Bioreactor and Microbial Growth

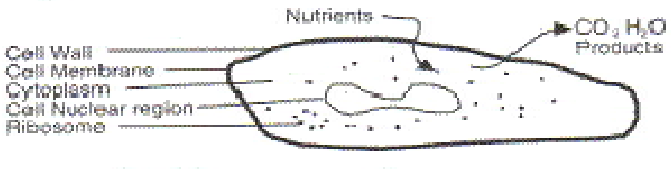
Bioreactor

- A reactor that sustains and supports life for cells and tissue cultures.
- Cellular reactions include:
  - Transformation of chemical energy
  - Construction, breakdown, and digestion of cellular components
- Enzymatic reactions are involved in the growth of microorganisms (microbial growth)
- Monod equation:
  - Describes the growth law for a number of bacteria
  - Similar to Michaelis-Menten equation

Bioconversion Advantages

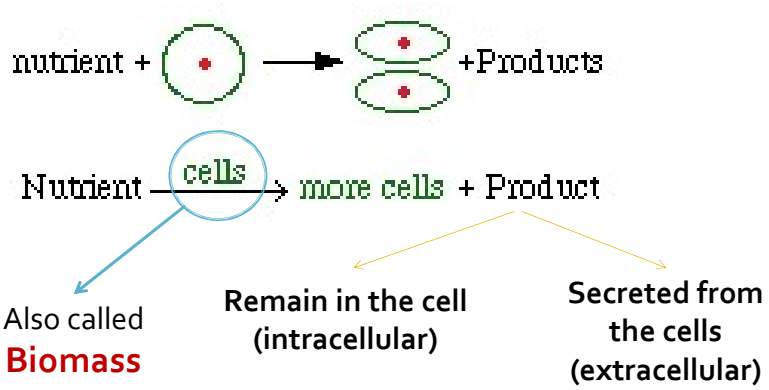
- Mild reaction conditions
- High yield
- Organisms contain several enzymes that can catalyze successive steps in a reaction
- Enzymes act as stereospecific catalyst

Schematic of Cell



- Cell wall: protect the cell from external influence
- Cell membrane: provides for selective transport of material into and out of the cell
- Cytoplasm: contains the ribosomes that contain ribonucleic acid (RNA)
- Nuclear region: contains deoxyribonucleic acid (DNA) which provides genetic information for the production of proteins and other cellular substances and structures.

Biosynthesis

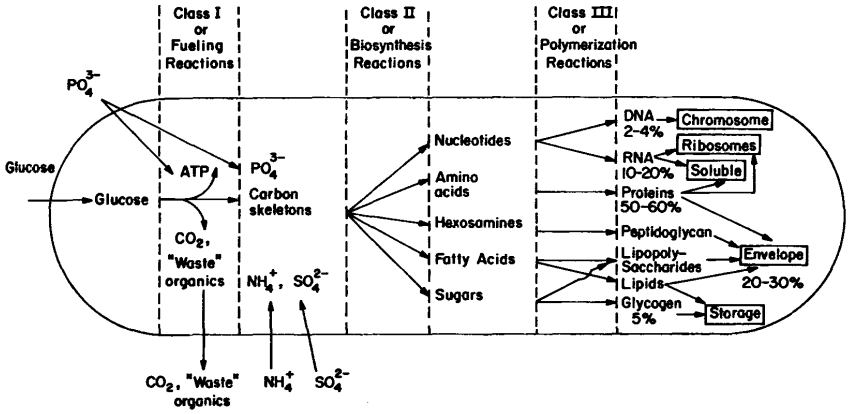


Reactions in the Cell

- All take place simultaneously and are classified as either:
  I. Nutrient degrading (fueling reactions)
  II. Synthesis of small molecules (amino acids)
  III. Synthesis of large molecules (RNA, DNA)

- **Autocatalytic reaction:** the rate of growth is proportional to cell concentration

Reactions in the Cell

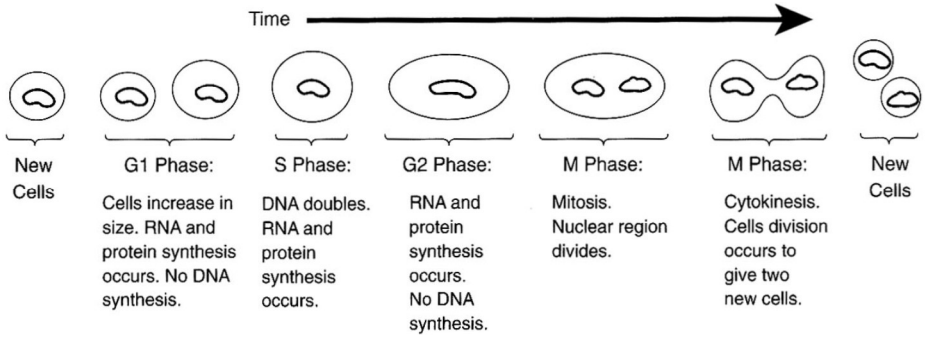


Reactions in the Cell

- ATP also transfers the energy it releases when it loses phosphonate group to form adenosine diphosphate (ADP)

