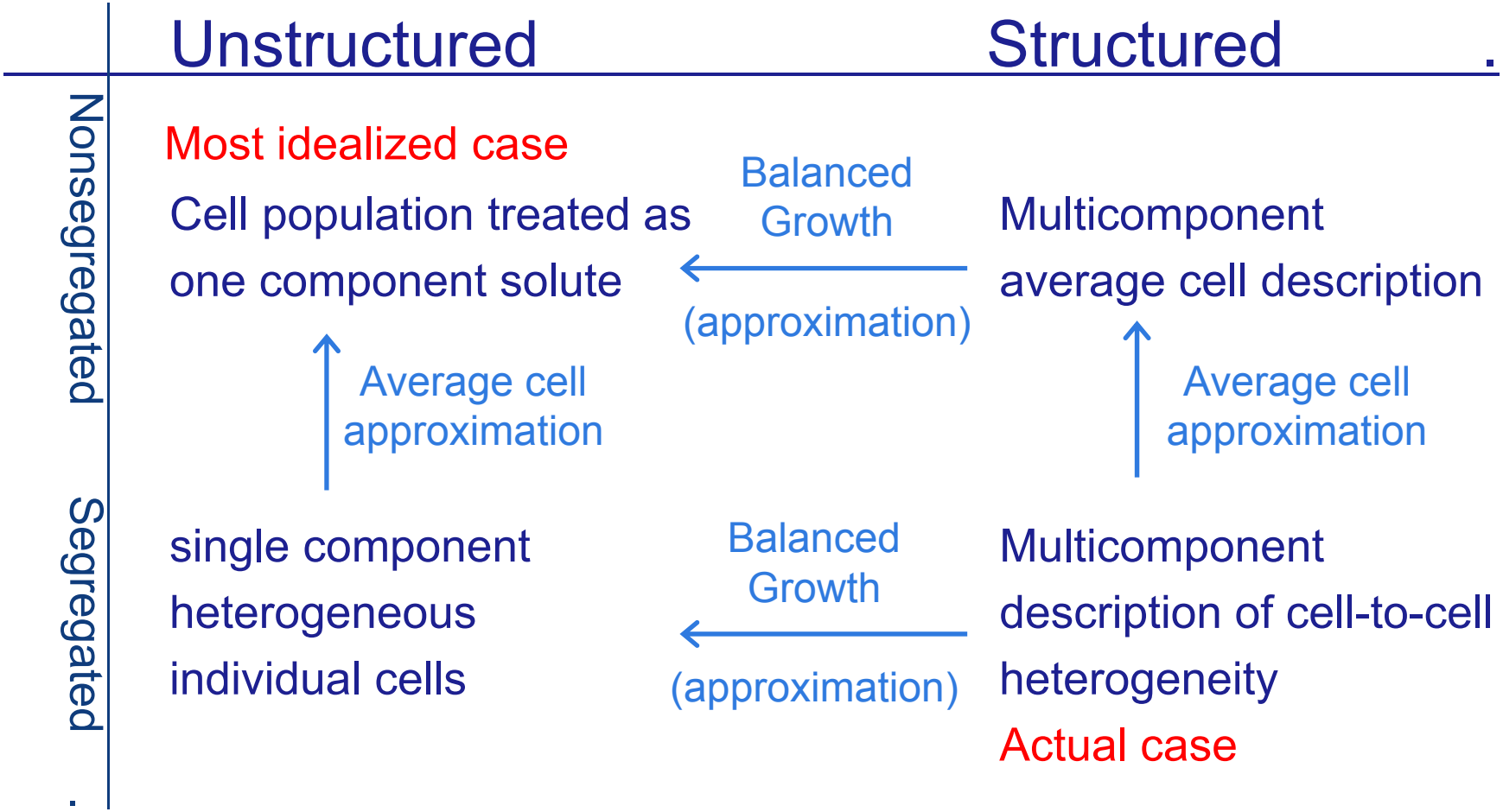
6.3. Quantifying Growth Kinetics

- Model



6.3.2. Using Unstructured Nonsegregated Models to Predict Specific Growth Rate

6.3.2.1. Substrate-limited growth

- Monod equation

$$\mu = \frac{\mu_m S}{K_s + S}$$

μ_m : maximum specific growth rate

K_s : saturation constant

- Other equations

Other equations for substrate-limited growth

Blackman equation:

$$\mu_g = \mu_m, \quad \text{iff } S \geq 2K_s$$

$$\mu_g = \frac{\mu_m}{2K_s} S, \quad \text{iff } S < 2K_s$$

Tessier equation:

$$\mu_g = \mu_m (1 - e^{-KS})$$

Moser equation:

$$\mu_g = \frac{\mu_m S^n}{K_s + S^n} = \mu_m (1 + K_s S^{-n})^{-1}$$

Contois equation:

$$\mu_g = \frac{\mu_m S}{K_{sx} X + S}$$

When more than one substrate is growth rate limiting

- Interactive or multiplicative

$$\mu/\mu_m = [\mu(s_1)]\,[\mu(s_2)]\,\ldots\,[\mu(s_n)]$$

- Additive

$$\mu/\mu_m = w_1\,[\mu(s_1)] + w_2\,[\mu(s_2)] + \ldots + w_N\,[\mu(s_n)]$$

- Noninteractive

$$\mu = \mu(s_1) \text{ or } \mu(s_2) \text{ or } \ldots\ \mu(s_n)$$

where the lowest value of $\mu(s_i)$ is used.

6.3.2.2. Models with growth inhibitors

- The inhibition pattern of microbial growth is analogous to enzyme inhibition.
  - Substrate inhibition
  - Product inhibition
  - Inhibition by toxic compound

Substrate Inhibition of Cell Growth

Noncompetitive substrate inhibition:

$$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{S}{K_I}\right)} \tag{6.39}$$

Or if $K_I \gg K_s$, then:

$$\mu_g = \frac{\mu_m S}{K_s + S + S^2/K_I} \tag{6.40}$$

For competitive substrate inhibition:

$$\mu_g = \frac{\mu_m S}{K_s\left(1+\frac{S}{K_I}\right)+S} \tag{6.41}$$

Enzyme Inhibition

Noncompetitive inhibition

$$v = \frac{V_m}{\left(1+\frac{[\mathrm{I}]}{K_1}\right)\left(1+\frac{K'_m}{[\mathrm{S}]}\right)} \qquad (3.26)$$

Competitive inhibition

$$v = \frac{V_m[\mathrm{S}]}{K'_m\left[1+\frac{[\mathrm{I}]}{K_I}\right]+[\mathrm{S}]}$$

Product Inhibition of Cell Growth

Competitive product inhibition:

$$\mu_g = \frac{\mu_m S}{K_s\left(1+\frac{P}{K_p}\right)+S} \tag{6.42}$$

Noncompetitive product inhibition:

$$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{P}{K_p}\right)} \tag{6.43}$$

Cell Growth in Ethanol Fermentation

Ethanol fermentation from glucose by yeasts is a good example of noncompetitive product inhibition, and ethanol is the inhibitor at concentrations above about 5%. Other rate expressions used for ethanol inhibition are

$$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)}\left(1-\frac{P}{P_m}\right)^n \tag{6.44}$$

where P_m is the product concentration at which growth stops, or

$$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)} e^{-P/K_p} \tag{6.45}$$

where K_p is the product inhibition constant.

Inhibition by Toxic Compounds

Competitive inhibition:
$$\mu_g = \frac{\mu_m S}{K_s\left(1+\frac{\mathrm{I}}{K_\mathrm{I}}\right)+S} \tag{6.46}$$

Noncompetitive inhibition:
$$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{\mathrm{I}}{K_\mathrm{I}}\right)} \tag{6.47}$$

Uncompetitive inhibition:
$$\mu_g = \frac{\mu_m S}{\left(\frac{K_s}{\left(1+\frac{\mathrm{I}}{K_\mathrm{I}}\right)}+S\right)\left(1+\frac{\mathrm{I}}{K_\mathrm{I}}\right)} \tag{6.48}$$

Enzyme Inhibition

Competitive inhibition

$$v = \frac{V_m[\mathrm{S}]}{K'_m\left[1+\frac{[\mathrm{I}]}{K_I}\right]+[\mathrm{S}]}$$

Noncompetitive inhibition

$$v = \frac{V_m}{\left(1+\frac{[\mathrm{I}]}{K_1}\right)\left(1+\frac{K'_m}{[\mathrm{S}]}\right)} \qquad (3.26)$$

Enzyme Inhibition

Uncompetitive inhibition

$$v = \frac{\dfrac{V_m}{\left(1+\dfrac{[\mathrm{I}]}{K_1}\right)}[\mathrm{S}]}{\dfrac{K'_m}{\left(1+\dfrac{[\mathrm{I}]}{K_1}\right)}+[\mathrm{S}]}$$

$$v = \frac{V_{m,\mathrm{app}}[\mathrm{S}]}{K'_{m,\mathrm{app}}+[\mathrm{S}]}$$

Inhibition by Toxic Compounds

In some cases, the presence of toxic compounds in the medium results in the inactivation of cells or death. The net specific rate expression in the presence of death has the following form:

$$\mu_g = \frac{\mu_m S}{K_s + S} - k_d' \tag{6.49}$$

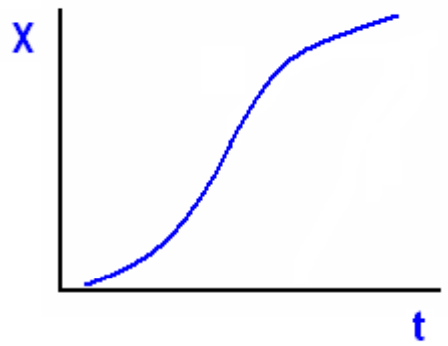
where k_d' is the death-rate constant (h^{-1}).

