)$$

$$(dN/dt)_{(t=0)}=A_1*\alpha$$

$$A_1=(1/\alpha)*(dN/dt)_{(t=0)}$$

Integrating equation (4) twice, gives the solution as

$$N=A_1*\sin(\alpha t)+A_2*\cos(\alpha t) \quad (5)$$

where  $A_1=(1/\alpha)*(dN/dt)_{(t=0)}$  and  $A_2=N_0$  are the constants of integration and are determined using initial values of cell numbers (N).

### ***Solutions of the Model Equations***

As explained earlier in the biological description section the culture experiment can be divided into three phases. We solve the equations (1), (2) and (4) for these three phases separately.

Constants  $K_{na}$  and  $K_{ng}$  are determined from equations (1) and (2) respectively as follows: discretize the equation using backward difference, substitute the values of A (or G) and N from the experiment. The constant for each phase is determined as the average value of  $K_{na}$ 's (or  $K_{ng}$ 's) in that phase. The constants thus determined are as follows:

Phase I  $K_{na}=2.99*10^{-12}$  ,  $K_{ng}=1.71*10^{-11}$

Phase II  $K_{na}=4.12*10^{-13}$  ,  $K_{ng}=2.93*10^{-12}$

Phase III  $K_{na}=8.30*10^{-14}$  ,  $K_{ng}=6.70*10^{-13}$

Comparison of the calculated nitrate and glucose concentrations using these constants with that of experimental values are shown in figure 3a and figure 3b. The best fit of the solution (4) to the experimental values of cell numbers is determined by searching the value of alpha for each phase (figure 3c). The values of alpha are 0.0001, 0.115 and 0.025 for phase I, II and III respectively.

The experimental process involves the complete reduction of  $15\mu\text{m}$  nitrate by a bacterial culture in the presence of 0.01% glucose in 46 hours. The mathematical model describes a bacterial process which captures the observed changes and a deterministic model measures the percentage of fit which could be employed to predict reactions within this experimental framework. The model could be extended to a larger framework as the present one involves only a few parameters like nitrate and glucose which limit bacterial growth and respiration in a batch culture. Although the simple mathematical model is able to capture the observed phenomenon reasonably well, considering light and phytoplankton, in this batch system would help understand how phytoplankton and bacteria compete for a given concentration of nitrate. Further experiments in chemostats or mesocosms would help understand and the growth kinetics or interactions in nature and add to the model.

### **Acknowledgements**

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## Legends to figures

Figure 1a. The culture reduces 15 $\mu$ m of nitrate to below detection limits by 28 hours.

Figure 1b. Nitrite detected were always  $\leq 1\mu$ m

Figure 1c. Glucose level comes down rapidly within 20 hours and reaches non-detectable levels in 46 hours.

Figure 1d. There is five fold increase in bacterial numbers by 24 hours.

Figure 2. Deviation of the predicted values from the observed values using different constant varying from 0.001 to 0.05. With a  $K_n = 0.05$  the percentage fit was 98% and with  $K_n=0.001$  it was 100%.

Figure 3a. Comparison of the calculated nitrate concentrations using these constants with that of experimental values

Figure 3b. Comparison of the calculated glucose concentrations using these constants with that of experimental values

Figure 3c. Best fit obtained by searching the value of alpha for each phase



Table 1. Rate of growth (cell number x 10<sup>7</sup>·ml<sup>-1</sup>·h<sup>-1</sup>)

| Hours of incubation | Glucose | Glucose+Nitrate | Contribution of Nitrate |
|---------------------|---------|-----------------|-------------------------|
| 0                   | 0       | 0               | 0                       |
| 4                   | 0.13    | 0.21            | 0.08(38%)               |
| 8                   | 0.25    | 0.29            | 0.04(14%)               |
| 12                  | 0.37    | 0.43            | 0.06(14%)               |
| 16                  | 1.07    | <b>1.06</b>     | <b>-0.01(.01%)</b>      |
| 20                  | 0.96    | 1.10            | 0.05(4.5%)              |
| 24                  | 0.97    | <b>0.93</b>     | <b>-0.04(0.04%)</b>     |
| 28                  | 0.82    | <b>0.81</b>     | <b>-0.01(0.01%)</b>     |
| 36                  | 0.49    | 0.61            | 0.12(20%)               |
| 46                  | 0.38    | 0.45            | 0.07(16%)               |

