EPFL

Laboratory of Computational Systems Biotechnology

Project 1: Enzymatic/Microbial kinetics
General reactor design
Batch operation
Yield coefficients

ChE-320 Bioreactor modeling and simulation

Vassily Hatzimanikatis

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Enzyme kinetics

Michaelis & Menten (1913) investigated the kinetics of an enzymatic reaction mechanism, invertase, that catalyzes the hydrolysis of sucrose into glucose and fructose:

$$S + E \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_2}{\Rightarrow} P + E$$

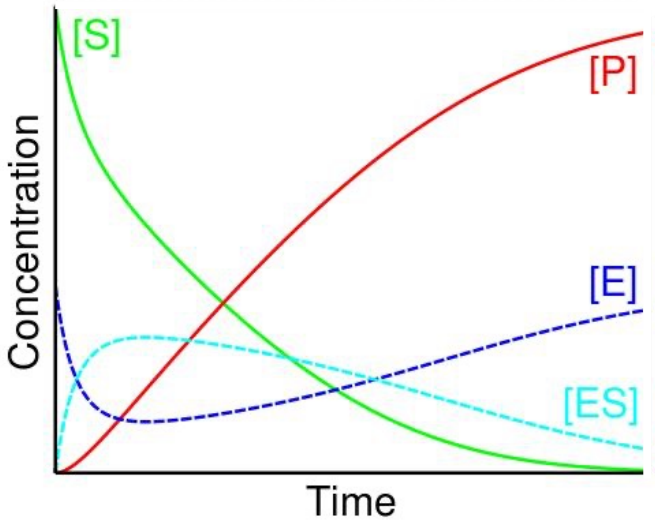
E: enzyme
S: substrate
P: product

Mass balances for batch reactor:

$$\frac{d[S]}{dt} = -k_1[S][E] + k_{-1}[ES]$$

$$\frac{d[ES]}{dt} = k_1[S][E] - (k_{-1} + k_2)[ES]$$

$$\text{Initial conditions @ t=0:} \begin{cases} [S] = S_o \\ [E] = E_o \\ [ES] = 0 \end{cases}$$



Quasi-Steady state approximation

Quasi-steady state(QSS) approximation for ES:

$$\frac{d[ES]}{dt} = 0$$

Valid when $[E] << S_O$

$$S + E \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_2}{\Rightarrow} P + E$$