$$ATP + H_2O \rightarrow ADT + P + H_2O + Energy$$

Phases of Cell Division



$$[\text{Cells}] + \left[\begin{matrix}\text{Carbon}\\ \text{source}\end{matrix}\right] + \left[\begin{matrix}\text{Nitrogen}\\ \text{source}\end{matrix}\right] + \left[\begin{matrix}\text{Oxygen}\\ \text{source}\end{matrix}\right] + \left[\begin{matrix}\text{Phosphate}\\ \text{source}\end{matrix}\right] + \cdots$$

$$[CO_2] + [H_2O] + [\text{Products}] + \left[\begin{matrix}\text{More}\\ \text{cells}\end{matrix}\right]$$

Culture media conditions (pH, temperature, etc.)

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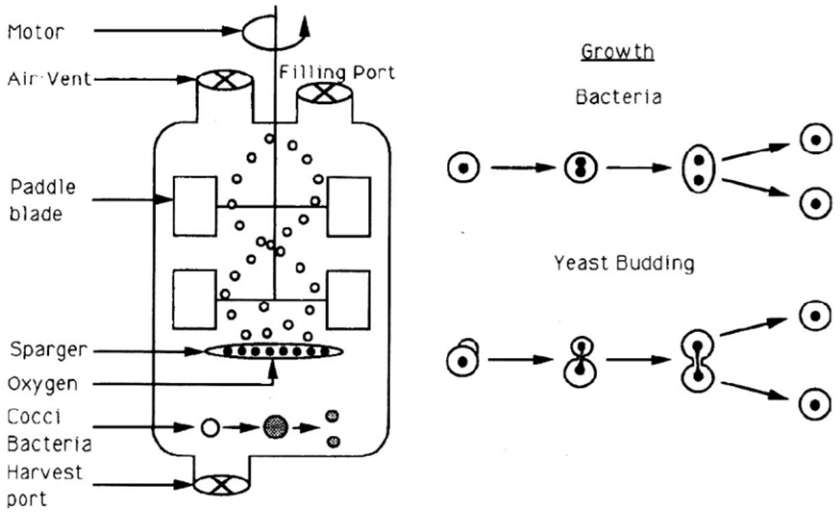
Batch Bioreactor



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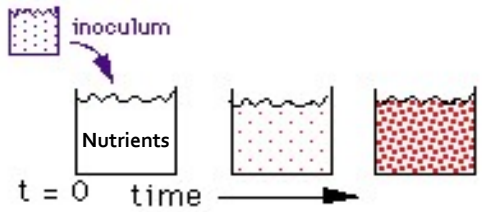
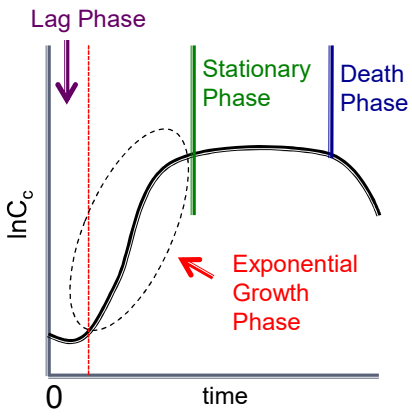
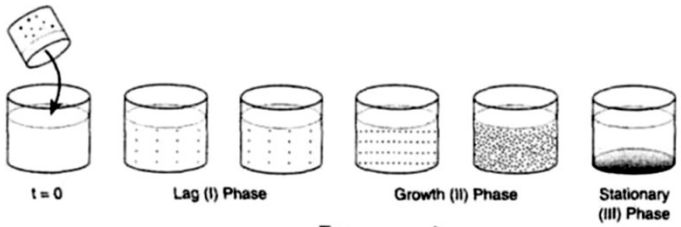
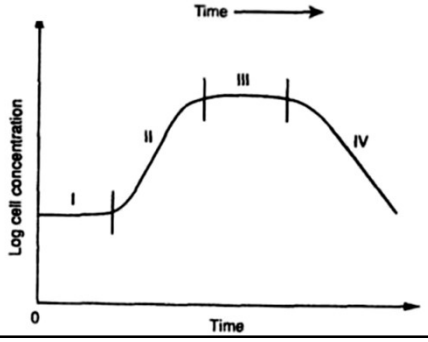
Phases of Bacteria Cell Growth