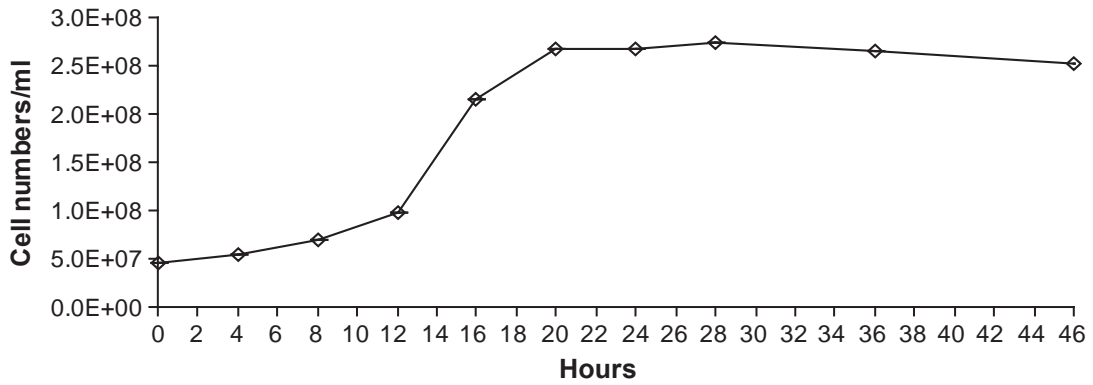
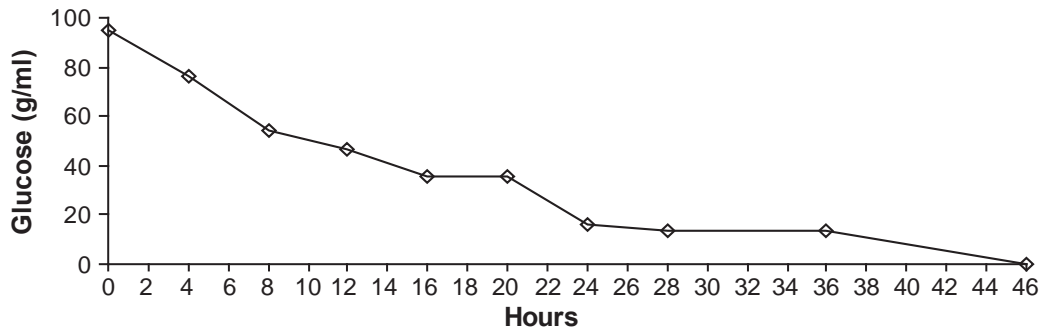
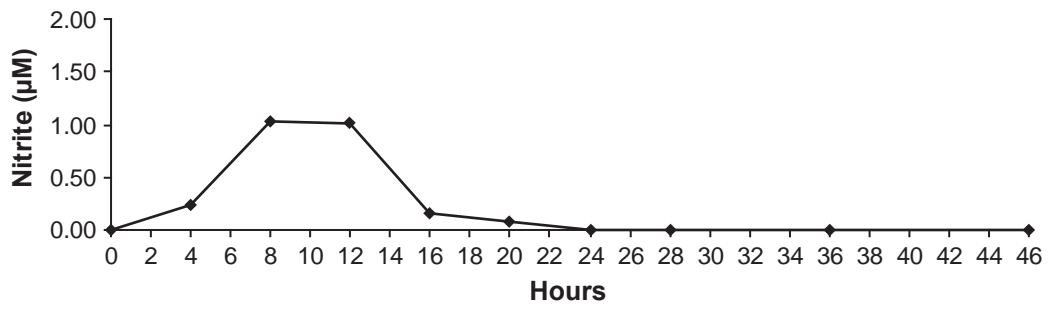
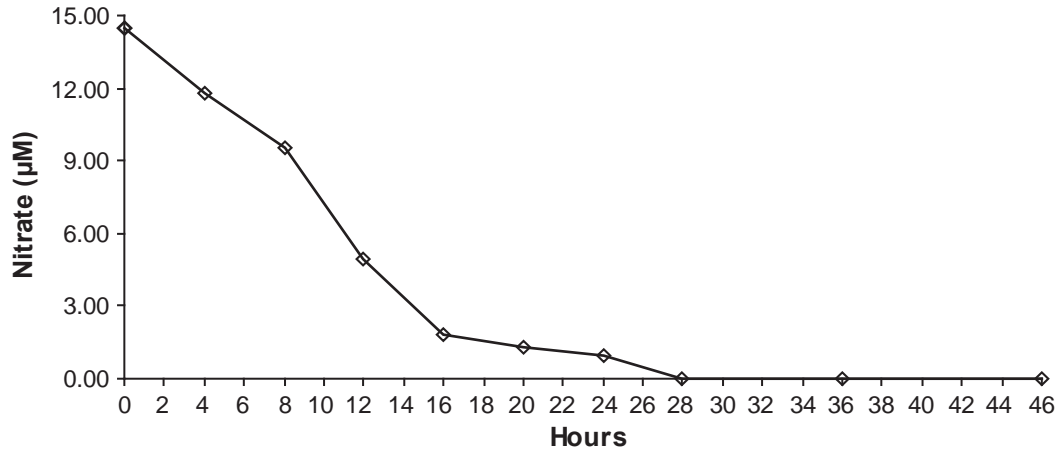


