6. How Cells Grow

$$\Sigma S + X \rightarrow \Sigma P + n X$$

Specific Growth Rate

$$\mu = \frac{1}{(XV)} \frac{d\,(XV)}{d\,t}$$

$$= \frac{1}{X} \frac{d\,X}{d\,t}$$

μ : specific growth rate (1/hr)

X : cell concentration (g/l)

6.2. Batch Growth

- No addition or removal
- Simple and widely used

6.2.2. Quantifying Cell Concentration

- 6.2.1.1. Determining cell number density
- 6.2.1.2. Determining cell mass concentration

6.2.1.1. Determining cell number density

- Hemocytometer
  - Usually used for animal cell count
- Plate count (colony count)
  - 25 generations are required to form an easily observable colony
- Particle counter (Fig. 6.1)
  - As cells pass through the orifice, the electrical resistance increases and causes pulses in current.
    - Number of pulse → number of cell
    - Height of pulse → size of cell

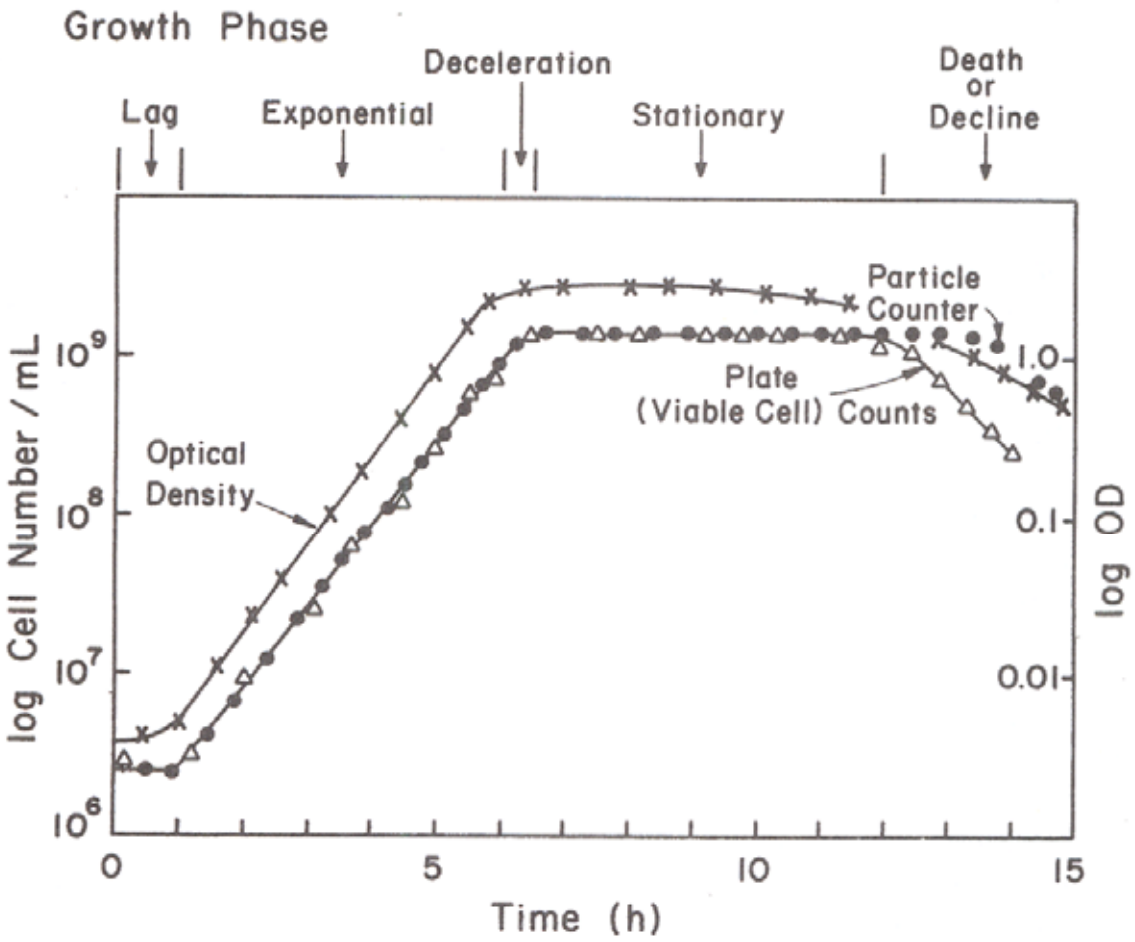
6.2.1.2. Determining cell mass concentration

- Direct methods
  - Dry cell weight
    - Centrifuge, wash, and dry at (80^{o}C for 24 h)
  - Packed cell volume
    - Centrifuge in a tapered graduated tube under standard conditions, and measure the cell volume
    - Rough estimation
  - Turbidity (or optical density) using a spectrophotometer
    - Fast, inexpensive, and simple
    - Correlation between OD and DCW/vol

Determining cell mass concentration

- Indirect methods
  - The measurement of substrate consumption and/or product formation (or CO_2 evolution)
  - The measurement of DNA or protein
    - DNA or protein/cell weight: fairly constant
    - RNA/cell weight varies significantly.
  - mg ATP/mg cells is approximately constant.
    - Luciferin + O_2 + ATP → light
      (in excess) luciferase

6.2.2. Growth Pattern and Kinetics in Batch Culture



Lag Phase

- Lag phase
  - Adaptation of cells to a new environment
  - To minimize lag time
    - Inoculating culture ~ active, in exponential phase
    - Small scale medium ≈ full-scale medium
    - Inoculum size: 5~10%
- Transfer of a small culture volume or inoculum to a large volume of medium will cause outward diffusion of the requisites (vitamins, cofactors, ions) for catalysis into the bulk medium if the new medium is lacking in these species or differs appreciably in ionic strength.
- Multi lag phase ~ e.g. Diauxic growth

Exponential Growth Phase

- Logarithmic growth phase
- This is a period of balanced growth.
  - All components of a cell grow with the same rate.
  - The average composition of a single cell remains approximately constant.
- Exponential Growth

$$X = X_0 e^{\mu_{net} t}$$

Exponential Growth

$$\frac{dX}{dt} = \mu_{net} X, \quad X = X_0 \quad \text{at} \quad t = 0$$

$$\ln \frac{X}{X_0} = \mu_{net}\, t, \quad \text{or} \quad X = X_0 e^{\mu_{net} t}$$

$$\tau_d = \frac{\ln 2}{\mu_{net}} = \frac{0.693}{\mu_{net}}$$

After Exponential Phase

- Deceleration growth phase
  - Due to either depletion of one or more essential nutrients or the accumulation of toxic by-products of growth
- Stationary phase
  - The net growth rate is zero. (= no cell division)
  - Or growth rate = death rate
- Death phase (decline phase)

$$\frac{dN}{dt} = -k'_d N \quad \text{or} \quad N = N_s e^{-k'_d t}$$

where N_s is the concentration of cells at the end of the stationary phase

Stationary Phase

- Primary metabolites are growth-related products.
- Secondary metabolites are nongrowth-related.
  - The production of antibiotics and some hormones is enhanced during the stationary phase.
- Endogeneous metabolism
  - During the stationary phase, the cell consumes cell substances (reserves) for new building blocks and for energy-producing monomers.
- Cryptic growth
  - Cells may grow on lysis products of lysed cells.