Phases of Bacteria Cell Growth





Rate Laws

- The **rate of microbial growth** is characterized by the net *specific growth rate*, μ_{net} (1/time), *defined* as

$$\mu_{\text{net}} \equiv \frac{1}{X}\frac{dX}{dt}$$

X : cell mass concentration

t: time

Rate Laws

$$\mu_{\text{net}} \equiv \frac{1}{X}\frac{dX}{dt}$$

$$\mu_{\text{net}} = \mu_g - k_d$$

μ_g : Gross specific growth rate

k_d : Rate of loss of cell mass due to cell death or endogenous metabolism

Rate Laws

$$\mu_R \equiv \frac{1}{N}\frac{dN}{dt} \equiv \text{net specific replication rate}$$

N : Cell number concentration (cell number /L)

$$\mu_R = \mu'_R - k_d$$

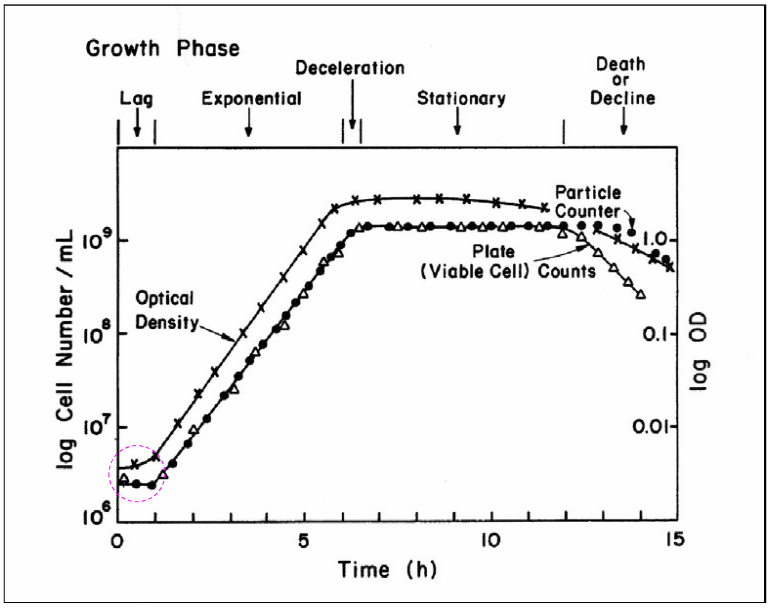
μ'_R : Gross specific replication rate (1/time)

k_d : Rate of cell death (1/time)

When cell death is unimportant, $\mu_R = \mu'_R$

Growth Kinetics

- In batch culture:
  - lag phase
  - logarithmic or exponential growth phase
  - deceleration phase
  - stationary phase
  - death phase



Typical growth curve for a bacterial population

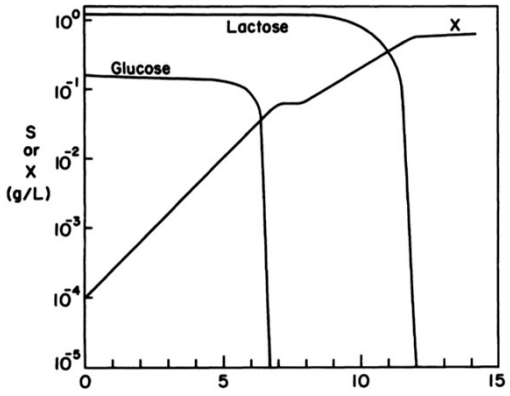
Batch Growth Kinetics

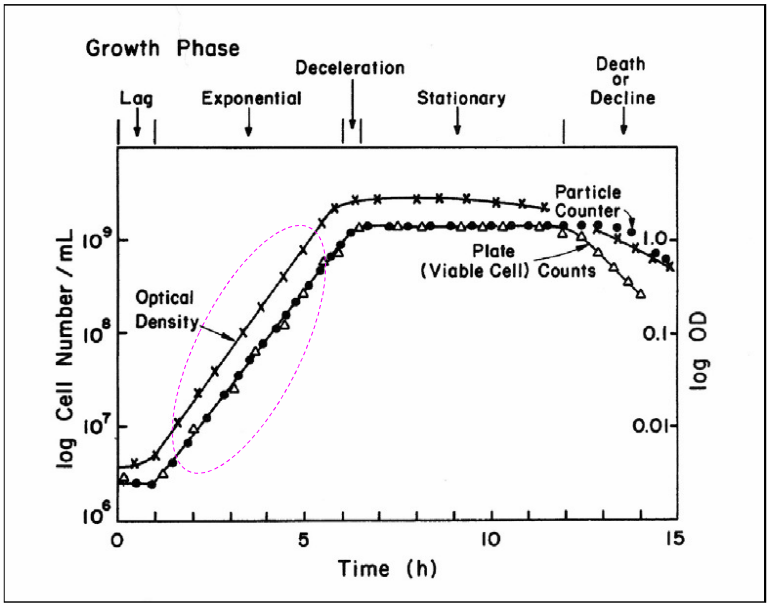
Lag phase

- A period of adaptation for the cells to their new environment
  - New enzymes are synthesized.
  - A slight increase in cell mass and volume, but no increase in cell number
  - Prolonged by low inoculum volume, poor inoculum condition (high % of dead cells), age of inoculum, nutrient-poor medium
  - Multiple lag phases (*diauxic growth*): medium contains more than one carbon source

