Chapter 6

Introduction

Cell growth is the primary response of viable cells to substrates and nutrients.

Substrates/nutrients + cells → products + more cells

specific growth rate (h^{-1}), $\mu \equiv \frac{1}{X}\frac{dX}{dt}$

X = cell mass concentration (g / L)

t = time (h)

Product formation is a secondary response.

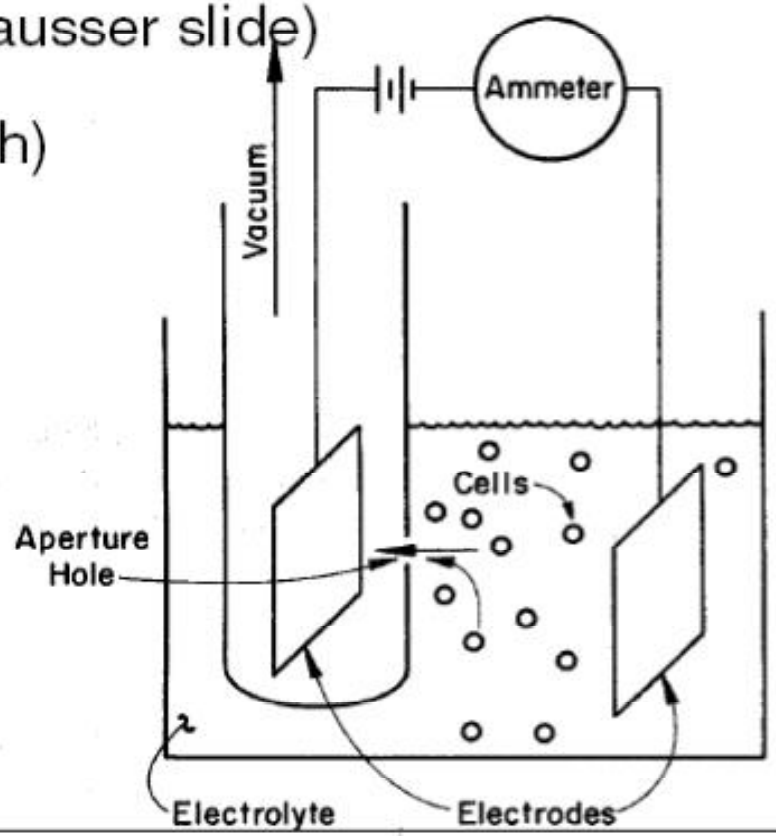
Determining cell concentration

1. Cell number concentration (cells/mL)

a) hemocytometer (Petroff-Hausser slide)

b) viable cell counts (petri dish)

c) electronic particle counter



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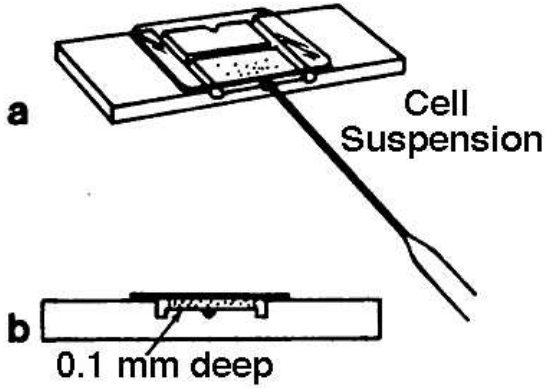
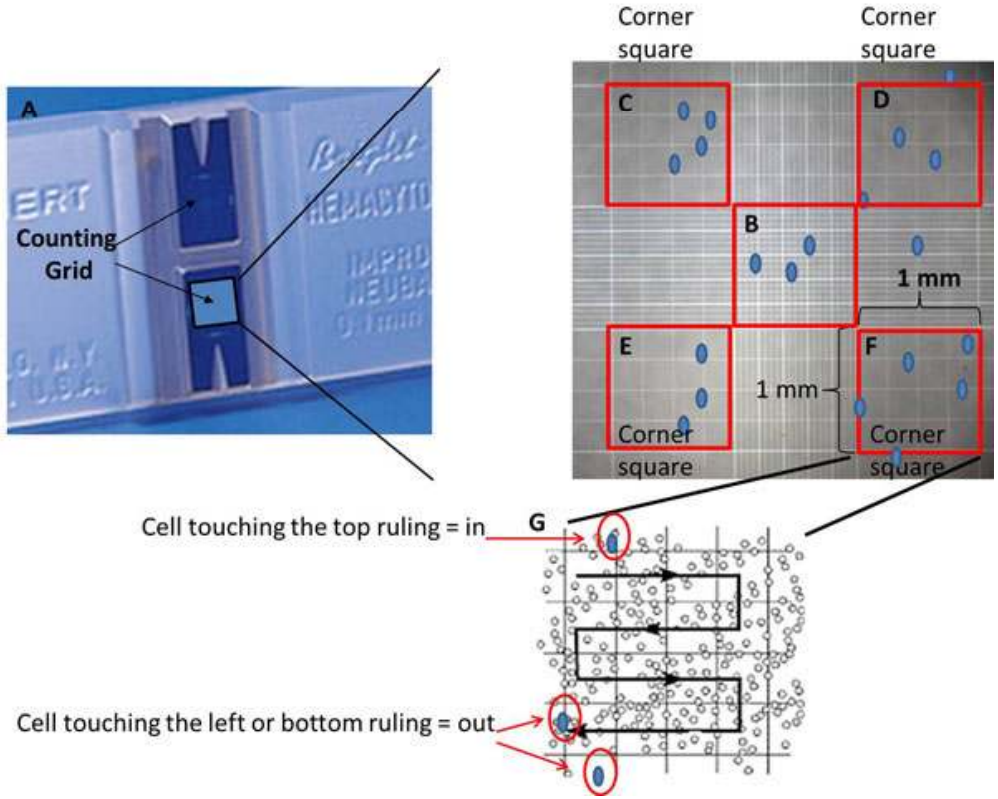
Determining cell concentration

