Experiment No. 9

Fed-batch operation to maintain exponential growth phase

Objective

To perform fed batch operation to maintain the exponential growth phase at a set specific growth rate

Introduction

Fed-batch fermentation is a semi-continuous fermentation in which only a small amount of substrate is available at the beginning of the process. During the process, substrate is added as required by the growth rate. The specific rate at which the substrate is used up, $q_{s/x}$, can be determined as the amount of substrate per cell and unit of time from the growth kinetics:

$$q_{S/X} = \frac{1}{Y_{X/S}} \mu \qquad \textbf{(1)}$$

Here in fed batch operation:

$$XV = X_0 V_0 e^{\mu t} \qquad \textbf{(2)}$$

where X and V are the biomass concentration and volume of culture at time t and X_0 and V_0 are the biomass concentration and volume of the growth medium in the reactor at time t = 0. μ is the specific growth of the organism.

The specific rate of substrate uptake $q_{S/X}$ (g/lh^{-1}) in a fed-batch culture is satisfied by addition on demand. The required volumetric feeding rate, Q_S, consists of $q_{S/X}$ and the cell density X:

$$Q_S = q_{S/X} X \qquad \textbf{(3)}$$

This must be identical to the feeding rate:

$$Q_S = \frac{FS_0}{V} \qquad \textbf{(4)}$$

where F is the rate of pumping (1/h) at the given time, S_0 is the concentration of the input and V is the volume of the reaction. The method of feeding can be either constant, which results in linear growth, or adjusted to increase exponentially so that S is maintained at an optimal level and results in exponential growth. The balance in a fed-batch fementer may be described as follows. Biomass:

$$\frac{d(VX)}{dt} = \mu XV \qquad \textbf{(4)}$$

from which: $$\frac{dX}{dt} = (\mu - D)X \qquad \textbf{(5)}$$

$$\frac{dVS}{dt} = 0$$

Since the volume increase as a result of the input is: $\frac{dV}{dt} = F$ **(6)**

D (the dilution rate as a result of input) is: $D = \frac{F}{V}$ **(7)**

For limiting substrate the following is valid: $\frac{d(VS)}{dt} = S_0 F - \left(\frac{\mu XV}{Y_{X/S}}\right) = 0$ **(8)**

Hence: $$F = \frac{\mu XV}{Y_{X/S} S_0} = \frac{\mu X_0 V_0 e^{\mu t}}{Y_{X/S} S_0} \qquad \textbf{(9)}$$

The initial OD at the end of bath operation is taken as corresponding to X_0 at time t = 0. Knowing the yield and the volume of the reactor at the end of batch we can find the flow rate required to maintain a given specific growth rate for a given substrate concentration. Hence, we can monitor the OD as well as concentration of substrate with time and verify whether the exponential phase is maintained.

Materials

A. Equipment

- Flasks
- Test tubes
- Eppendorfs
- Graduated cylinder
- Centrifuge
- Oven, 100 ºC
- Balance
- Spectrophotometer
- Fermentor
- Orbital Incubator shaker

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

Media: Mineral medium used previously for batch cultivation of *B. licheniformis*
Inoculum: 10% of fresh *B. licheniformis* culture grown on Mineral medium

Procedure:

Batch cultures are started in the reactor by incoluating the bacteria and the OD was measured at regular time intervals and supernatant stored for glucose estimation. The samples are drawn at 1-2 hours time interval.

A feeding rate F according to equation 9 is obtained by substituting values of X_0, V_0 and S_0, S in the fermenter of 1.5 g/L and μ as determined previously from batch kinetics. Samples are withdrawn every one hour and analyzed for glucose and biomass concentration. Feeding will be started at the end of exponential growth phase in the reactor.

Task Required

1. Calculation of feed rate F
2. Time profile of biomass and glucose concentration in the reactor under batch and fed batch mode
3. Maintenance of exponential growth phase