Diauxic growth curve for *E. coli* on Glucose and Lactose

- At 2 h after inoculation, cells are growing rapidly, glucose is being consumed, and lactose is not being utilized.
- At 7 h, cell mass accumulation is zero. All the glucose has been consumed.
- At 10 h the culture is growing and lactose is being consumed, but the rate of growth (cell mass accumulation) is less than at 2 h.





Typical growth curve for a bacterial population

Batch Growth Kinetics

Exponential growth phase

- In this phase, the cells have adjusted to their new environment and multiply rapidly (exponentially)
  - ***Balanced growth*** – all components of a cell grow at the same rate.
  - Growth rate is independent of nutrient concentration, as nutrients are in excess.

Batch Growth Kinetics

Exponential growth phase

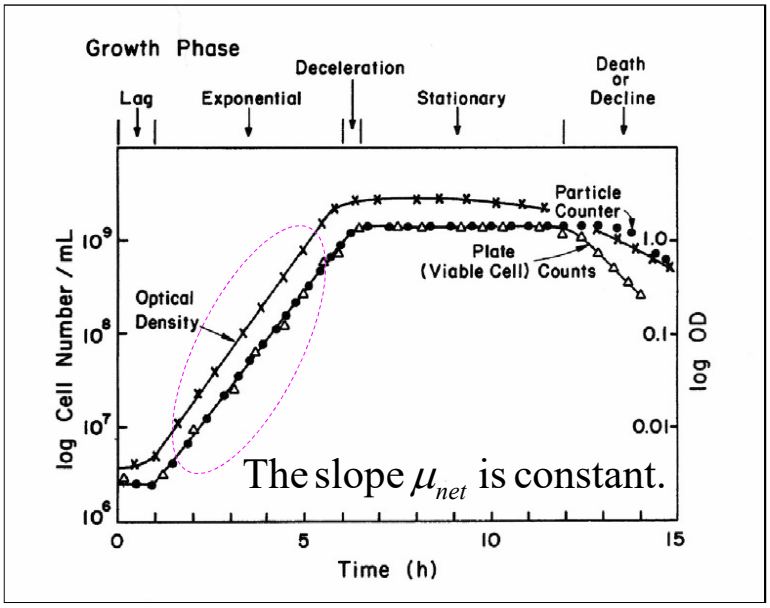
The balance of cell mass in a batch culture gives:

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

Integration of the above equation yields:

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

X and X_0 are cell concentrations at time t and $t = 0$



Typical growth curve for a bacterial population

Batch Growth Kinetics

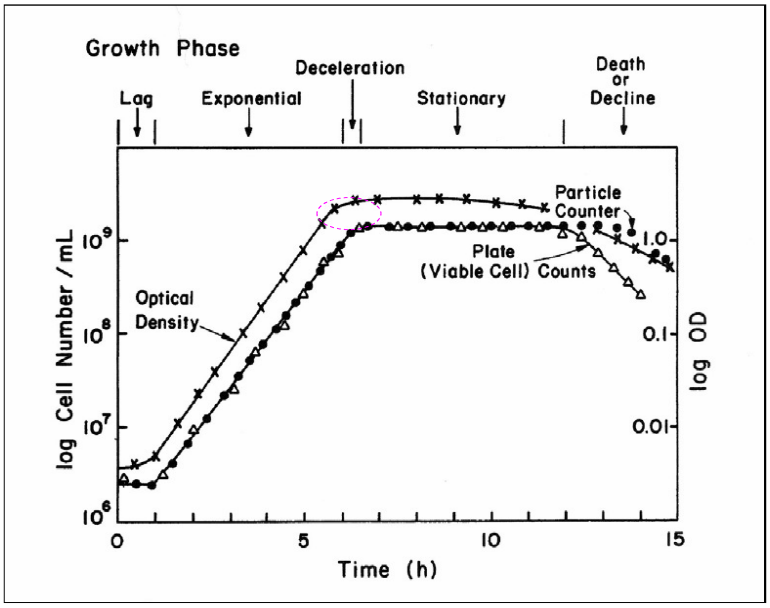
Exponential growth phase

$$\mu_{net} = \mu_R = \mu_m$$

μ_m is the maximum specific growth rate (1/time)