$$0 = k_1[S][E] - (k_{-1} + k_2)[ES]$$

$$0 = k_1[S](E_O - [ES]) - (k_{-1} + k_2)[ES]$$

$$k_1[S]E_O = k_1[S][ES] + (k_{-1} + k_2)[ES]$$

$$[S]E_O = [S][ES] + \underbrace{\frac{k_{-1} + k_2}{k_1}}_{K_M}[ES]$$

$$K_M[=]\frac{mol}{vol}$$

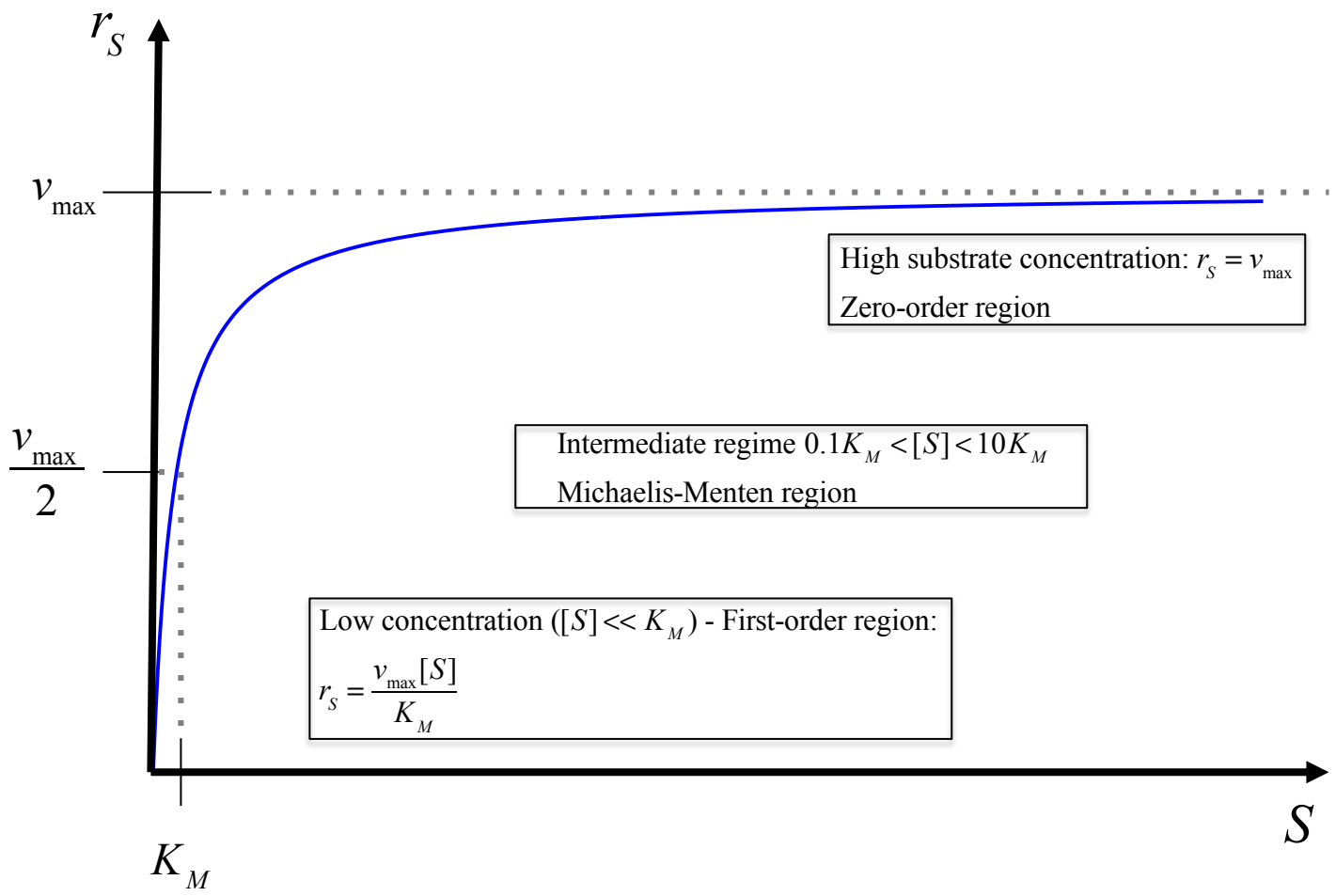
$$[ES] = \frac{E_O[S]}{K_M + [S]}$$

$$\frac{d[P]}{dt} = k_2[ES] = \frac{k_2 E_O[S]}{K_M + [S]} = \frac{v_{\max}[S]}{K_M + [S]}$$

