

CH40001 Biochemical Engineering

Chapter 5. Microbial Growth Models

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Algal Cultivation



Pictures of Bubble Column Algal Photobioreactors



Day 1



Day 2



Day 3



Day 4



Day 5

Fig 1: Growth of algae with time in bubble column pilot plant photobioreactor in mixotrophic conditions at atmospheric CO₂ concentrations

General reaction resulting in cell growth

- When cells are grown on ammonia :



$CH_a O_b N_c$ is the elemental composition of the cell.

$CH_l O_m$ is the elemental composition of the carbon source.

$CH_p O_q N_r$ is the elemental composition of the elemental products.

- In the process of cell growth,
 - ATP (“energy currency” of the cell),
 - NADPH (employed for cellular electron transport)are generated based on the oxidation of the carbon source $CH_l O_m$.

MICROBIAL GROWTH

- ▶ Cell Cycle – Various events that occur during the growth of a single cell from its inception till its time of division into daughter cells are referred to as the cell cycle.
 1. **M-phase** : Nucleus division (mitosis) occurs.
 2. **Inter-phase** : Daughter cells formed from cell division(mitosis) enter G phase.
 3. **G₁-phase** : High rate of biosynthesis.
 4. **S-phase** : DNA synthesis occurs till the DNA content of the cell has doubled.
 5. **G₂-phase**: Initiation of mitosis.
(next: repeat the sequence from step 1)
- ▶ Nomenclature:
 r_X : rate of cell growth (volumetric rate of increase of cell concentration X)
X : Cell concentration (usually dry cell weight per volume)
 μ : specific rate = r_X/X

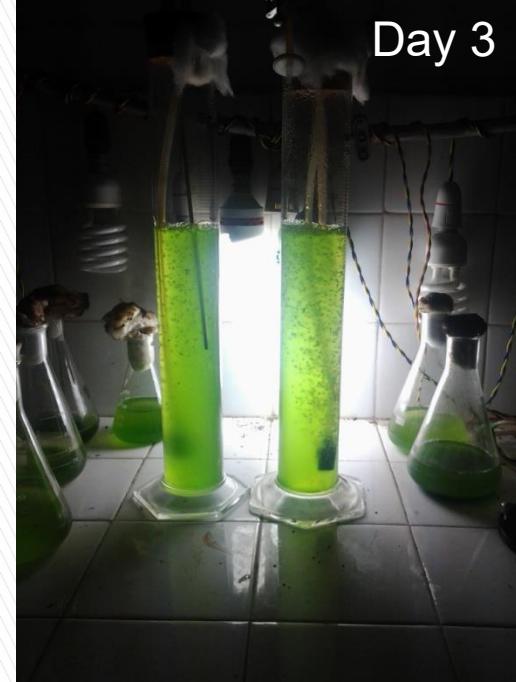
Day 1



Day 2



Day 3



Multiple Substrates = Acetic Acid + Carbon dioxide,

Mixotrophic Growth (= Autotrophic + Heterotrophic)