- ***Doubling time of cell mass (τ_d):*** the time required to double the microbial mass:

$$\tau_d = \frac{\ln X / X_0}{\mu_{net}} = \frac{\ln 2}{\mu_{net}} = \frac{0.693}{\mu_{net}}$$

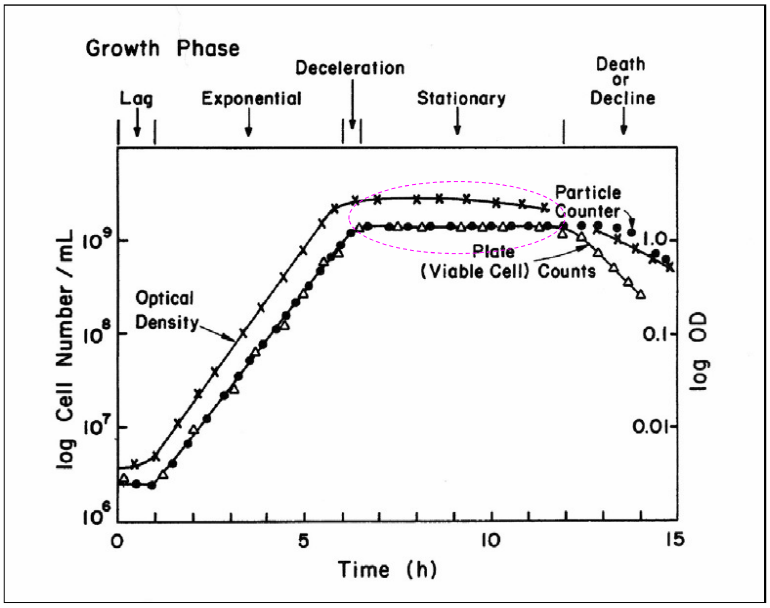


Typical growth curve for a bacterial population

Batch Growth Kinetics

Deceleration growth phase

- Very short phase, during which growth decelerates due to either:
  - Depletion of one or more essential nutrients
  - The accumulation of toxic by-products of growth (e.g. Ethanol in yeast fermentations)
  - Period of unbalanced growth: Cells undergo internal restructuring to increase their chances of survival



Typical growth curve for a bacterial population

Batch Growth Kinetics

Stationary Phase:

- With the exhaustion of nutrients (S ≈ 0) and build-up of waste and secondary metabolic products
  - Net growth rate = 0 (no cell division).
  - growth rate = death rate
  - Cells are still metabolically active and produce secondary metabolites.

- ***Primary metabolites*** are growth-related: ethanol by *S. cerevisae.*
- ***Secondary metabolites*** are non-growth-related: antibiotics.

Batch Growth Kinetics

Stationary phase

- One or more of the following phenomena may take place:
  1. Total cell mass concentration may stay constant, but the number of viable cells may decrease.
  2. Cell lysis may occur and viable cell mass may drop. A second growth phase may occur and cells may grow on lysis products of lysed cells (cryptic growth).
  3. Cells may not be growing but may have active metabolism to produce secondary metabolites.

Batch Growth Kinetics

Stationary phase

- ***Endogenous metabolism*** occurs by catabolizing cellular reserves for new building blocks and energy-producing monomer (maintenance energy).
- The rate describing the conversion of cell mass into maintenance energy or the loss of cell mass due to cell lysis:

$$\frac{dX}{dt} = -k_d X$$

k_d is the rate constant for endogenous metabolism

Batch Growth Kinetics

Death Phase:

- The living organism population decreases with time, due to a lack of nutrients and accumulation of toxic metabolic by-products.
- The rate of death usually follows first order kinetics:

$$\frac{dN}{dt} = -k'_d N \qquad or \qquad N = N_S e^{-k'_d t}$$

N_S is the concentration of cells at the end of the stationary phase

k'_d is the first-order death rate constant

Batch Growth Kinetics

- Yield coefficients: defined based on the amount of consumption of another material.

$$\text{Growth yield}: \quad Y_{X/S} \equiv -\frac{\Delta X}{\Delta S}$$

$$\text{Product yield}: \quad Y_{P/S} \equiv -\frac{\Delta P}{\Delta S}$$

$$\text{Growth yield based on consumption of oxygen}:$$

$$Y_{X/O_2} \equiv -\frac{\Delta X}{\Delta O_2}$$

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

Batch Growth Kinetics

- Yield coefficients: defined based on the amount of consumption of another material.
- For most bacteria and yeast:

$$Y_{X/S} = 0.4 - 0.6 \text{ g/g glucose}$$

$$Y_{X/O_2} = 0.9 - 1.4 \text{ g/g } O_2$$

- At the end of the batch growth period, the measured yields are apparent as endogenous metabolism occurring, $K_d > 0$, which changes the metabolic pathways of the substrate.