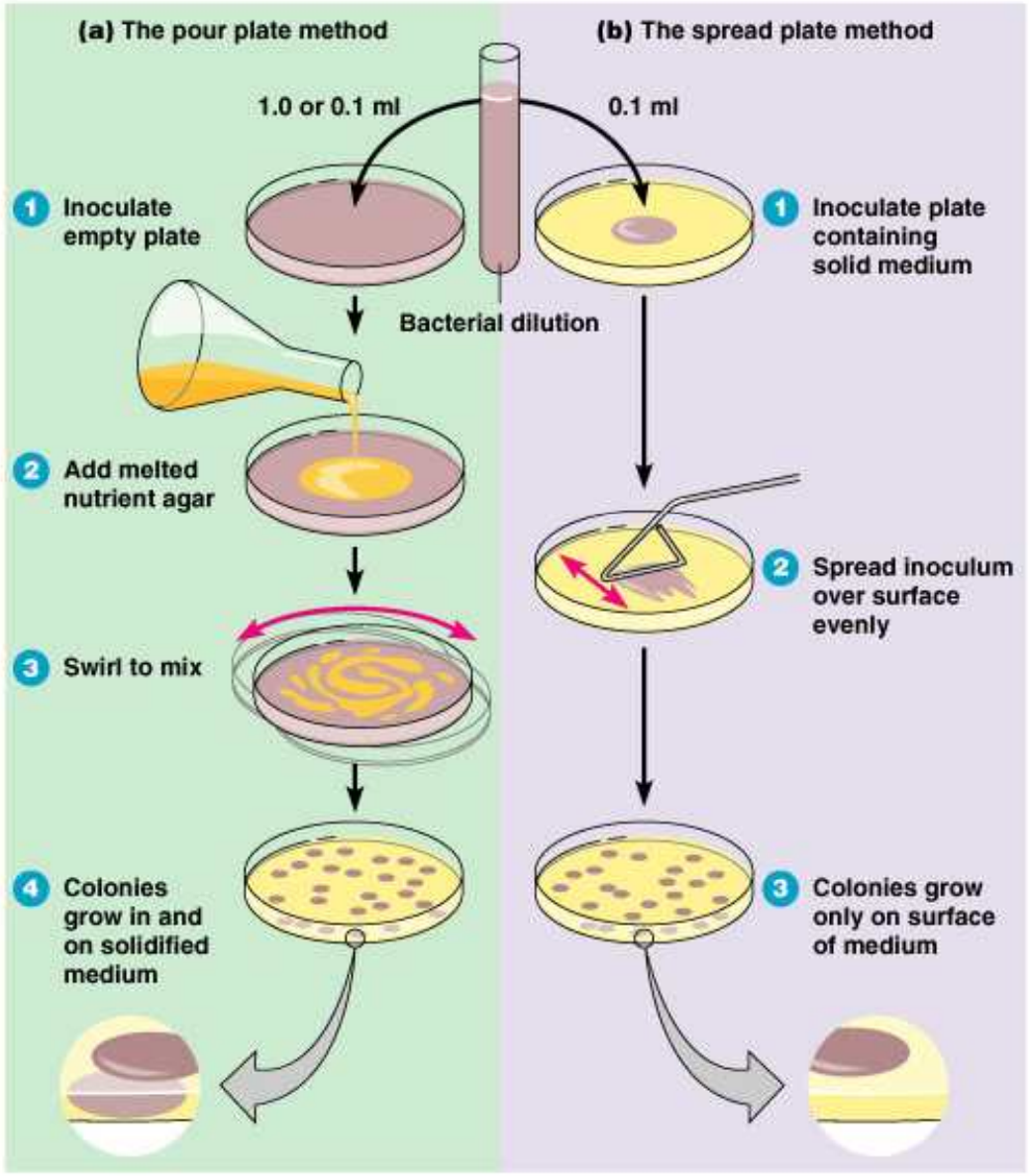
Hemocytometer

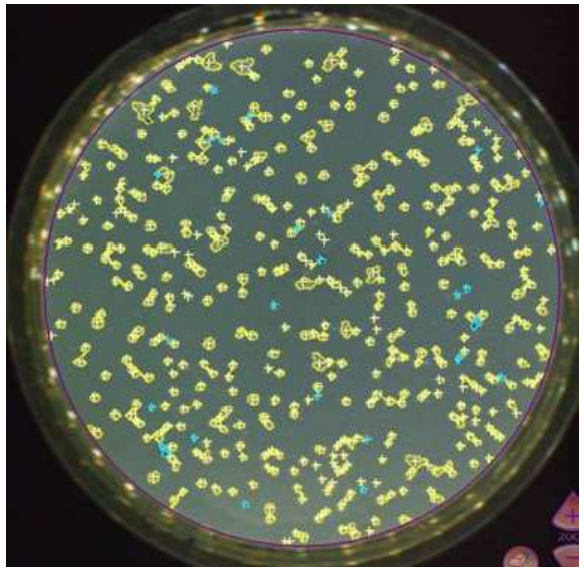




Determining cell concentration

Bacteria





Determining cell concentration (cont.)

2. Cell mass concentration (g/mL)

