# 6. How Cells Grow

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

# Specific Growth Rate

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

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

$\mu$ : specific growth rate (1/hr)

X : cell concentration (g/l)

# 6.2. Batch Growth

- No addition or removal
- Simple and widely used

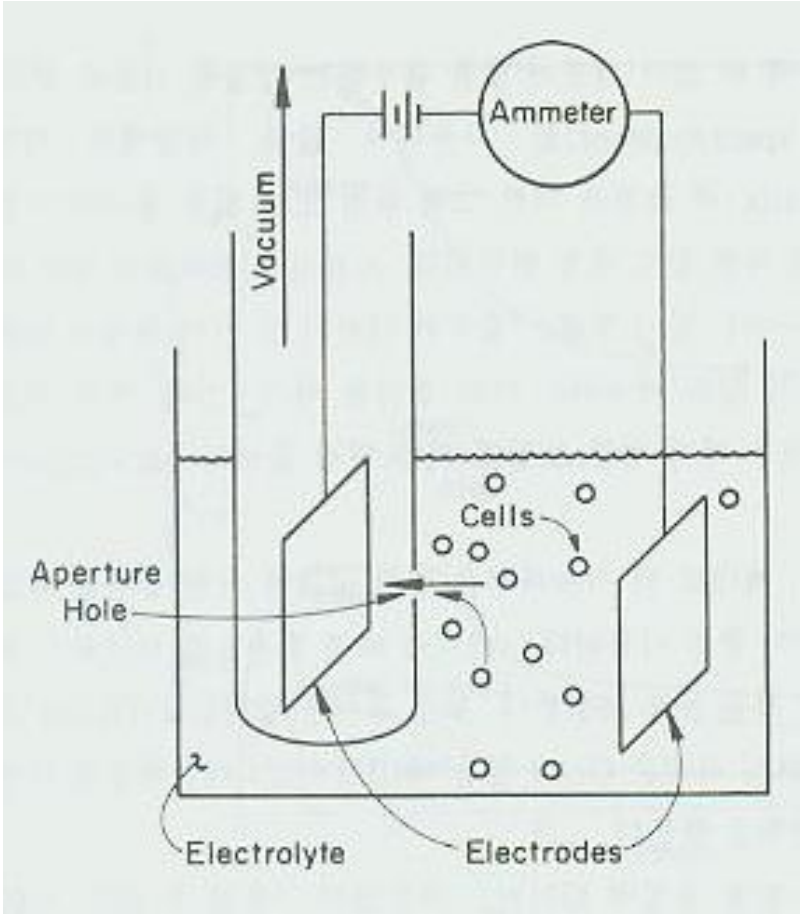
## 6.2.2. Quantifying Cell Concentration