Figure :1 (a-d)

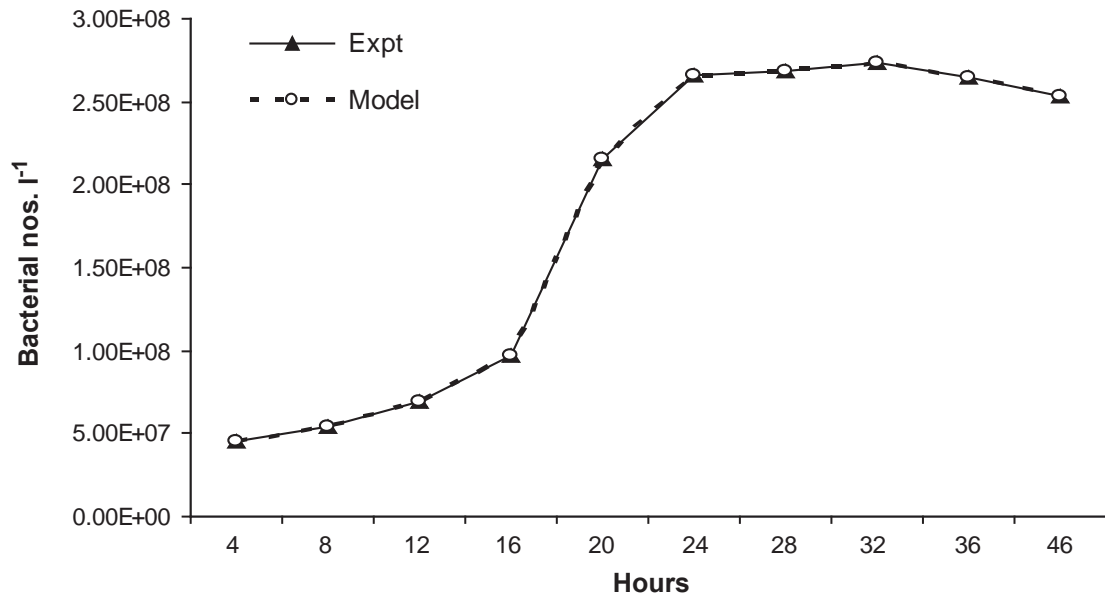