a) direct methods

→ dry weight (filtration or centrifugation)

→ packed cell volume (centrifugation)

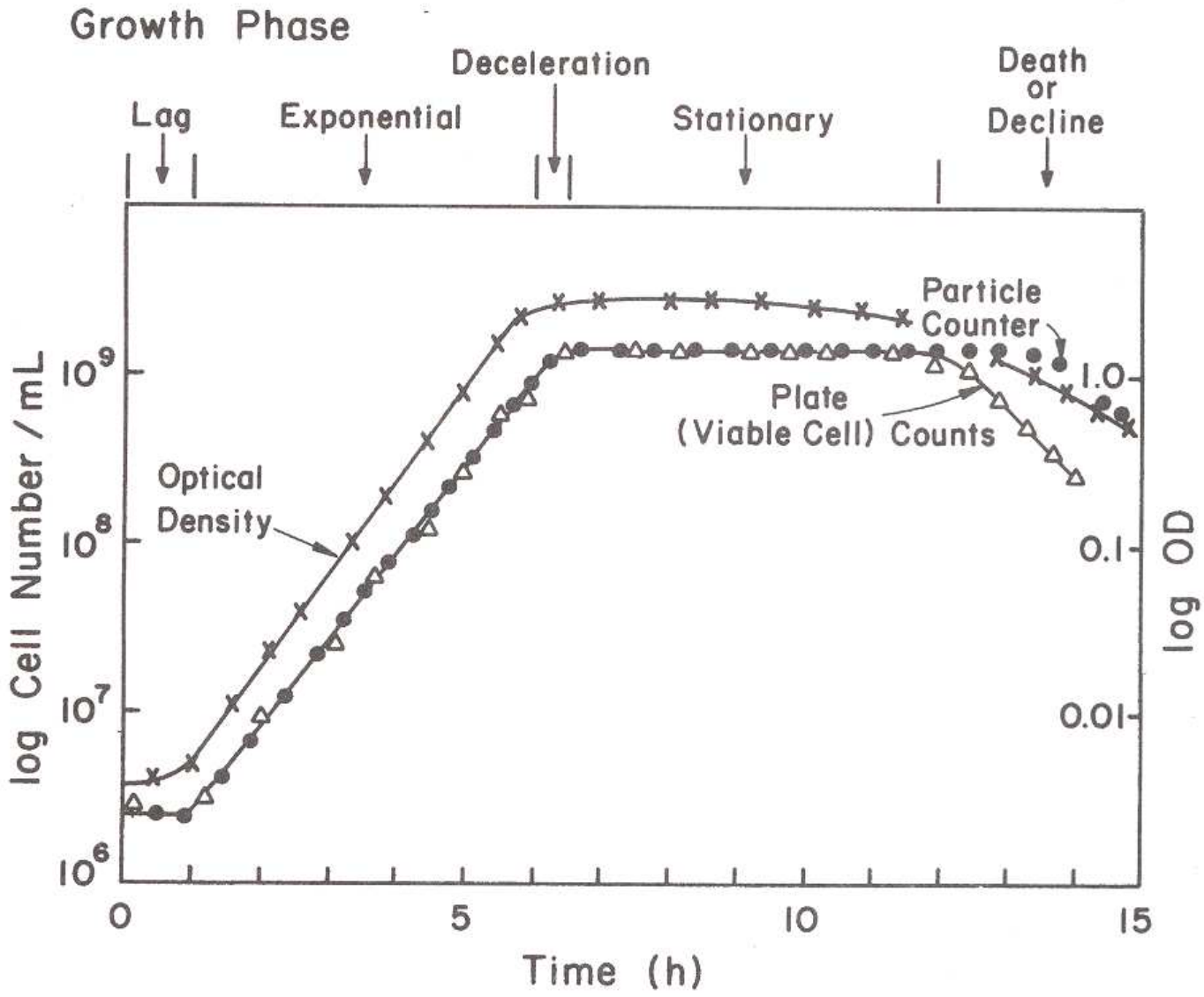
→ optical density (light scattering, 600-700 nm)