Day 4



Day 5



Day 6



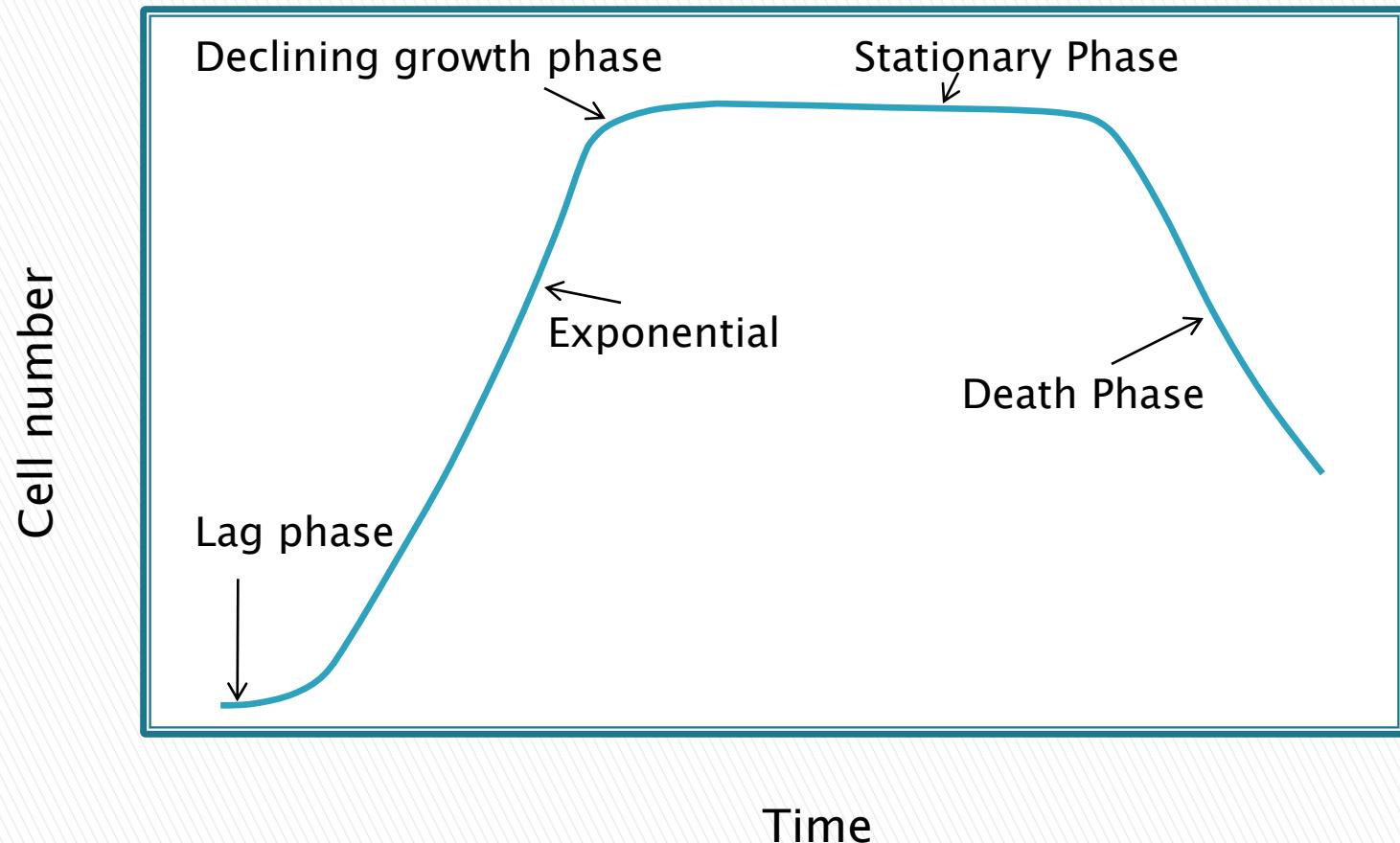
Types of Growth Kinetic Models

- ▶ Structured model: recognizing the components in cells in response to the environment.
- ▶ Unstructured model: assuming fixed cell composition (balanced-growth).
- ▶ Segregation model: segregating the culture into individual units (cells) that may differ from each other.
- ▶ Nonsegregation model: cells are the same in the culture.

Unstructured Models

- 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).
- Nonsegregation model: assuming all cells are the same in the culture.
 - Satisfactory under most circumstances.