$$Y_{X/S}^{M} > Y_{X/S}^{App}$$

How Environmental Conditions Affect Growth Kinetics

- Patterns of microbial growth and product formation are influenced by environmental conditions such as
  - Temperature
  - pH
  - Dissolved-oxygen concentration

How Environmental Conditions Affect Growth Kinetics

Temperature

- An important factor affecting the performance of cells
- According to their temperature optima, organisms can be classified in three groups:
  1. Psychrophiles (T_{opt} < 20°C)
  2. Mesophiles (20° < T_{opt} < 50°C)
  3. Thermophiles (T_{opt} > 50°C)
- As the temperature is increased toward optimal growth temperature, the growth rate approximately doubles for every 10°C increase in temperature.
- Above the optimal temperature range, the growth rate decreases and thermal death may occur.

How Environmental Conditions Affect Growth Kinetics

Temperature

- The net specific replication rate can be expressed by the following equation for temperature above optimal level:

$$\frac{dN}{dt} = (\mu'_R - k'_d)N$$

$$\mu'_R = Ae^{-E_a/RT}, \quad k'_d = A'e^{-E_d/RT}$$

- At high temperatures, the thermal death rate exceeds the growth rate, which causes a net decrease in the concentration of viable cells.

How Environmental Conditions Affect Growth Kinetics

Temperature

- Temperature also affects:
  - product formation
    - the temperature optimum for growth and product formation may be different
  - yield coefficient
  - rate-limiting step in a fermentation process
    - At high temperatures, the rate of bioreaction might become higher than the diffusion rate, and diffusion would then become the rate-limiting step (for example, in an immobilized cell system)

How Environmental Conditions Affect Growth Kinetics

Hydrogen-ion concentration (pH)

- pH affects the activity of enzymes and therefore the microbial growth rate
- The optimal pH for growth may be different from that for product formation.
- Different organisms have different pH optima:
  - for many bacteria: pH = 3 to 8
  - for yeast: pH = 3 to 6
  - for molds: pH = 3 to 7
  - for plant cells: pH = 5 to 6
  - for animal cells: pH = 6.5 to 7.5

How Environmental Conditions Affect Growth Kinetics

Hydrogen-ion concentration (pH)

- Many organisms have mechanisms to maintain intracellular pH at a relatively constant level in the presence of fluctuations in environmental pH.
- When pH differs from the optimal value, the maintenance-energy requirements increase.
- One consequence of different pH optima is that the pH of the medium can be used to select one organism over another.

How Environmental Conditions Affect Growth Kinetics

Dissolved oxygen (DO)

- Aerobic fermentation requires oxygen
- Oxygen gas is sparingly soluble in water
- If DO is below a *critical oxygen concentration*, growth or respiration approaches a first-order rate dependence on the DO concentration.
- Above a *critical oxygen concentration*, growth rate becomes independent of DO concentration.
- Oxygen is a growth-rate-limiting factor when the DO level is below the *critical DO concentration*.

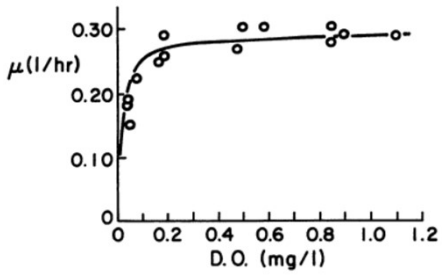
How Environmental Conditions Affect Growth Kinetics

Dissolved oxygen (DO)

- *Critical oxygen concentration for*
  - bacteria and yeast: 5%-10% of the saturated DO
  - mold: 10%-50% of the saturated DO
- The saturated DO in aqueous solution is 7 ppm at 25oC and 1 atm.
- Factors affecting the saturation value
  - the presence of dissolved salts and organics can alter the saturation value
  - increasingly high temperatures decrease the saturation value

Growth-rate dependence on DO

AZOTOBACTER VINELANDII
(STRICTLY AEROBIC ORGANISM)



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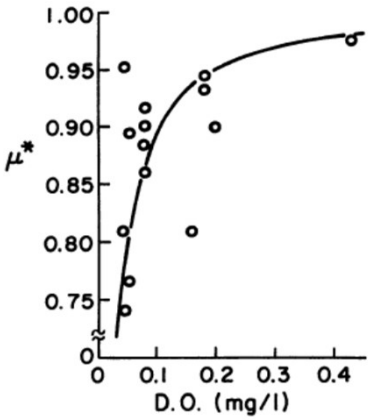


Environmental Conditions

Dissolved oxygen (DO)