b) indirect methods

→ measure biomolecule concentration and correlate to dry cell mass concentration.

(DNA, protein, ATP, NADH, product formation)

Cell growth kinetics in batch culture



Lag phase

→ no increase in cell numbers

→ induction of enzymes to utilize ↓ substrate(s)

→ very important to decrease lag period to ↑ productivity

i. Inoculate with exponential phase cells

ii. Pre-acclimate inoculum in growth media

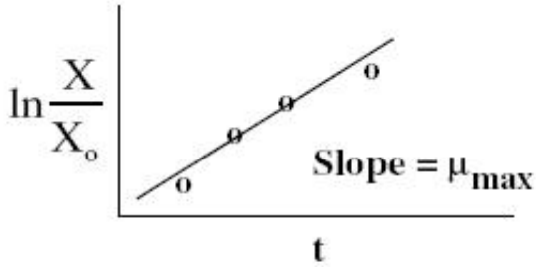
iii. Use high cell inoculum size

Exponential growth phase

1. Nutrient and substrate concentrations are high
2. Growth rate is independent of nutrient and substrate conc.
3. Cell number and mass concentrations increase exponentially

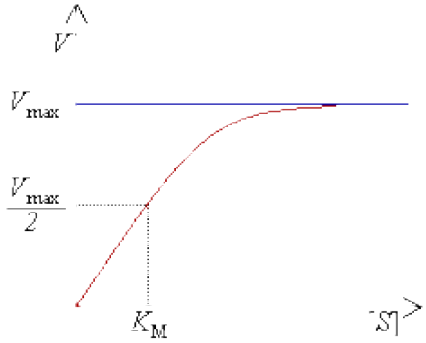
$$\frac{dX}{dt} = \mu_{max} X,\ X = X_o \text{ at } t = 0$$

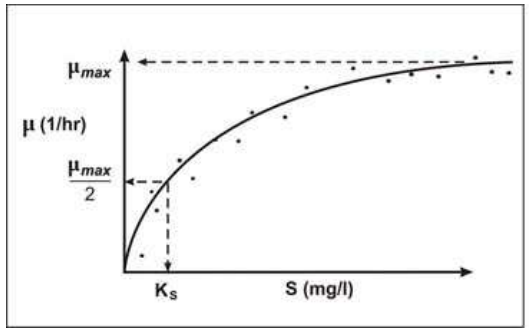
$$X = X_o e^{\mu_{max} t} \text{ or } \ln\frac{X}{X_o} = \mu_{max} t$$



doubling time of cells (t_d), $\frac{X}{X_o} = 2 \Rightarrow \ln(2) = \mu_{max} t_d$

$$t_d = \frac{\ln 2}{\mu_{max}} \quad \text{or} \quad \mu_{max} = \frac{\ln 2}{t_d}$$