Typical Microbial growth in a batch reactor



Malthusian Model for Cell Growth

- ▶ During exponential phase in a batch reactor,

$$\frac{dX}{dt} = \mu X \quad \mu = \text{growth rate of cells}$$

Initial Condition: $X = X_0, t = t_{lag}$

$$X = X_0 e^{\mu(t - t_{lag})}$$

$$\text{or, } \ln\left(\frac{X}{X_0}\right) = \mu(t - t_{lag})$$

$$t_d = \frac{\ln 2}{\mu} \quad (\text{Doubling time})$$

$$\nu = \frac{1}{N} \frac{dN}{dt} \quad \text{or,} \quad \mu = \frac{1}{X} \frac{dX}{dt}$$

Monod Growth model

- Monod Growth Equation: unstructured and non-segregation model

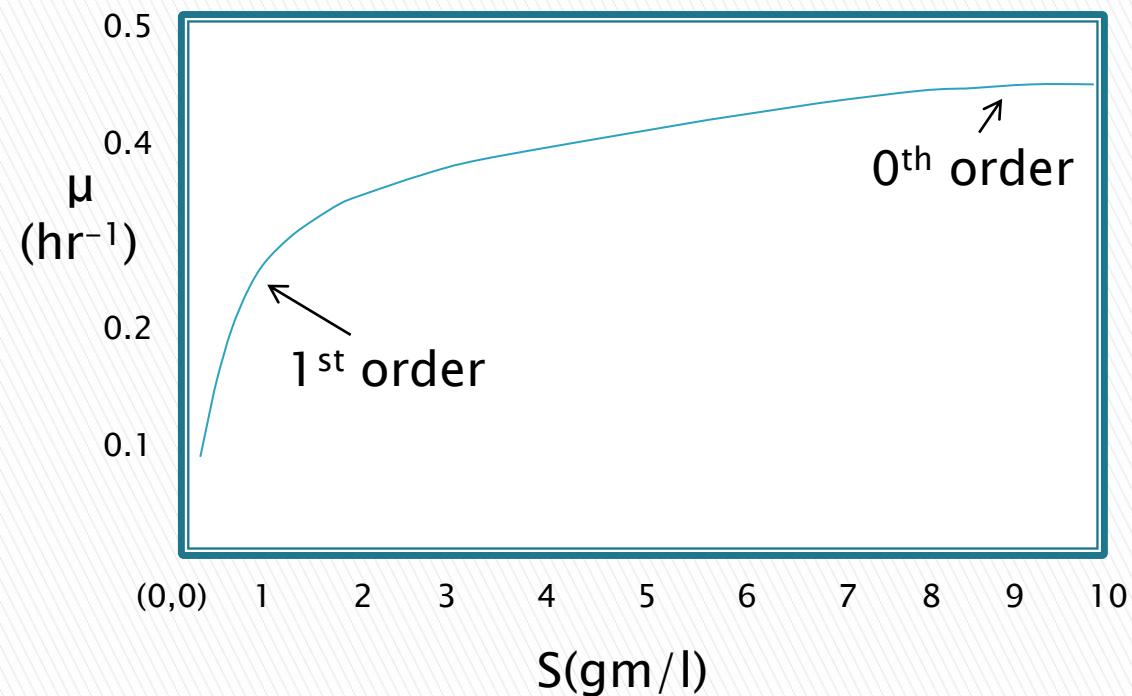
Assumption:

- 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 sufficient 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.

Applicable when growth is slow and population density is low.

iii. Monod model (J Monod, Ann. Inst. Pasteur:79,390 (1950))

$$\mu = \frac{\mu_{\max} S}{K_S + S} \quad (\text{specific growth rate is substrate dependent})$$



$$\frac{dX}{dt} = \mu X = \frac{\mu_{\max} S X}{K_S + S}$$

μ has a Michaelis-Menton kinetics

$$\begin{aligned} \mu &= \frac{\mu_{\max} S}{K_S} && (1^{\text{st}} \text{ order}) \text{ for } S \ll K_S \\ &= \mu_{\max} && (0^{\text{th}} \text{ order}) \text{ for } S \gg K_S \end{aligned}$$

Values of μ_{\max} and K_s for various organisms and substrates (at optimum temperature)