- 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

# Particle Counter



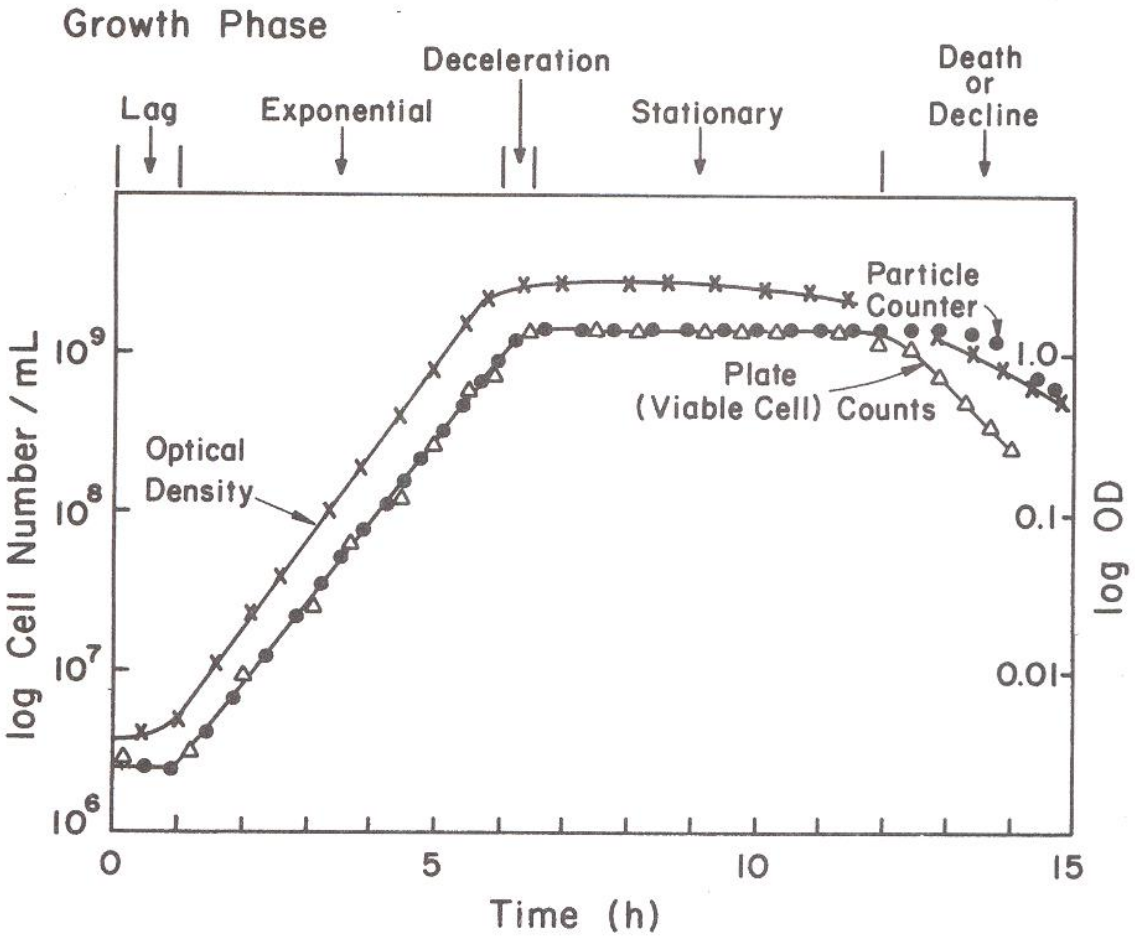
# 6.2.1.2. Determining cell mass concentration

- 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

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

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

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

# 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.

# Yield Coefficient

$$Y_{X/S} = \frac{\Delta X}{\Delta S} = -\frac{dX}{dS}$$

$$\Delta S = \Delta S_{\text{assimilation into biomass}} + \Delta S_{\text{assimilation into an extracellular product}} + \Delta S_{\text{growth energy}} + \Delta S_{\text{maintenance energy}}$$

$$Y_{X/O2} = \frac{\Delta X}{\Delta O_2} \qquad Y_{P/S} = \frac{\Delta P}{\Delta S}$$

# Growth Yield

- For organisms growing aerobically on glucose
  - $Y_{x/s}$ = 0.4 – 0.6 g/g
  - $Y_{x/o2}$ = 0.9 – 1.4 g/g

- In most cases the yield of biomass on a carbon-energy source is 1.0 $\pm$ 0.4 g biomass/g of carbon consumed.

# Maintenance Coefficient

$$m \equiv \frac{[dS/dt]_m}{X}$$

- Maintenance
  - To repair damaged cellular components
  - To transfer some nutrients and products
  - For motility
  - To adjust the osmolarity

# Product Formation

- Growth-associated product formation
  - Specific rate of product formation

$$q_p = \frac{1}{X}\frac{dP}{dt} = Y_{p/x}\,\mu$$

$$\frac{dP}{dt} = Y_{p/x}\frac{dX}{dt}$$

- Nongrowth-associated product formation

$$q_p = \beta = \text{constant}$$

  - Ex) many secondary metabolites such as antibiotics

# Product Formation

- Mixed-growth-associated product formation
  - Specific rate of product formation

$$q_p = \alpha \mu + \beta$$

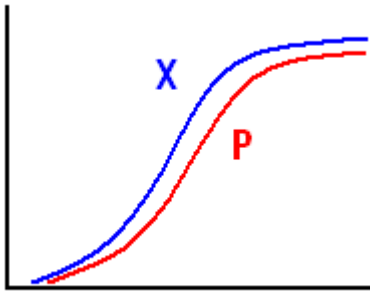
$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X$$