where $v_{\max} = k_2 E_O[=]\frac{mol}{vol \cdot time}$

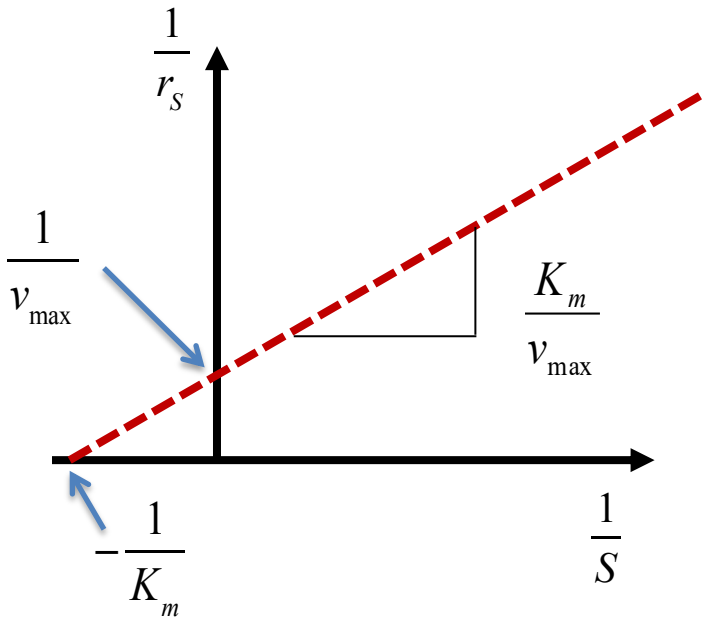
Reaction rate phases

$$r_S = v_{\max} \frac{S}{K_M + S}$$



Lineweaver-Burk plot

$$\frac{r_S}{v_{\max}} = \frac{[S]}{K_M + [S]} \Rightarrow \frac{1}{r_S} = \frac{K_M}{v_{\max}} \frac{1}{[S]} + \frac{1}{v_{\max}}$$



Parameters $v_{\max}$ and K_M can be determined from experimental data.

Typical values in Michaelis - Menten kinetics :

k_1 $10^5 - 10^9 L/mol \cdot s$

k_{-1} $10 - 10^4 \ 1/s$

k_2 $1 - 10^6 \ 1/s$

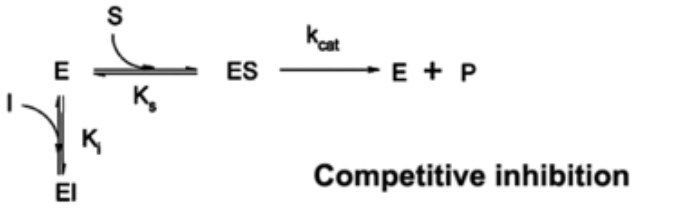
K_M $10^{-6} - 10^{-1} \ mol/L$

Inhibitory kinetics

Inhibition type

Competitive $E + I \Leftrightarrow EI$

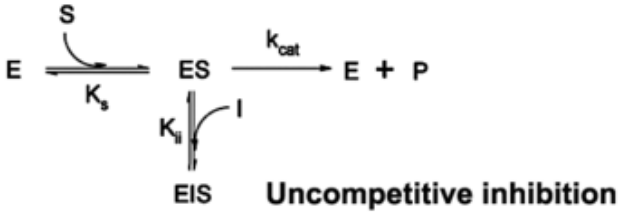
$$\frac{v_{max} S}{K_M \left(1 + I / K_I\right) + S}$$



Competitive inhibition

Uncompetitive $ES + I \Leftrightarrow ESI$

$$\frac{v_{max} S}{K_M + S\left(1 + I / K_I\right)}$$



Uncompetitive inhibition

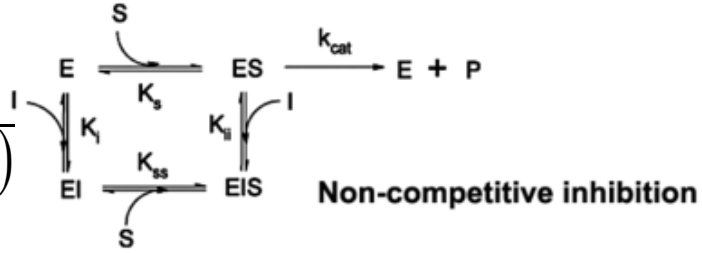
Non-competitive

$E + S \Leftrightarrow ES \rightarrow P$

$E + I \Leftrightarrow EI \Leftrightarrow ESI$

$ESI \Leftrightarrow ES \rightarrow P$

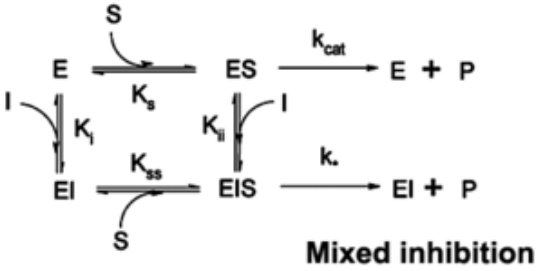
$$\frac{v_{max} S}{\left(1 + I / K_I\right)\left(K_M + S\right)}$$



Non-competitive inhibition

Double Michaelis-Menten Kinetics (two substrates involved):

$$r_S = \frac{v_{max}[S_1][S_2]}{(K_{M1} + [S_1])(K_{M2} + [S_2])}$$



Mixed inhibition

Allosteric effect

It was originally formulated by Archibald Hill in 1910 to describe the sigmoidal O_2 binding curve of hemoglobin.