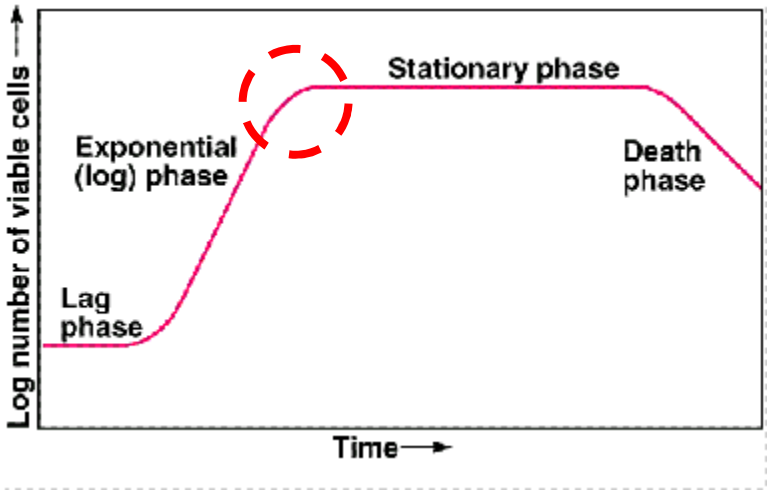




Deceleration phase

→ depletion of one or more nutrients

→ accumulation of toxic byproducts of growth



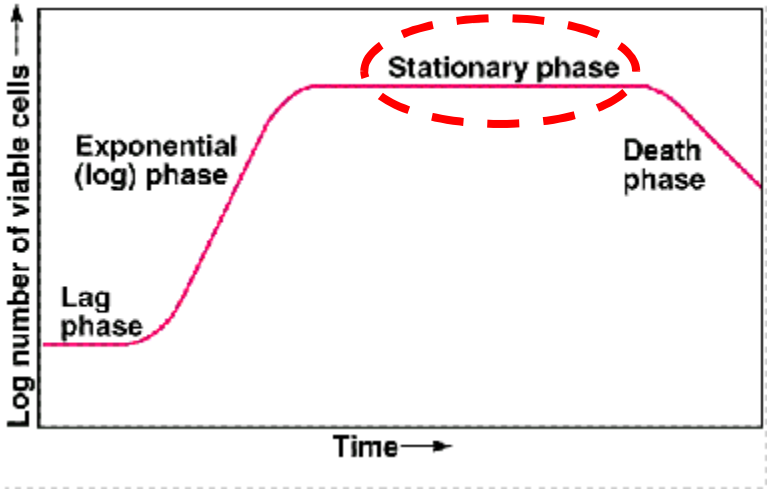
Stationary phase

→ no net growth of cell numbers or cell mass (no cell division)

→ cell growth rate = cell death rate

→ *secondary metabolites* (products) produced

→ removal of inhibitory compounds will result in further growth if additional substrate is provided



Death phase

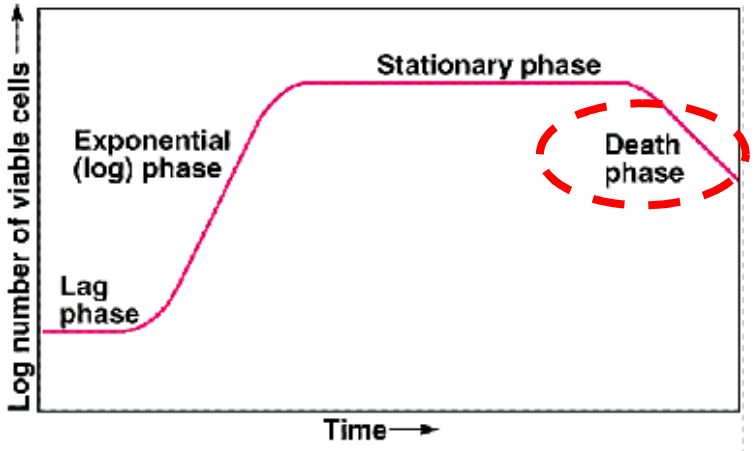
1. Cell lysis (spillage) may occur
2. Rate of cell decline is first-order