(Luedeking-Piret Equation)

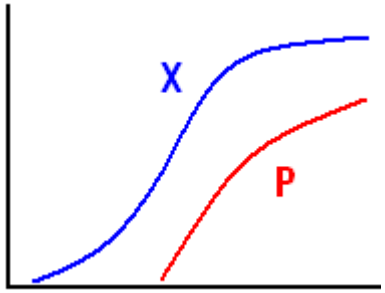
- Ex) lactic acid, xanthan gum, some secondary metabolites

# Kinetic Pattern of Product Formation

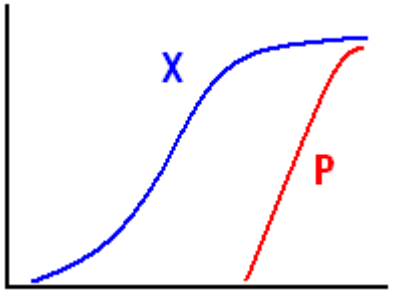
- Fig. 6.6




(a) growth-associated




(b) mixed-growth-associated




(c) nongrowth-associated

# 6.2.3. How Environmental Conditions Affect Growth Kinetics

- Effect of Temperature
  - Eqs. (6.21) and (6.22)

$$\frac{dN}{dt} = (\mu_R' - k_d')N$$

$$\mu_R' = Ae^{-E_a/RT}, \quad k_d' = A'e^{-E_d/RT}$$

(Eq. for temperature above optimal level)

- $E_a$: activation energy for growth (10-20 kcal/mol)
- $E_d$: activation energy for thermal death (60-80 kcal/mol)

  - Thermal death is more sensitive to temperature changes than microbial growth.

# 6.2.3. How Environmental Conditions Affect Growth Kinetics

- Effect of Temperature (Fig. 6.7)