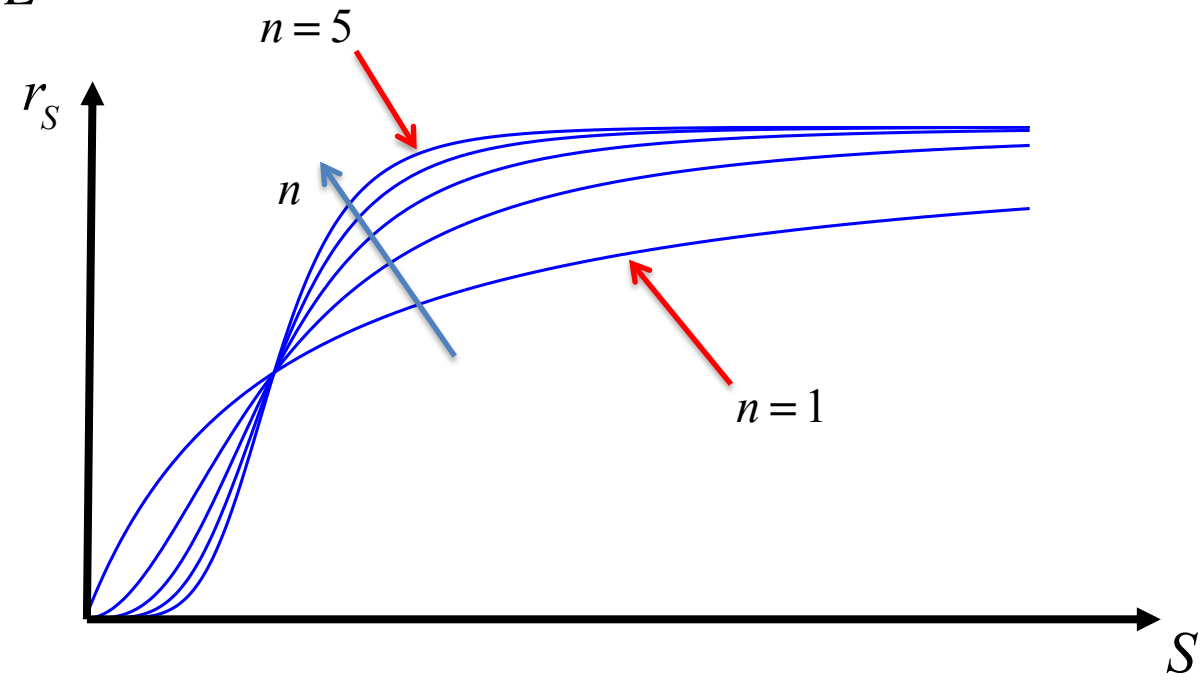
Allosteric Kinetics

Enzyme can bind to more than one substrate molecule.

$$nS + E \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES_n$$

$$ES_n \xrightarrow{k_2} nP + E$$

$$r_S = \frac{v_{\max}[S]^n}{K_M^n + [S]^n} \quad \text{Hill kinetics}$$



Microbial kinetics

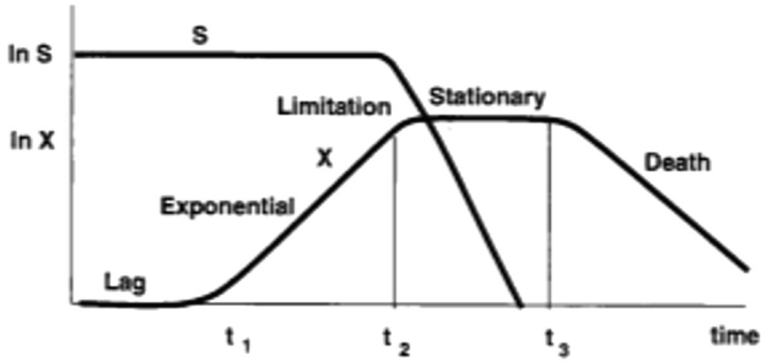


Figure 3.6. Biomass and substrate concentrations during batch growth.

