ABE 304
Bioprocess Engineering Laboratory

PRELAB: FERMENTATION

BACKGROUND

The focus of this lab is the kinetics of batch cell growth or fermentation of *Xanthamonas campestris* to produce xanthan gum. Temperature has a significant effect on the rate and yield of xanthan gum produced. Higher temperature can result in higher xanthan production rate while lower temperatures increase cell growth (Shu & Yang, 1990). Cell growth in a batch system follows the scheme shown in Figure 1. After the growth medium has been inoculated, the cells experience a lag phase while they adapt to their new environment. They then have an acceleration period followed by a growth phase where they consume the substrate. Once the nutrients have been depleted the cells experience a decline and enter the stationary phase eventually reaching the death phase.

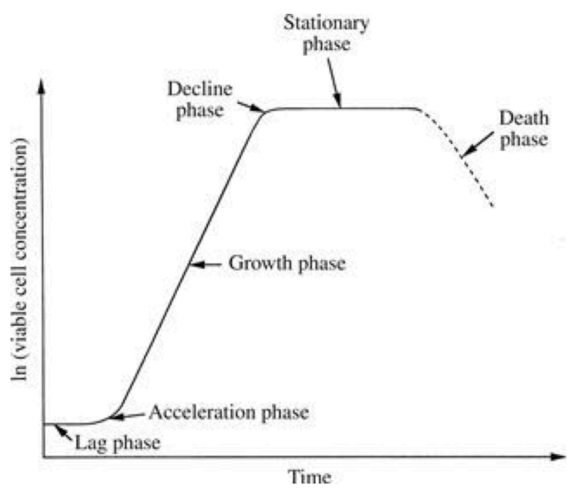

Figure 1. Idealized growth curve of bacteria in a closed, batch culture.
Note that the y-axis is the natural log of x.
Figure from (Doran, 2013). *(pg. 636). Bioprocess Engineering Principles*

During the growth phase the rate appears to be exponential – note that the y axis is plotted as a natural log. The rate of cell growth, r_x, is explained by Equation 1.

$$r_x = \mu x \qquad (1)$$

Where μ is the specific growth rate [time^{-1}] and x is the concentration of cells [mass/volume]. Cell growth is a first-order autocatalytic reaction. Assuming a constant volume, well mixed (homogeneous) reactor under batch (closed) conditions we might therefore predict a rate of growth that is proportional to the number of starting cells, shown in Equation 2..

$$r_x = \frac{dx}{dt} \qquad (2)$$

During the growth phase the specific growth rate can be considered to be a constant. Equations 1 and 2 can be combined and integrated using the initial conditions of $x = x_0$ at time zero to get Equation 3.

$$\ln x = \ln x_0 + \mu\, t \qquad (3)$$

Equation 3 can be rearranged to give a simple exponential growth equation shown by Equation 4.

$$x = x_0 e^{\mu t} \qquad (4)$$

However, this simple exponential growth model cannot account for the initial delay in growth (lag phase), the period of plateau when the bacteria are becoming nutrient limited (stationary phase), nor cell death (death phase). The exponential growth rate constant, μ, therefore represents a maximum growth rate that occurs during the "growth" phase of the bacteria. As an alternative, other models of cell growth exist such as the Monod, logistic, or Luedeking-Piret (Weiss & Ollis, 1980), each with their own strengths and limitations as well.

PRE-LAB QUESTIONS

1. Consider that you start an *X. campestris* culture in a batch reactor with an initial inoculum such that you get a typical growth curve similar to Figure 1. What type of plot would be best to determine which portion of the plot is the growth phase (i.e. semi-log, log-log, linear)? Explain your answer.

2. The growth media for the fermentation consists of:
   a. Glucose
   b. KH_2PO_4
   c. $MgSO_4 \cdot 7H_2O$
   d. Antifoam A

   What is the purpose of each of these components during the fermentation?

3. Yield is a measure of how much of the substrate is converted into the product. Explain the difference between theoretical yield and observed yield for a microbial process.
4. What will the concentration of xanthan gum be at each point on your calibration curve?
5. Glucose will not need a calibration curve to determine the concentration. Why is calibration not necessary for glucose but is for xanthan?

This assignment is due on Blackboard by 5 PM on Sunday February 11.

You will need to make tables for your temperature and responses in your lab notebook prior to the start of lab. Include a table for the calibration curve. The TA will check your notebook for the appropriate tables.

REFERENCES

1. Doran, P. M. (2013). *Bioprocess Engineering Principles*. Academic Press.
2. Shu, C. H., & Yang, S. T. (1990). Effects of temperature on cell growth and xanthan production in batch cultures of Xanthomonas campestris. *Biotechnology and Bioengineering*, *35*, 454–468. http://doi.org/10.1002/bit.260350503
3. Weiss, R. M., & Ollis, D. F. (1980). Extracellular microbial polysaccharides. I. Substrate, biomass, and product kinetic equations for batch xanthan gum fermentation. *Biotechnology and Bioengineering*, *22*(4), 859–873. http://doi.org/10.1002/bit.260220410