$$\frac{dX}{dt} = -k_d' X, \Rightarrow X = X_s \text{ at } t = 0$$

k_d; specific cell death rate

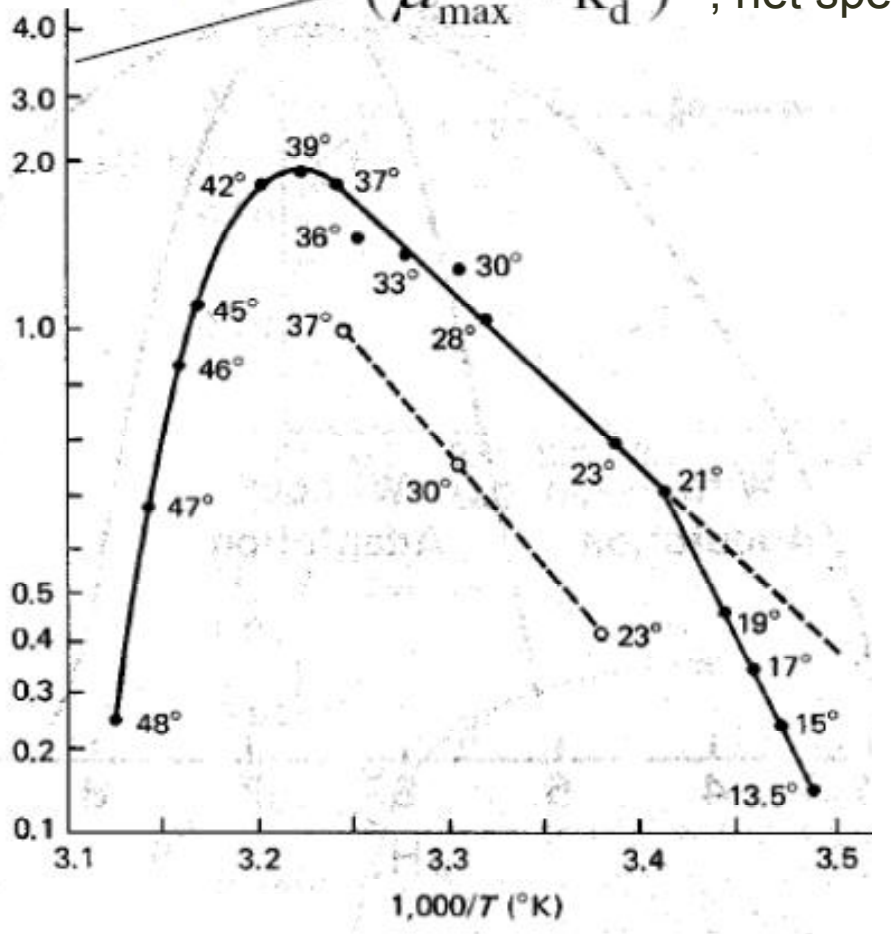
$$X = X_s e^{-k_d' t} \quad \text{or} \quad \ln\frac{X}{X_s} = -k_d' t$$