Exponential phase:

$$\frac{dX}{dt} = \mu X$$

$$\frac{X}{X_O} = e^{\mu t}$$

$\mu \equiv$ specific growth rate (slope of curve between t_1 and t_2)

Death phase (lack of nutrients, toxicity, cell aging,etc) :

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

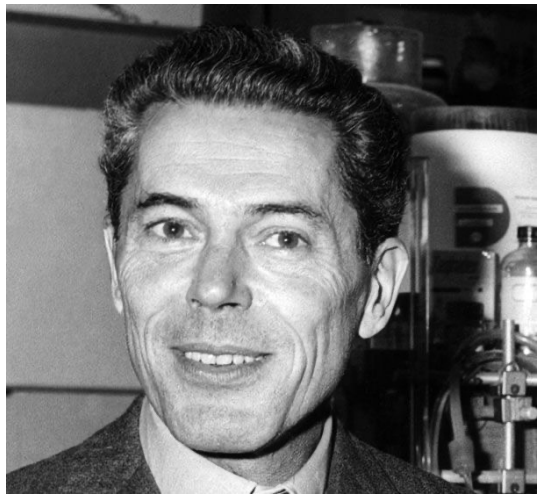
The exponential and limiting regions can be described by a single Monod equation.

The Monod equation

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

$\mu_{\max} \equiv$ maximum specific growth rate

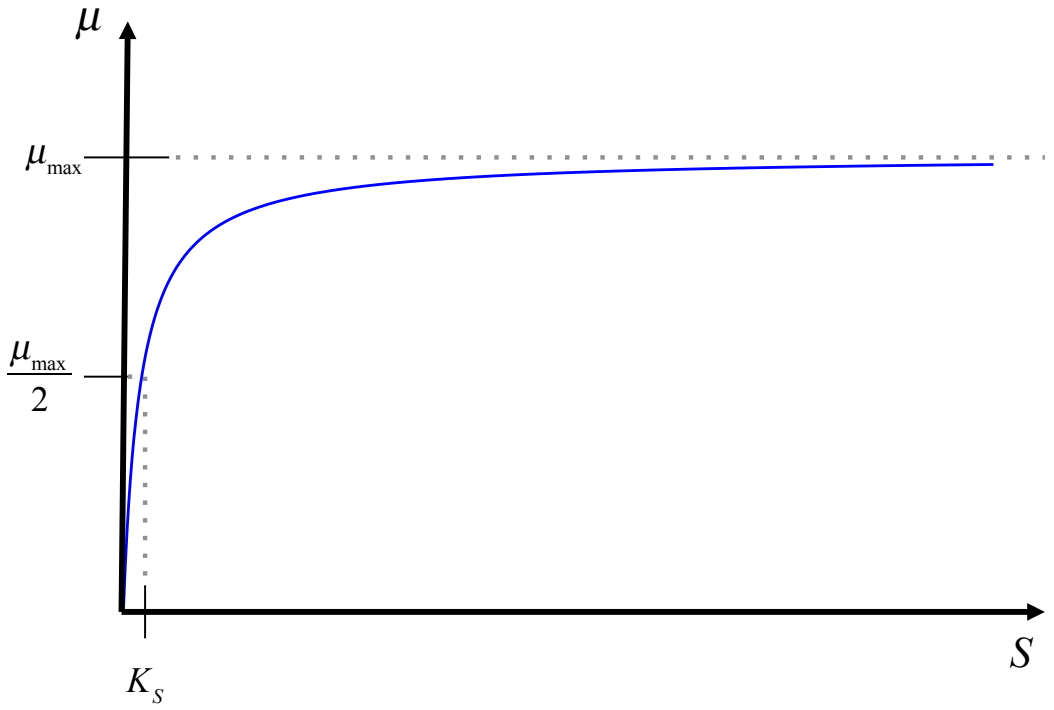
$K_S \equiv$ saturation(Monod) constant

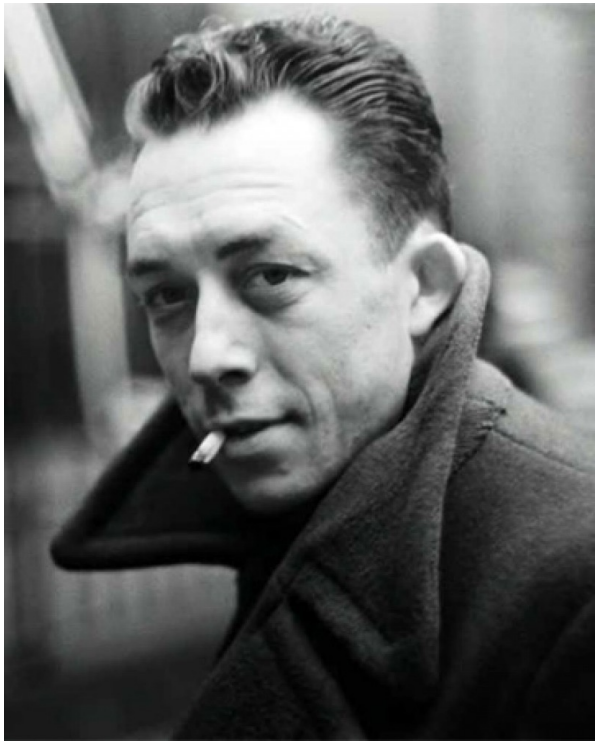


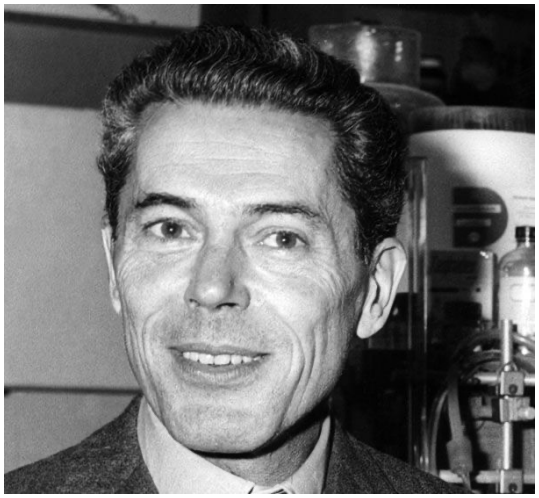
$$[S] \to 0, \mu \to \frac{\mu_{\max}[S]}{K_S}$$