6.3.2.3. The logistic equation

$dX/dt = \mu X$

$dX/dt = k (1-X/X_\infty) X, \qquad X(0)=X_0$

$$X = \frac{X_0 e^{kt}}{1- (X_0/X_\infty) (1 - e^{kt})}$$



Sigmoidal

(stationary phase involved)

6.3.2.4. Growth model for filamentous organisms

- In suspension culture : formation of microbial pellet
- On solid medium : colony (long and highly branched cells

- In the absence of mass transfer limitations

$R \propto t$ R: radius of a microbial pellet
radius of a mold colony

$dR/dt = k_p$ = constant Eq(1)

Growth model for filamentous organisms

- M : mass of pellet

$$dM/dt = \rho\,(4\pi R^2)\,(dR/dt)$$

$$= k_p\,\rho\,(4\pi R^2) \qquad \text{(by Eq(1))}$$

$$= k_p\,(36\pi\rho)^{1/3}\,(4/3\,\pi R^3\rho)^{2/3}$$

$$= \gamma\,M^{2/3} \qquad \text{Eq(2)}$$

where $\gamma = k_p\,(36\pi\rho)^{1/3}$

$M = 4/3\,\pi R^3\rho$

Growth model for filamentous organisms

- Eq(2)

$$dM/dt = \gamma M^{2/3} \qquad \text{Eq(2)}$$

$$M^{-2/3}\, dM = \gamma\, dt$$

$$3\,[M^{1/3}]_{M_0}^{M} = \gamma\, t$$

$$M = (M_0^{1/3} + (\gamma\, t)/3)^3$$

$$\approx (\gamma\, t\, /3)^3$$

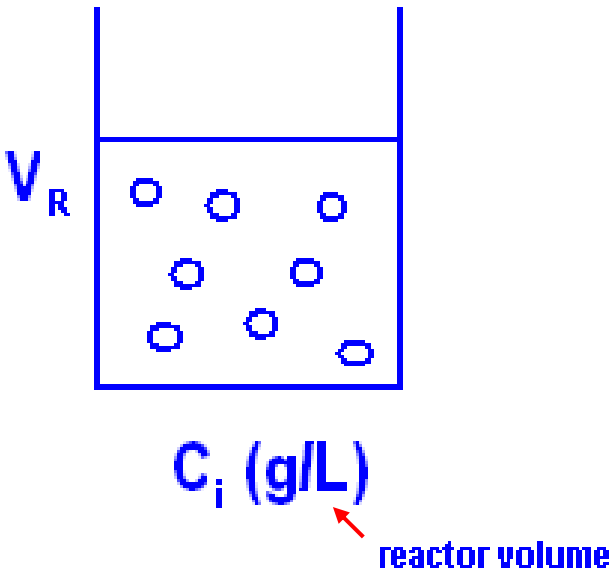
M0 (the initial biomass) is usually very small compared to M.

6.3.3. Chemically Structured Model

- Since it is not practical to write material balances on every cell component, we must select skillfully the key variables and processes of major interest in a particular application when formulating a structured kinetic model.

- Mass balance inside the cell

- Batch
  - **V_R : total volume of liquid in the reactor**
  - **C_i : extrinsic concentration of component i**
  - **X : extrinsic concentration of biomass**



Intrinsic and Extrinsic Concentrations

- Intrinsic concentration
  - The amount of a compound per unit cell mass (or cell volume)
- Extrinsic concentration
  - The amount of a compound per unit reactor volume

Intrinsic and Extrinsic Concentrations

$$\underbrace{\frac{d[V_R C_i]}{dt}}_{\substack{\text{rate of change}\\ \text{in amount of } i\\ \text{in the reactor}}} = \underbrace{V_R X}_{\substack{\text{total}\\ \text{biomass}\\ \text{in reactor}}} \times \underbrace{r_{fi}}_{\substack{\text{rate of}\\ \text{formation}\\ \text{of } i \text{ per}\\ \text{unit biomass}\\ \text{based on intrinsic}\\ \text{concentrations}}} \qquad (6.60)$$

where V_R is the total volume in the reactor, X is the extrinsic biomass concentration, and C_i is the extrinsic concentration of component i.