Organism &Growth Temperature	Limiting Nutrient	μ_{\max} (hr ⁻¹)	K_s (mg/lit)
Escherichia Coli (37°C)	Glucose	0.8–0.14	2–4
Escherichia Coli (37°C)	Glycerol	0.87	2
Escherichia Coli (37°C)	Lactose	0.8	20
Sacromyces Cerevisiae (30°C)	Glucose	0.5–0.6	25
Candida Tropicallis (30°C)	Glucose	0.5	25–75
Klebsiella Aerogenes	Glycerol	0.85	9

Values of activation energy for various microorganisms

Organism	Temp($^{\circ}$ C)	E_A , Kcal/mole
Aspergillus Nidulans	20-37	14
E. Coli	23-37	13.1
Klebsiella Aerogenes	20-40	14.2
Psychrophillic Pseudomonad	2-12	23.8

Models for cell-growth

i. Malthusian Model:

$$r_X = \mu X = \frac{dX}{dt} \text{ (for batch reactor)}$$

$$X = X_0 e^{\mu(t-t_{lag})}$$

Shortcoming: predicts unlimited growth.

ii. Logistic model:

To overcome this shortcoming , Verlhulst (1844) and Pearl & Reed (1920) proposed the addition of a cell-concentration dependent second term:

$$r_X = kX(1 - \beta X)$$

For a batch system,

$$\frac{dX}{dt} = kX(1 - \beta X) \text{ with } X = X_0 \text{ at } t = 0.$$

$$\Rightarrow X = \frac{X_0 e^{kt}}{1 - \beta X_0 (1 - e^{kt})} \text{ (logistic eqn)}$$

iv. Modified Monod Model:

It is found experimentally the rate of growth decreases at high values of initial substrate concentration S_0 .

$$\mu = \frac{\mu_{\max} S}{K_S + K_S S_0 + S}$$

v. Konak Model(1974):

$$\frac{d\mu}{dS} = k(\mu_{\max} - \mu)^p$$

where p,k are adjustable parameters.

when p=1,

$$\mu = \mu_{\max} (1 - e^{ks}) \quad \text{Tiessier equation}$$

for p ≠ 1,

$$\mu_{\max}^{1-p} - (\mu_{\max} - \mu)^{1-p} = (1 - p)kS$$

Above eqn. → Monod model for p=2

$$\mu = \frac{\mu_{\max} S}{\frac{\mu_{\max}}{k} + S}$$

Other Types of Growth Kinetics

► Substrate Inhibition

$$\mu = \frac{\mu_{\max} S}{K_S + S + S^2 / K_I}$$

$$\frac{dX}{dt} = \mu X = \frac{\mu_{\max} SX}{K_S + S + S^2 / K_I}$$

Here, K_I is the Haldane or substrate inhibition coefficient

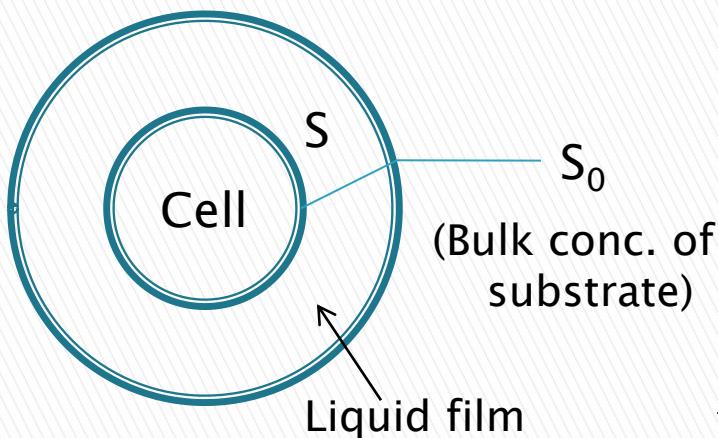
► Product Inhibition

$$\mu = \frac{\mu_{\max} S}{K_S + S} \left[1 - \left(\frac{[P]}{[P^*]} \right)^n \right],$$

Where $[P]$ is the ethanol conc., n is a determinable constant, $[P]^*$ is the critical ethanol conc. above which cells cease to grow (~ 10 gm/lit for *S. cerevisiae*).