$$[S] \to \infty, \mu \to \mu_{\max}$$

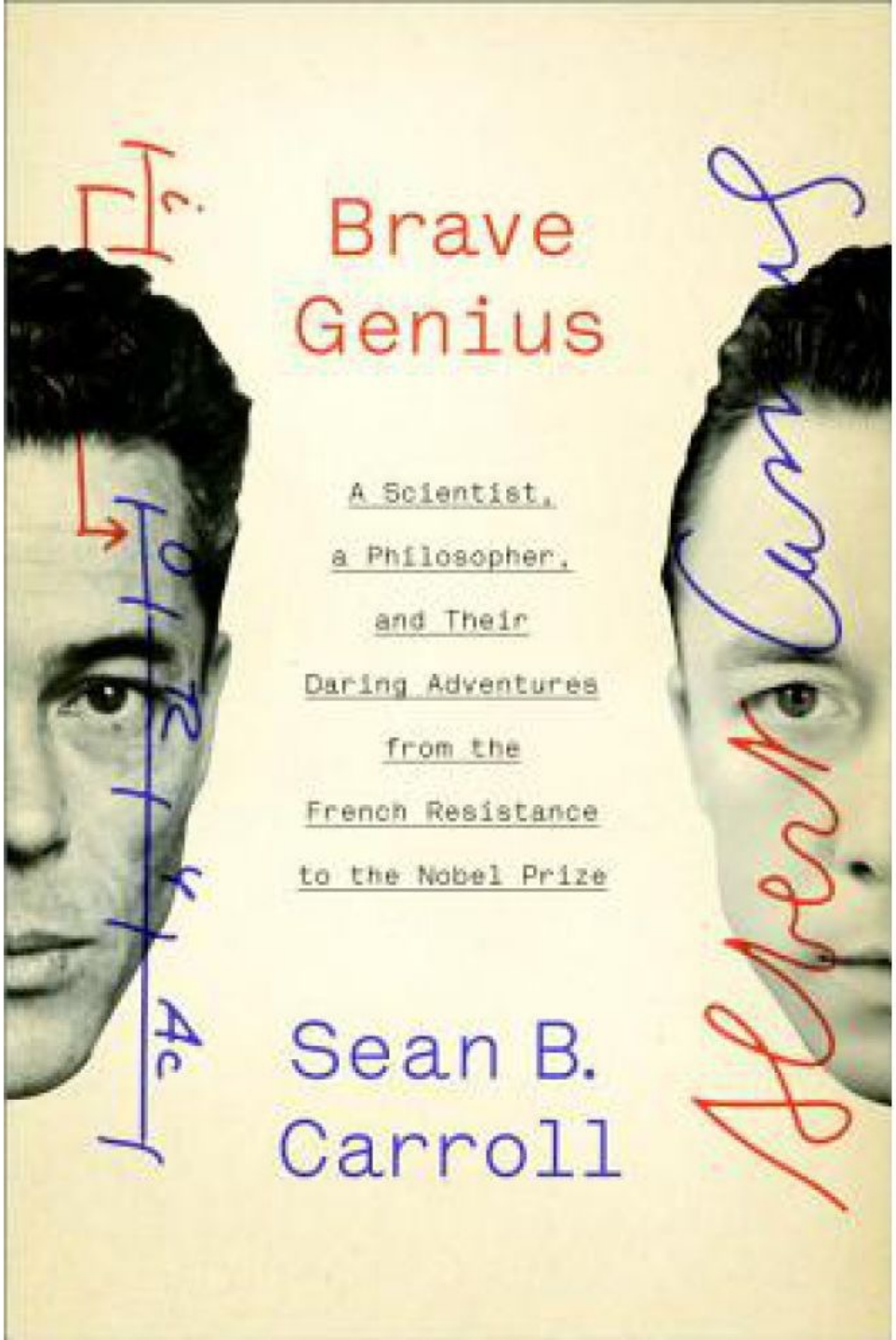
$$[S] = K_S, \mu = \frac{\mu_{\max}}{2}$$







Brave Geniuses

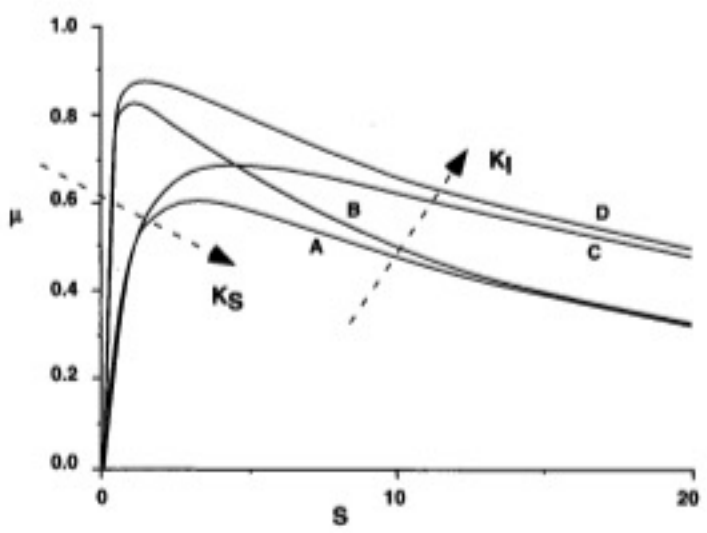


Substrate inhibition of growth

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

$$\text{Inhibition term} \sim \frac{[S]^2}{K_I}$$

** High values of K_I => decreased effect of substrate inhibition



$$[S] = K_S \text{ or } K_I,$$

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

Maximum μ occurs at $[S] = \sqrt{K_S K_I}$ and

$$\mu = \frac{\mu_{\max}}{2\sqrt{K_S / K_I} + 1}$$

Figure 3.7. Substrate inhibition kinetics for various values of K_S and K_I. The parameters used are as follows: For all curves $\mu_m = 1.0$ 1/h. Curve A: $K_S = 1$ and $K_I = 10$, Curve B: $K_S = 0.1$ and $K_I = 10$; Curve C: $K_S = 1$ and $K_I = 20$; Curve D: $K_S = 0.1$ and $K_I = 20$. The units of K_S, K_I and S are g/m^3.