- Oxygen is usually introduced to fermentation broth by sparging air through the broth.
- Oxygen transfer from gas bubbles to cells is usually limited by oxygen transfer through the liquid film surrounding the gas bubbles.
- The **rate of oxygen transfer** from the gas to liquid phase (OTR) is given by

$$OTR = N_{O_2} = k_L a\,(C^* - C_L)$$

k_L : the oxygen transfer coefficient (cm/h)
a : the gas-liquid interfacial area (cm^2/cm^3)
$k_L a$: the volumetric oxygen transfer coefficient (h^{-1})
C^* : saturated DO concentration (mg/l);
C_L : the actual DO concentration (mg/l);
N_{O_2} : the rate of oxygen transfer (mg O_2/l-h)

Environmental Conditions

Dissolved oxygen (DO)

- **Rate of oxygen uptake** (OUR) is oxygen consumption rate by microbes. If the maintenance requirement of O2 is negligible compared to growth, then

$$OUR = q_{o_2} X = \frac{\mu_g X}{Y_{X/O_2}} \ (\text{mg } O_2/\text{h})$$

$$q_{o_2} \text{ is the specific rate of } O_2 \text{ consumption } (\text{mg } O_2/\text{g cells - h})$$

- When oxygen transfer is the rate-limiting step, at steady state, the rate of oxygen consumption is equal to the rate of oxygen transfer.

$$\frac{\mu_g X}{Y_{X/O_2}} = k_L a(C^* - C_L) \quad \text{OR} \quad \frac{dX}{dt} = Y_{X/O_2} k_L a(C^* - C_L)$$

Sufficient oxygen supply: OTR ≥ OUR

Environmental Conditions

Dissolved oxygen (DO)

Question:

- Oxygen is to be supplied for yeast production. If oxygen uptake rate (OUR) is 15 g/l medium-h for a required yeast growth, and the oxygen transfer rate (OTR) is 10 g/l medium-h. Is such oxygen transfer rate sufficient to maintain the required yeast growth? If the required growth has to be maintained, how to improve the oxygen transfer rate?

Answers:

OUR = 15g/l medium-h > OTR = 10 g/l medium-h

➔ insufficient oxygen supply rate

➔ Oxygen transfer rate is limiting

Increase ($k_L a$) so that $\frac{\mu_g X}{Y_{X/O_2}} = k_L a(C^* - C_L)$

Environmental Conditions

Ionic Strength (I)

- Ionic strength of a medium (I) affects
  - the transport of certain nutrients in and out of cells
  - the metabolic functions of cells,
  - the solubility of certain nutrients (dissolved oxygen and minerals)

$$I = \frac{1}{2}\Sigma C_i Z_i^2$$

C : the concentration of an ion

Z : its charge

Environmental Conditions Effect

- **High substrate concentration**: substrate inhibition
- Substrate inhibition can be overcome by

  intermittent addition of the substrate to the medium.

Exercise (P6.2)

- The growth of a microbial population is a function of pH and is given by the following equation:

$$\mu_g = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_m S}{K_s\left(1+\frac{H^+}{k_1}\right)+S}$$

A. With a given set of experimental data (X and S versus t), describe how you would determine the constants μ_m, K_S, and k_1.
B. How would the double-reciprocal plot $1/\mu_g$ versus $1/S$ change with pH (or H^+ concentration)?

Batch Growth

Heat generation by microbial growth

- About 40% to 50% of the energy stored in a carbon and energy source is converted to biological energy (ATP) during aerobic metabolism, and the rest of the energy is released as heat.
- For actively growing cells, the maintenance requirement is low, and heat evolution is directly related to growth.

Batch Growth

Heat generation by microbial growth

- The heat of combustion of the substrate is equal to the sum of the metabolic heat and the heat of combustion of the cellular material.

$$\frac{\Delta H_S}{Y_{X/S}} = \Delta H_C + \frac{1}{Y_H}$$

ΔH_S : the heat of combustion of the substrate (kJ/g substrate)

$Y_{X/S}$: the cell mass yield per substrate consumption (g cell/g substrate)

ΔH_c : the heat of combustion of cells (kJ/g cells)

$\frac{1}{Y_H}$: the metabolic heat evolved per gram of cell mass produced (kJ/g cells)

Batch Growth

Heat generation by microbial growth

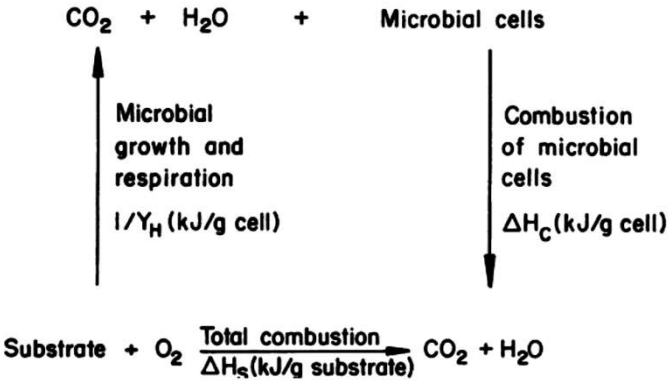


Figure 6.10. Heat balance on microbial utilization of substrate.