Coupling of Mass Transfer & Monod Kinetics

$$\text{Rate of substrate transport} = k_L a' \left(\frac{X}{\rho_{cell}} \right) (S_0 - S)$$



$$\text{Rate of substrate uptake} = qX = \frac{q_{max}SX}{K_s + S}$$

Equating these two rates:

$$\frac{q_{max}SX}{K_s + S} = k_L a' \left(\frac{X}{\rho_{cell}} \right) (S_0 - S)$$

$$\text{Noting that, } q = \frac{q_{\max} S}{K_s + S} \Rightarrow S = \frac{q K_s}{q_{\max} - q}$$

The above equation could be solved for S_0 in terms of q and transport properties as

$$S_0 = \frac{q K_s}{q_{\max} - q} + \frac{q \rho_{cell}}{k_L a'}$$

Note : $Y_{X/S}$ = yield of $X/S = \mu/q$

$$S_0 = \frac{\mu K_s}{\mu_{\max} - \mu} + \frac{\mu \rho_{cell}}{k_L a' Y_{X/S}}$$

The last equation is a quadratic which could be solved for μ if determinant > 0 .

$$\Rightarrow 4S_0 \left(\frac{\mu_{\max} \rho_{cell}}{k_L a' Y_{X/S}} \right) < \left[S_0 + K_s + \left(\frac{\mu_{\max} \rho_{cell}}{k_L a' Y_{X/S}} \right) \right]^2$$

When the above condition is satisfied ,

μ (after binomial expansion and considering the first term)

$$\mu_{MT} = \mu_{\max} \frac{S_0}{K_s + S_0 + \frac{\mu_{\max} \rho_{cell}}{k_L a' Y_{X/S}}} = \mu_{\max} \frac{S_0}{K_{app} + S_0}$$

$$\text{where, } K_{app} = K_s + \frac{\mu_{\max} \rho_{cell}}{k_L a' Y_{X/S}}$$

In the absence of mass transfer resistance (Monod model)

$$\mu_M = \mu_{\max} \frac{S_0}{K_s + S_0}$$

$$\text{Now, } \because K_{app} > K_s$$

$$\therefore \mu_M > \mu_{MT}$$

Growth of Fungal Colony

- ▶ Fungi growth often show a constant rate of increase of radius of the mold colony, which could be expressed as

$$\frac{dr}{dt} = K, \text{ where } K \text{ is a constant.}$$

At any instant 't', the volume of the colony is given by (for *cylindrical mold*)

$$X = \pi r^2 h \rho$$

$$\frac{dX}{dt} = 2\pi rh\rho \frac{dr}{dt} = 2\pi \sqrt{\frac{X}{\pi h\rho}} h\rho K = 2\sqrt{\pi h\rho X} K$$

At $t = 0, X = X_0,$

$$\text{Integrating, } X = (\lambda t + X_0^{1/2})^2 \quad \text{Where, } \lambda = K\sqrt{\pi h\rho}$$

► Spherical Mold:

$$X = \frac{4}{3} \pi r^3 \rho$$

$$\frac{dX}{dt} = 4\pi r^2 \rho \frac{dr}{dt} = \frac{4}{3} \pi \rho K \left(\frac{3X}{4\pi\rho} \right)^{\frac{2}{3}}$$

At $t = 0, X = X_0,$

Integrating, $X = \left(\frac{\gamma t}{3} + X_0^{\frac{1}{3}} \right)^3$ Where, $\gamma = K(36\pi\rho)^{\frac{1}{3}}$

Diffusion of oxygen/nutrients in the fungal pellet

$$\frac{1}{r} \frac{d}{dr} \left(r \frac{dC_{O_2}}{dr} \right) = \frac{R^2 \left(\frac{\nu_{\max}}{K_M D_{eff}} \right) C_{O_2}}{1 + \beta C_{O_2}} \quad (\text{dimensionless equation})$$