3. Growth can be re-established by transferring to fresh media



Effects of temperature on cell growth

$(\mu_{max} - k_d)$; net specific cell growth rate



Similar to enzyme reaction rate dependence on T

pH effects

→ acceptable pH is ± 1 to 2 pH units

→ pH range varies by organism

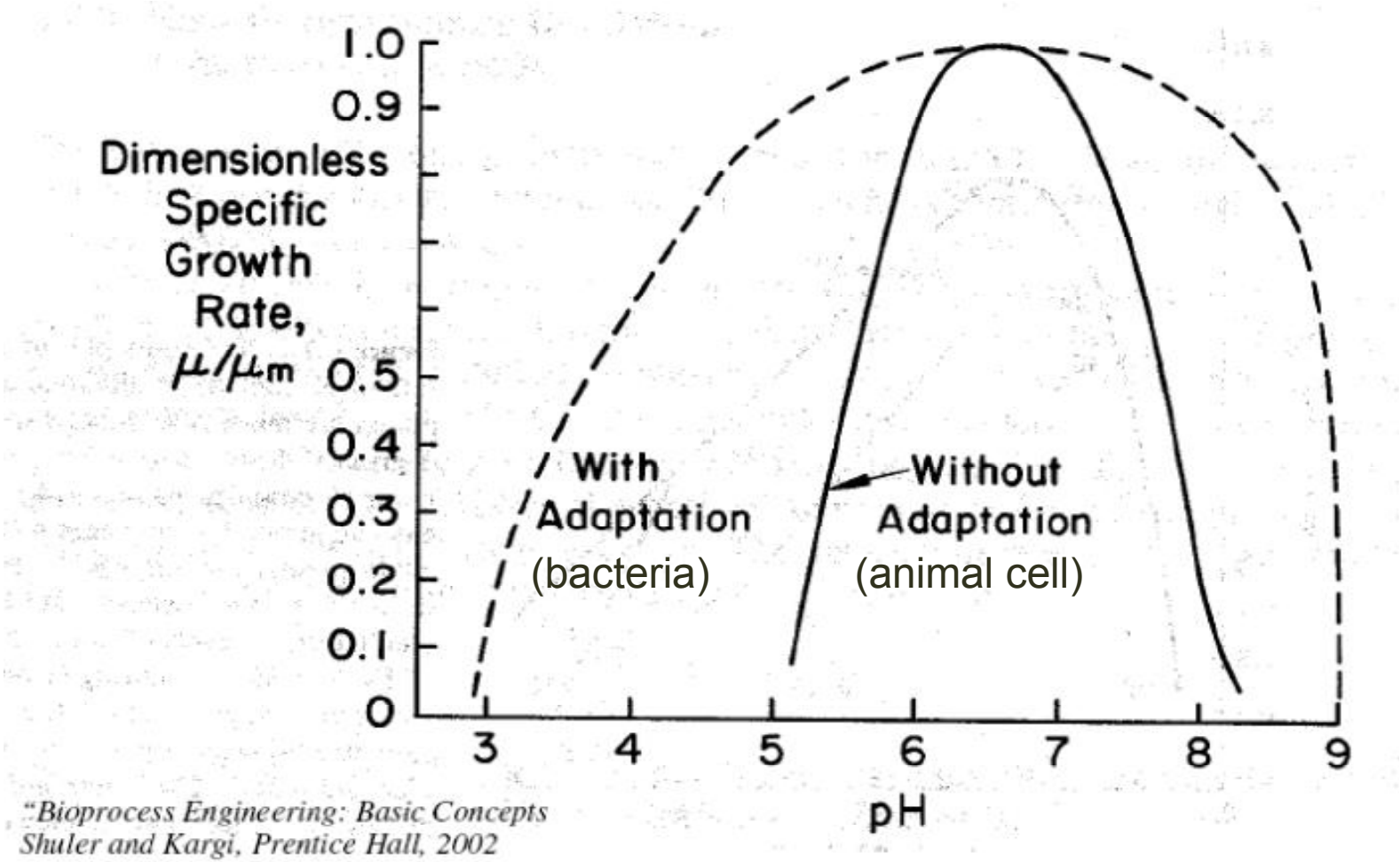
bacteria (most)	pH = 3 to 8
yeast	pH = 3 to 6
plants	pH = 5 to 6
animals	pH = 6.5 to 7.5

→ microorganism have the ability to control pH inside the cell, but this requires maintainance energy

→ pH can change due to

- utilization of substrates; NH_4^+ releases H^+, NO_3^- consumes H^+
- production of organic acids, amino acids, CO_2, bases

pH effects (cont.)



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Effects of dissolved O_2 (DO)

→ O_2 may be a limiting substrate for aerobic fermentation, since O_2 is sparingly soluble in water

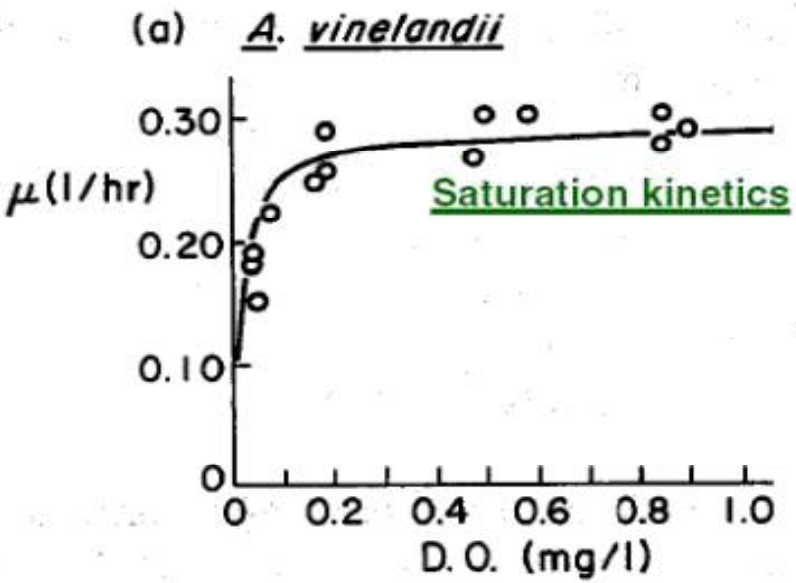
→ critical O_2 concentration

5 to 10% of saturation (≈ 7 mg O_2/L) for bacteria/yeast

→ growth exhibits saturation kinetics with respect to O_2 concentration (see next page)

Effects of dissolved O_2 (cont.)