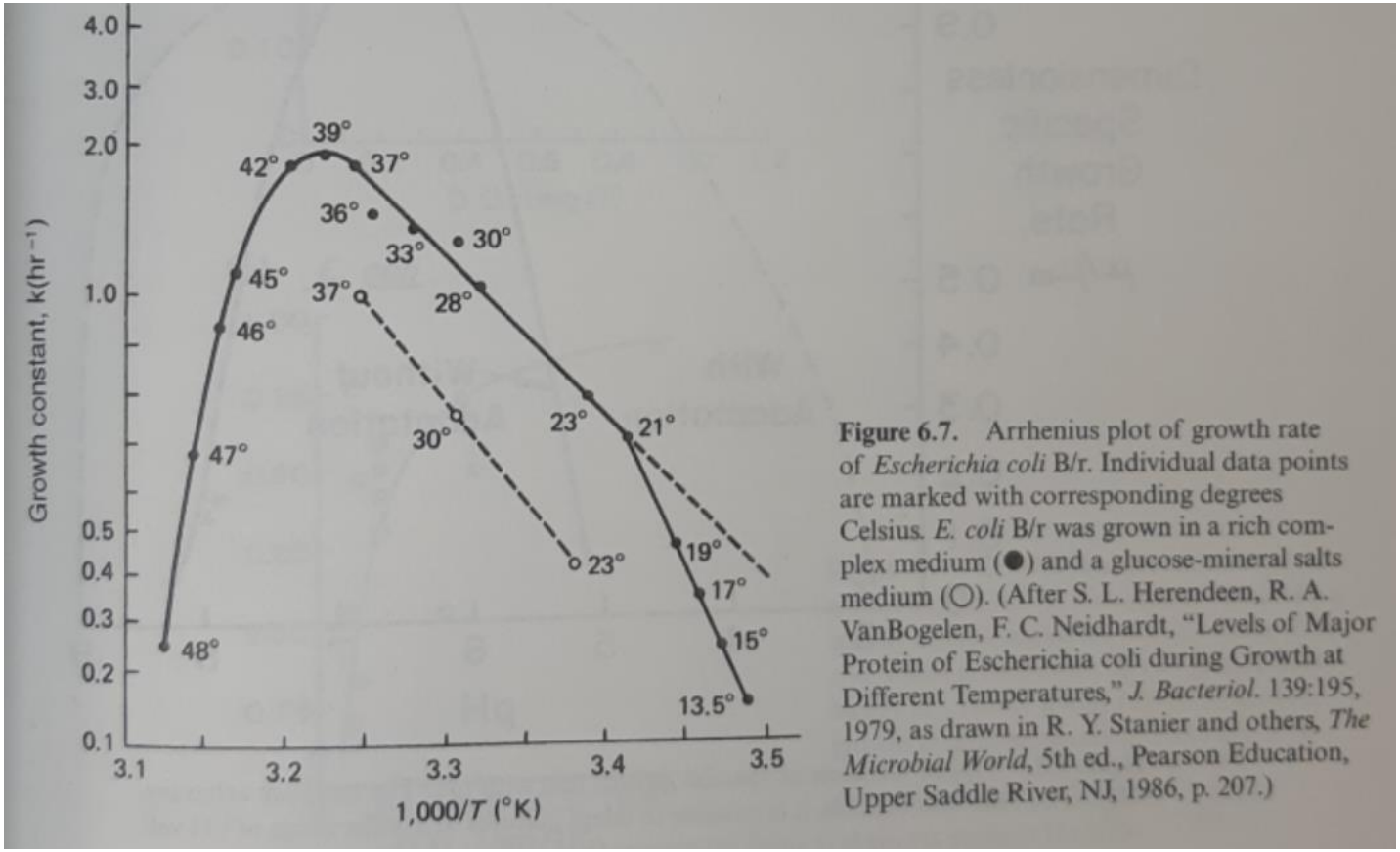



**Figure 6.7.** Arrhenius plot of growth rate of *Escherichia coli* B/r. Individual data points are marked with corresponding degrees Celsius. *E. coli* B/r was grown in a rich complex medium (●) and a glucose-mineral salts medium (○). (After S. L. Herendeen, R. A. VanBogelen, F. C. Neidhardt, "Levels of Major Protein of Escherichia coli during Growth at Different Temperatures," *J. Bacteriol.* 139:195, 1979, as drawn in R. Y. Stanier and others, *The Microbial World*, 5th ed., Pearson Education, Upper Saddle River, NJ, 1986, p. 207.)

# Effect of pH

- pH optimum
  - Bacteria: 3~8
  - Yeast: 3~6
  - Mold: 3~7
  - Plant cell: 5~6
  - Animal cell: 6.5~7.5
- Fig. 6.8
- pH controller