If, $C_{O_2} \gg K_M$ (nutrients present in abundance)

$$\frac{1}{r} \frac{d}{dr} \left(r \frac{dC_{O_2}}{dr} \right) = \frac{R^2 \nu_{\max}}{S_0 D_{eff}}$$

with B.C.s $C_{O_2}(\bar{r} = 1) = 1, \frac{dC_{O_2}}{d\bar{r}}(\bar{r} = 0) = 0.$

Solution:

$$C_{O_2} = 1 - \frac{R^2 v_{\max}}{6S_0 D_{eff}} \left[1 - (\bar{r})^2 \right]$$

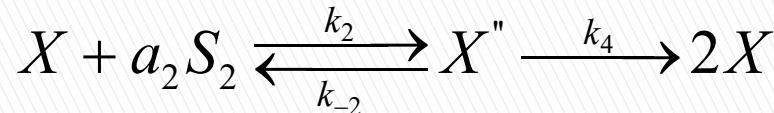
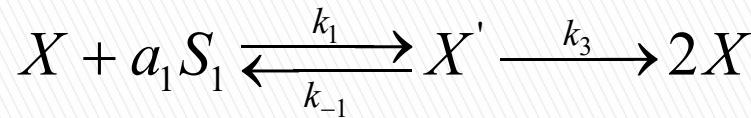
- ▶ Please note the number 6 must be replaced by 4 in all equations on this slide.
- ▶ At critical radius (R_{crit}), when the center of the pellet is depleted of oxygen, growth stops.

When $R = R_{crit}$, $C_{O_2} = 0$ at $r = 0$

$$\frac{R_{crit}^2 v_{\max}}{6S_0 D_{eff}} = 1 \quad \Rightarrow \quad R_{crit} = \sqrt{\frac{6S_0 D_{eff}}{v_{\max}}}$$

Multiple Substrates and Models

- ▶ Multiple Substrates:



Where, $Y_{X/S_1} = \frac{1}{a_1}$, $Y_{X/S_2} = \frac{1}{a_2}$

Balance Equations:

$$\frac{dX'}{dt} = k_1 X S_1 - k_{-1} X' - k_3 X'$$

$$\frac{dX''}{dt} = k_2 X S_2 - k_{-2} X'' - k_4 X''$$

Constraint Equation: $X_T = X' + X''$

Pseudo-steady state approximation: $\frac{dX'}{dt} = 0, \frac{dX''}{dt} = 0.$

$$\frac{dX}{dt} = \frac{dX_T}{dt} = k_3 X' + k_4 X''$$

$$\therefore \frac{dX'}{dt} = 0 \Rightarrow X' = \frac{k_1 X S_1}{k_{-1} + k_3}$$

$$\frac{dX''}{dt} = 0 \Rightarrow X'' = \frac{k_2 X S_2}{k_{-2} + k_4}$$

$$X_T = X \left[1 + \frac{k_1 S_1}{k_{-1} + k_3} + \frac{k_2 S_2}{k_{-2} + k_4} \right]$$

$$\begin{aligned}
\frac{dX_T}{dt} &= k_3 X' + k_4 X'' = X \left[\frac{k_3 k_1 S_1}{k_{-1} + k_3} + \frac{k_2 k_4 S_2}{k_{-2} + k_4} \right] \\
&= X_T \left[1 + \frac{k_1 S_1}{k_{-1} + k_3} + \frac{k_2 S_2}{k_{-2} + k_4} \right]^{-1} \left[\frac{k_3 k_1 S_1}{k_{-1} + k_3} + \frac{k_2 k_4 S_2}{k_{-2} + k_4} \right] \\
&= \mu X_T
\end{aligned}$$