Obligate aerobic cells



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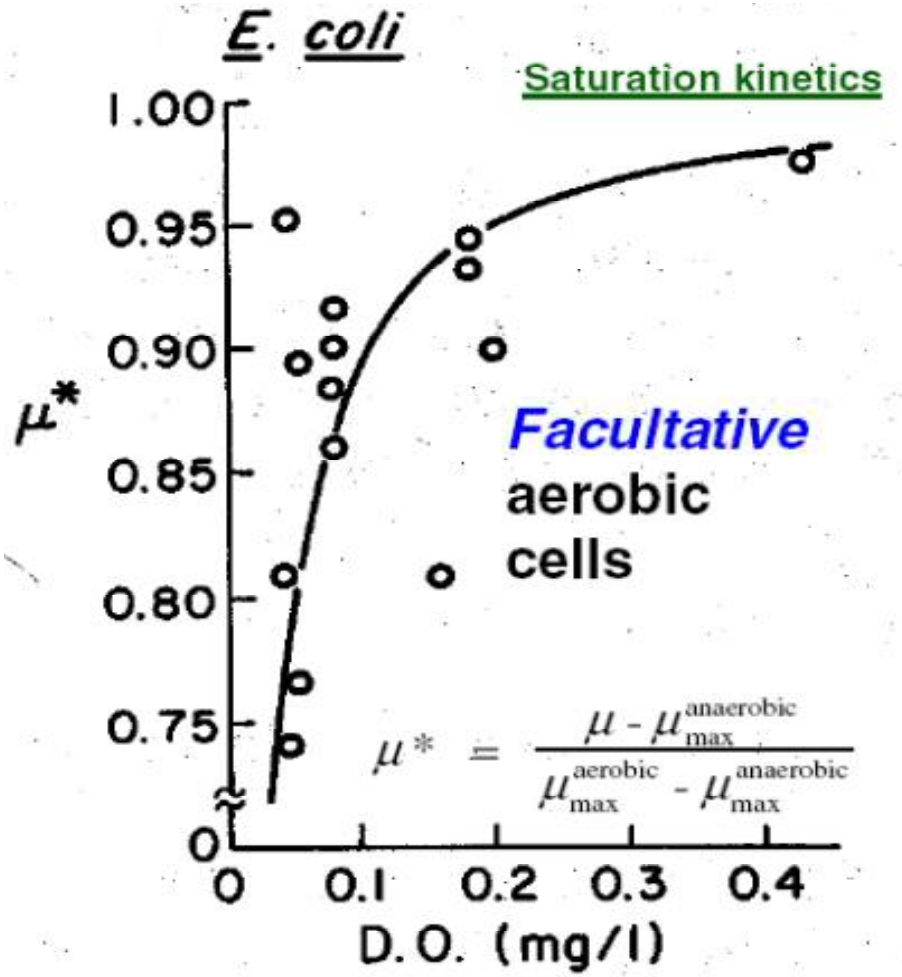
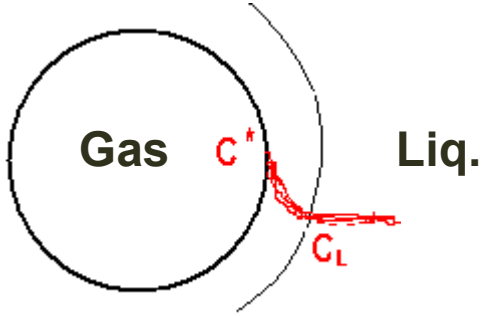


Oxygen transfer rate (OTR)

- **Oxygen transfer from gas to liquid**



- **OTR = k_L a (C^* - C_L)**
  - OTR [mg O_2 / L / h]
  - k_L : oxygen transfer coefficient (cm/h)
  - a : gas-liquid interfacial area per unit vol. (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**

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

  - OUR [mg O_2 / L / h]
  - 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,**

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

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

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

$$Y_{X/O2} \cdot OTR = \text{cell growth rate}$$

Other effects on cell growth

→ dissolved CO_2 (DCO_2); to high or low DCO_2 is toxic

→ ionic strength (I); too high dissolved salts is inhibitory to membrane function (membrane transport of nutrients, osmotic pressure)

$$I = 1/2 \ \Sigma \ C_i Z_i^2$$

C_i = molar concentration of ion i

Z_i = ion charge

→ maximum non-inhibitory concentrations of substrates, products

glucose (100 g/L), ethanol (10 g/L), NH_4^+ (5 g/L), ..