

Experiment No. 4

Growth Kinetics at Various Substrate Concentrations

Objective

To study kinetics of growth under batch conditions

Apply simple unstructured growth models and obtain the kinetic parameters

Introduction:

The general goal in making a medium is to support good growth and/or high rates of product synthesis. Contrary to intuitive expectation, this does not necessarily mean that all nutrients should be supplied in great excess. For one thing, excessive concentration of a nutrient can inhibit or even poison cell growth. Moreover, if the cells grow too extensively, their accumulated metabolic end products will often disrupt the normal biochemical processes of the cells. Consequently, it is common practice to limit total growth by limiting the amount of one nutrient in the medium. If the concentration of one essential medium constituent is varied while the concentrations of all other medium components are kept constant, the growth rate typically changes in a hyperbolic fashion.

One of the simplest models which includes the effect of nutrient concentration is the model developed by Jacques Monod based on observations of the growth of *E. coli* at various glucose concentrations. It is assumed that only one substrate (the growth-limiting substrate, S) is important in determining the rate of cell proliferation. The form of the Monod equation is similar to that of Michaelis-Menten enzyme kinetics and is given by:

$$\frac{dX}{dt} = \frac{\mu_{\max} SX}{K_s + S}$$

where μ_{\max} is the maximum specific growth rate of the cells and K_s is the value of the limiting nutrient concentration which results in a growth rate of half the maximum value.

List of Reagents and Instruments

A. Equipment: Flasks, Spectrophotometer, Sample tubes, Micropipette, Centrifuge

B. Reagents

- Antrone reagent: Dissolve 2 g Antrone in 1000 ml of concentrated sulphuric acid

C. Organism

- *Bacillus licheniformis* NRRL B-642

D. Media composition

- **For culture maintenance (Slant and/or Plate)**

- Nutrient agar medium, 28 g/l

- **For Growth media in flask (Minimal Salt medium)**

Chemical name	Composition (g/l)
Glucose	2.0
Potassium Dihydrogen Phosphate	0.2
Di -Potassium hydrogen phosphate	0.8
Magnesium Sulphate Hepta hydrate	0.5
Ammonium Sulphate	1.0
Calcium Chloride	0.05

Procedure

Inoculate freshly grown bacterial culture into a series of 500 ml Erlenmeyer flasks containing 150 ml of nutrient media with different glucose concentrations: 0.5, 1, 2, 3 and 4 g/L.

Take 2 ml of samples from each flask at following time intervals: 0 hr, 4 hrs, 6hrs, 8hrs, 10 hrs, 12hrs, 14 hrs, 16 hrs, 18hrs, 20 hrs, 22 hrs and 24 hrs.

Estimate the biomass concentration in the sample, by optical density measurement method by use of calibration curve obtained earlier.

Estimate glucose concentrations in the sample, by antrone method.

Task Required

Calculation of specific growth rate of the microorganism at different concentrations of glucose in the medium.

Estimation of kinetic parameters by application of simple unstructured growth models.