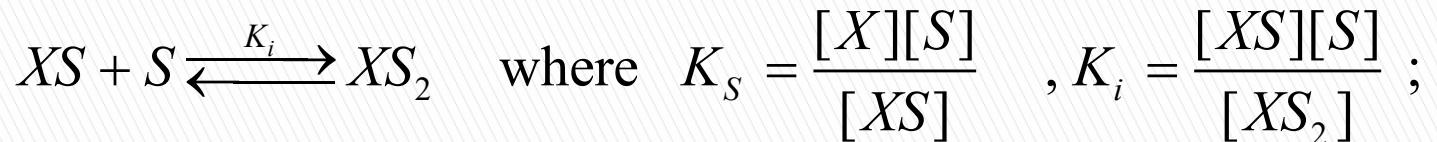
$$\therefore \mu = \frac{\mu_{\max 1} S_1}{K_1 + S_1 + \alpha_2 S_2} + \frac{\mu_{\max 2} S_2}{K_2 + S_2 + \alpha_1 S_1}$$

where, $\alpha_2 = \left(\frac{k_2}{k_1} \right) \left(\frac{k_{-1} + k_3}{k_{-2} + k_4} \right) = \frac{K_1}{K_2}$, $\alpha_1 = \frac{1}{\alpha_2}$

and, $K_1 = \frac{k_{-1} + k_3}{k_1}$, $K_2 = \frac{k_{-2} + k_4}{k_2}$,

$$\mu_{\max 1} = k_3, \quad \mu_{\max 2} = k_4$$

Effect of Inhibitory Substrates



$$\frac{d[X_T]}{dt} = K[XS] = \frac{K}{K_S}[X][S]$$

$$\begin{aligned} [X_T] &= [X] + [XS] + [XS_2] \\ &= [X] + \frac{[X][S]}{K_S} + \frac{[X][S]^2}{K_i K_S} \\ &= [X] \left(1 + \frac{[S]}{K_S} + \frac{[S]^2}{K_i K_S} \right) \end{aligned}$$

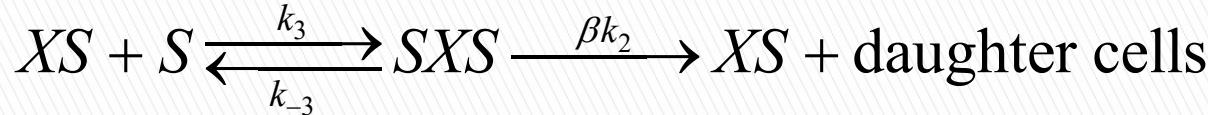
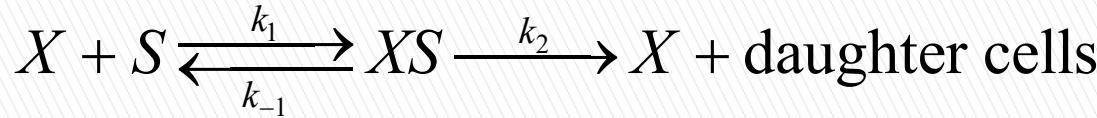
$$\frac{d[X_T]}{dt} = \frac{K}{K_S}[X][S] = \frac{K}{K_S} \frac{[S]}{\left(1 + \frac{[S]}{K_S} + \frac{[S]^2}{K_i K_S}\right)} [X_T]$$

$$\mu = \frac{1}{[X_T]} \frac{d[X_T]}{dt} = \frac{\mu_{\max} [S]}{\left(1 + \frac{[S]}{K_S} + \frac{[S]^2}{K_i K_S}\right)}$$

This rate of growth reaches a maximum value at $S = S_{crit}$ beyond which it declines. To find the maximum specific growth:

$$\frac{d\mu}{dS} = 0 \quad \Rightarrow \quad S_{crit} = \sqrt{K_i K_S}$$

Allosteric Inhibition



Same Procedure as previous case,

$$\frac{d[X_T]}{dt} = k_2[XS] + \beta k_2[SXS]$$

$$\mu = \mu'_{\max} \frac{S \left(1 + \frac{\beta S}{K_M} \right)}{K_M + S + \frac{S^2}{K'_M}}$$

$$\text{where, } K_M = \frac{k_{-1} + k_2}{k_1} \quad \& \quad K'_M = \frac{k_{-3} + \beta k_2}{k_3}$$