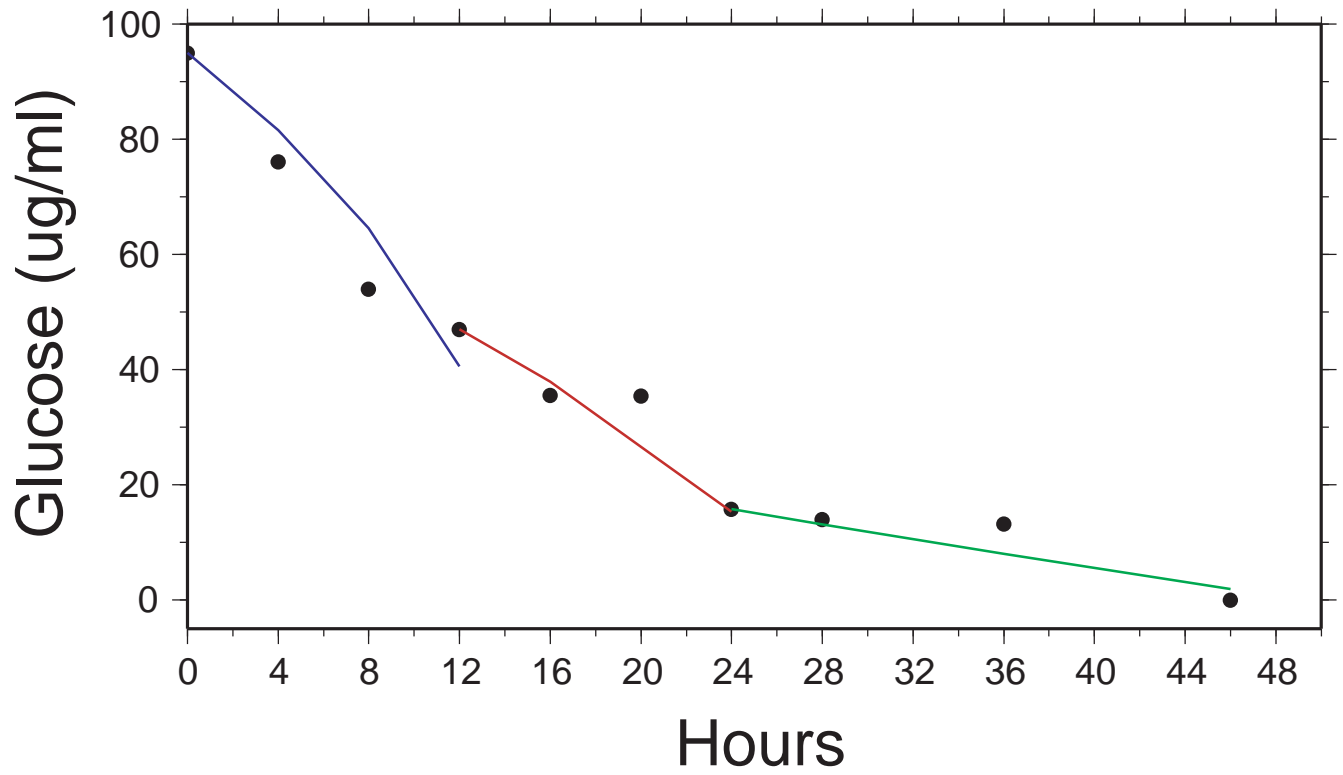
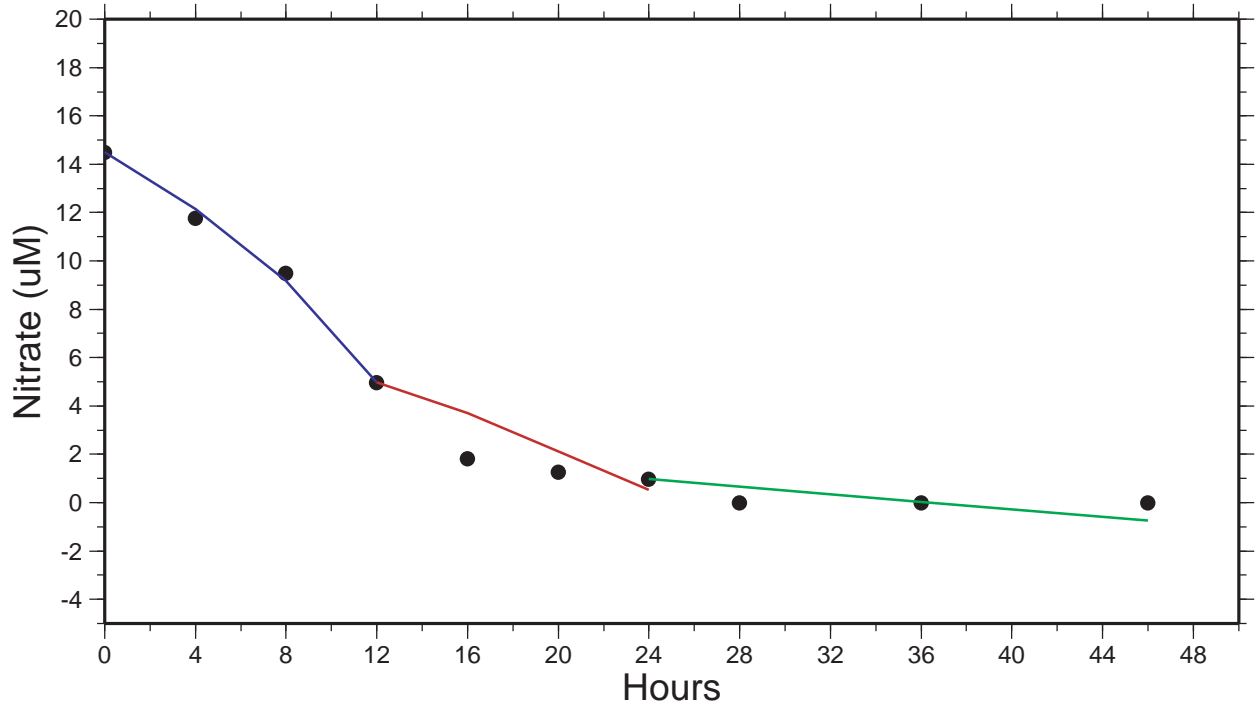