Intrinsic and Extrinsic Concentrations

$$\frac{d(C_i/X)}{dt} = \left(\frac{1}{X}\frac{dC_i}{dt}\right) - \left(C_i/X\frac{dX/dt}{X}\right) \tag{6.61}$$

Recalling

$$\mu = \frac{1}{X}\frac{dX}{dt} \tag{6.2a}$$

we have

$$\frac{d(C_i/X)}{dt} = \left(\frac{1}{X}\frac{dC_i}{dt}\right) - \mu C_i/X \tag{6.62}$$

and, substituting eq. 6.60 for $(1/X)(dC_i/dt)$ after dividing eq. 6.60 by $V_R X$ and assuming V_R is a constant,

$$\frac{d(C_i/X)}{dt} = r_{fi} - \frac{\mu_{net} C_i}{X} \tag{6.63}$$

In eq. 6.63, the r_{fi} term must be in terms of intrinsic concentrations and the term $\mu_{net}C_i/X$ represents dilution by growth. These concepts are illustrated in Example 6.3.

Single-cell model for *E. coli* by Shuler and colleagues

- Fig. 6.14

- The model contains the order of 100 stoichiometric and kinetic parameters, almost all of which can be determined from previous literature an the biochemistry of *E. coli* growth.

Single-cell model for *E. coli* by Shuler and colleagues

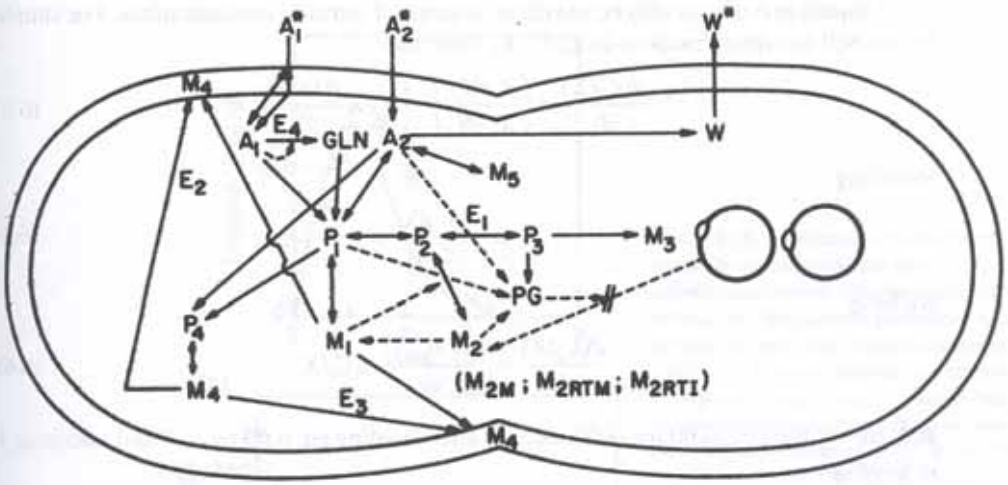


Figure 6.14. An idealized sketch of the model *E. coli* B/rA growing in a glucose–ammonium salts medium with glucose or ammonia as the limiting nutrient. At the time shown the cell has just completed a round of DNA replication and initiated cross-wall formation and a new round of DNA replication. Solid lines indicate the flow of material, while dashed lines indicate flow of information. The symbols are: A_1, ammonium ion; A_2, glucose (and associated compounds in the cell); W, waste products (CO_2, H_2O, and acetate) formed from energy metabolism during aerobic growth; P_1, amino acids; P_2, ribonucleotides; P_3, deoxyribonucleotides; P_4, cell envelope precursors; M_1, protein (both cytoplasmic and envelope); M_{2RTI}, immature "stable" RNA; M_{2RTM}, mature "stable" RNA (*r*-RNA and *t*-RNA—assume 85% *r*-RNA throughout); M_{2M}, messenger RNA; M_3, DNA; M_4, nonprotein part of cell envelope (assume 16.7% peptidoglycan, 47.6% lipid, and 35.7% polysaccharide); M_5, glycogen; PG, ppGpp; E_1, enzymes in the conversion of P_2 to P_3; E_2, E_3, molecules involved in directing cross-wall formation and cell envelope synthesis; GLN, glutamine; E_4, glutamine synthetase; * indicates that the material is present in the external environment. (With permission, from M. L. Shuler and M. M. Domach, in *Foundations of Biochemical Engineering*, H. W. Blanch, E. T. Papoutsakis, and G. Stephanopoulos, ed., ACS Symposium Series 207, American Chemical Society, Washington, DC, 1983, p. 93.)