Batch Growth

Heat generation by microbial growth

Re-arrange the above equation:

$$\frac{1}{Y_H} = \frac{\Delta H_S}{Y_{X/S}} - \Delta H_C$$

The higher degree of oxidation of the substrate has lower amounts of heat released:

	$1/Y_H$
Glucose:	2.38 kcal/g cell produced
ethanol:	5.55 kcal/g cell produced
Methanol:	8.33 kcal/g cell produced
Methane:	16.34kcal/g cell produced

Batch Growth

Heat generation by microbial growth

$$\frac{1}{Y_H} = \frac{\Delta H_S}{Y_{X/S}} - \Delta H_C$$

The total rate of heat evolution in a batch fermentation Q_{GR} (kJ/h) is

$$Q_{GR} = V_L \mu_{net} X \frac{1}{Y_H}$$

X : cell mass concentration (g/L); V_L : liquid volume (L)

Batch Growth

Heat generation by microbial growth

- In aerobic fermentations, the rate of metabolic heat evolution Q_{GR} (kcal/h)can roughly be correlated to the rate of oxygen uptake Q_{O_2}(millimoles of O2/h), since oxygen is the final electron acceptor.

$$Q_{GR} \cong 0.12 Q_{O_2}$$

- Heat removal: by circulating cooling water through a cooling coil or cooling jacket in the fermentation.

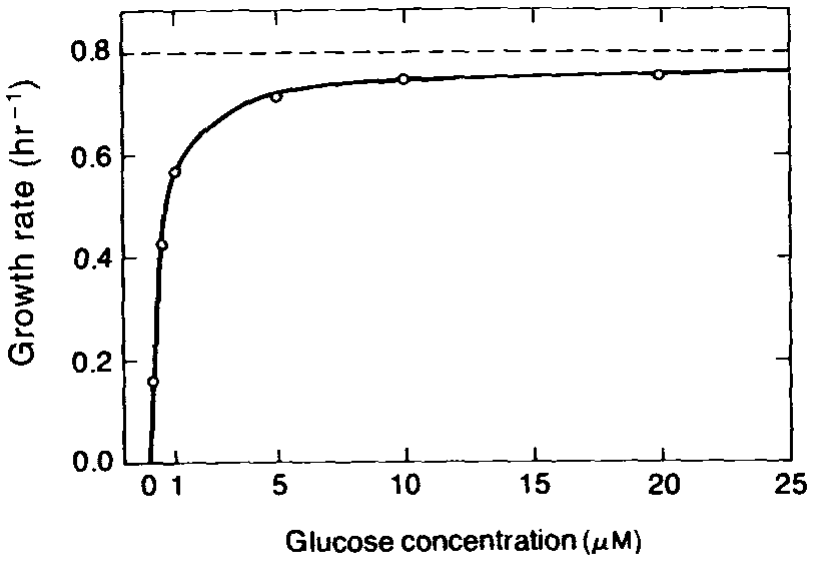
Quantifying Growth Kinetics
Monod equation: Unstructured Nonsegregated Model

- **Unstructured model:** assuming fixed cell composition.
  Applicable to balanced-growth condition:
  - exponential growth phase in batch culture
  - single-stage, steady state continuous culture
  - cell response is fast compared to external changes
  - the magnitude of the external changes is not too large
    (e.g. 10% - 20% variation from initial conditions).
- **Nonsegregated model:** assuming all cells are the same in the culture.

Quantifying Growth Kinetics
Monod equation: Unstructured Nonsegregated Model

Assumption:

- a single enzyme system with Michaelis-Menten kinetics is responsible for uptake of substrate S, and the amount of that enzyme or its activity is sufficiently low to be growth-rate limiting.
- the relationship of specific growth rate to substrate concentration assumes the form of saturation kinetics.
- a single chemical species is growth-rate limiting while changes in other nutrient concentrations have no effect.



Monod type saturation growth kinetics

Quantifying Growth Kinetics
Monod equation

- When Monod equation is applied to cellular systems, the gross specific growth rate μ_g (1/time) is described by:

$$\mu_g = \frac{\mu_m S}{K_S + S}$$

μ_m: maximum specific growth rate (1/time).
K_S : saturation constant or half-velocity constant (g/l)

- If endogenous metabolism is unimportant, $\mu_g = \mu_{net}$
- When the substrate concentration S>> K_S (exponential growth phase), $\mu_g = \mu_m$
- When the substrate concentration S<< K_S, $\mu_g = (\mu_m/K_S)S$