Figure: 2



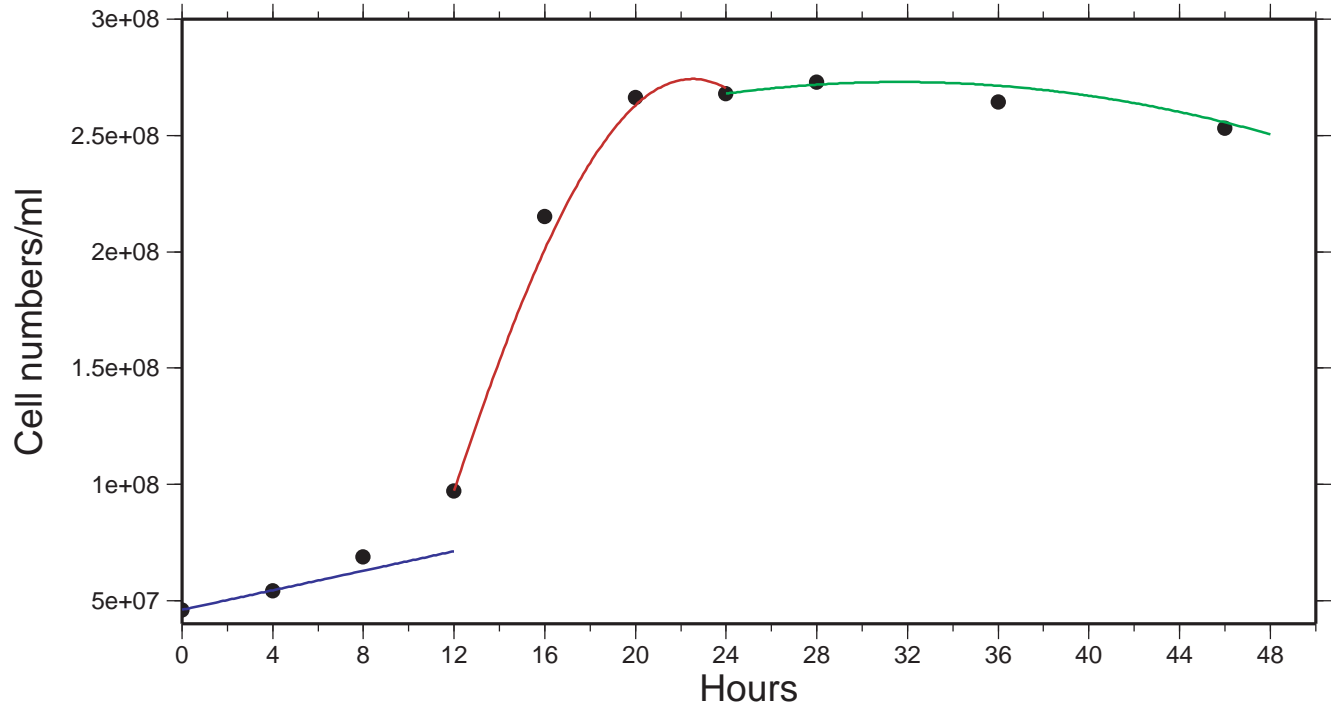


Figure: 3