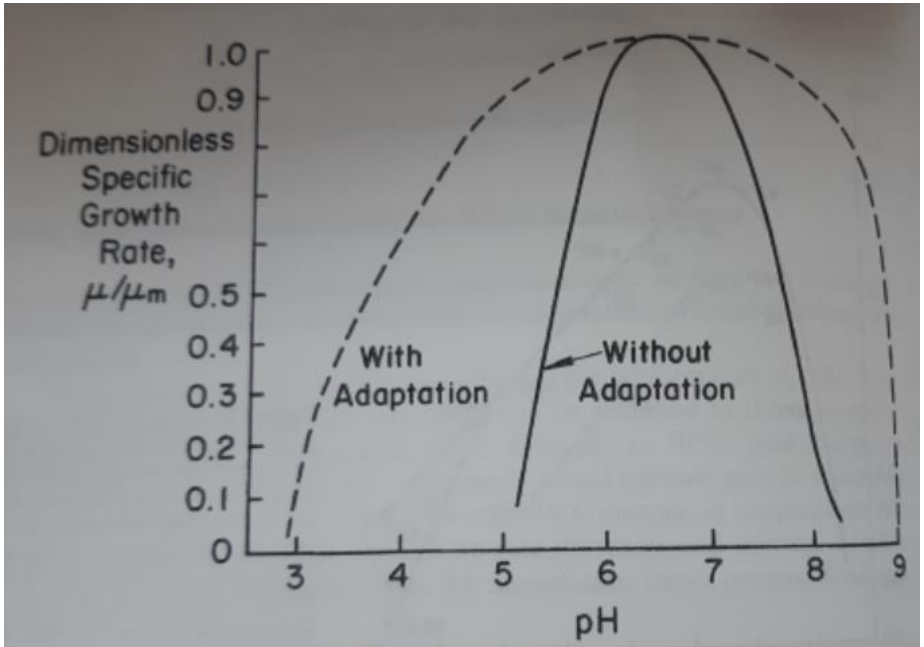
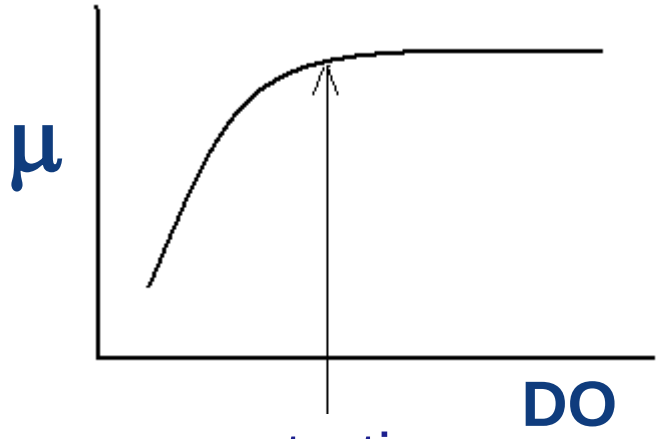



**Figure 6.8.** Typical variation of specific growth rate with pH. The units are arbitrary. With some microbial cultures, it is possible to adapt cultures to a wider range of pH values if pH changes are made in small increments from culture transfer to transfer.

# Effect of DO (dissolved oxygen)

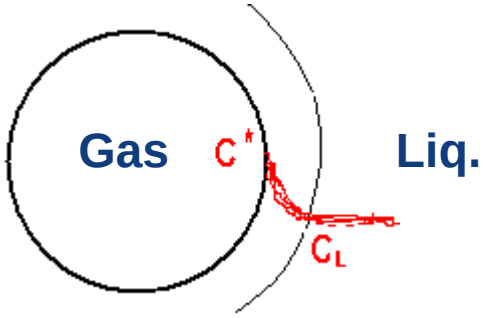
- Fig. 6.9




- Critical oxygen concentration
  - The growth rate is independent of DO above a critical oxygen concentration.
    - Bacteria and yeasts: 5%~10% of the saturated DO
    - Molds: 10~50% of the saturated DO
      (depending on the pellet size of molds)
  - Saturated DO in $H_2O$ at 25$^oC$ and 1 atm ≈ 7ppm
    - Salts and organic material alter the saturation value.

# Oxygen Transfer Rate (OTR)

- OTR from gas to liquid




$$OTR = N_{O2} = k_L\, a\, (C^* - C_L)$$

- $N_{O2}$ : OTR (mg $O_2$ / L / h)
- $k_L$ : oxygen transfer coefficient (cm/h)
- a : gas-liquid interfacial area ($cm^2/cm^3$)
- $k_L$ a : volumetric oxygen transfer coefficient (1/h)
- C* : saturated DO concentration (mg/L)
- $C_L$ : DO concentration in the broth (mg/L)

# Oxygen Uptake Rate (OUR)

- OUR from liquid to cell

  $OUR = q_{O2} X = (\mu X) / Y_{X/O2}$

  - $q_{O2}$ : specific rate of oxygen consumption (mg $O_2$/g cell/h)
  - $Y_{X/O2}$ : oxygen yield coefficient (g cell/g $O_2$)

- When oxygen transfer is the rate-limiting step,


$OTR (\rightarrow) = OUR (\rightarrow)$

$(\mu X) / Y_{X/O2} = k_L a (C^* - C_L)$

$d X / dt = Y_{X/O2} k_L a (C^* - C_L)$