Other microbial kinetics

Product inhibition :

$$\mu = \frac{\mu_{\max}[S]}{K_S + [S] + [P]\ /K_I}$$

Two - substrate Monod kinetics :

$$\mu = \mu_{\max} \frac{[S_1]}{K_1 + [S_1]} \frac{[S_2]}{K_2 + [S_2]}$$

Double - Monod kinetics :

$$\mu = \mu_{\max} \left(\frac{k_1[S_1]}{K_1 + [S_1]} + \frac{k_2[S_2]}{K_2 + [S_2]} \right) \frac{1}{k_1 + k_2}$$

Diauxic Monod :

$$\mu = \mu_{\max,1} \frac{[S_1]}{K_1 + [S_1]} + \mu_{\max,2} \frac{[S_2]}{K_2 + [S_2] + [S_2]^2 / K_I}$$

Yield coefficients

- Defined based on the amount of consumption of another material

$$Y_{X/S} = -\frac{\Delta X}{\Delta S}$$ X: biomass S: substrate

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

- Yield coefficients can also be based on other substrates or product formation:

$$Y_{X/O2} = -\frac{\Delta X}{\Delta O_2}$$

$$Y_{P/S} = -\frac{\Delta P}{\Delta S}$$ P: product

For aerobic growth on glucose:

$Y_{X/S}$ typically 0.4 to 0.6 g/g for yeast and bacteria

Y_{X/O_2} 0.9 to 1.4 g/g

For anaerobic growth: smaller yield coefficents

- In this course, in general we assume a constant for yield coefficient

Maintenance

Uptake of substrate by microorganisms is considered to be related to either:

(1) Growth (increase in biomass) , or

(2) Cell maintenance

Substrate consumed for cell maintenance is proportional to the total quantity of cells.

$$r_S = -\frac{r_X}{Y_{X/S}} - m \cdot [X]$$

$$m \text{ (maintenance factor) } [=] \frac{mass\ substrate}{mass\ cells \cdot\ time}$$

General Mass Balance equation

Main difficulties arise from the uncertainties in the kinetic rate expression and the reaction stoichiometry.

General mass balance form:

$$\text{Rate of accum} = \dot{\text{in}} - \dot{\text{out}} + \dot{\text{gen}} - \dot{\text{consume}}$$

Total mass balance:

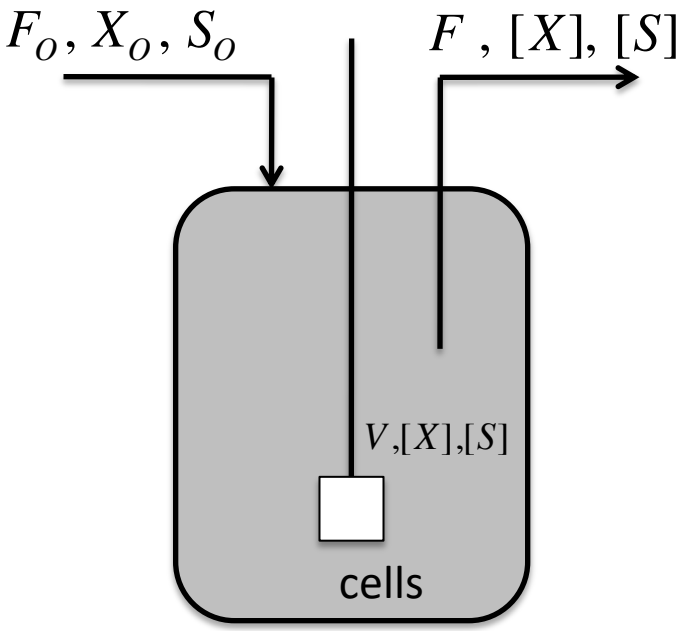
$$\frac{d(V\rho)}{dt} = \rho(F_O - F)$$

Substrate balance:

$$\frac{d(V \cdot [S])}{dt} = F_O S_O - F \cdot [S] - r_S \cdot V$$

Biomass balance:

$$\frac{d(V \cdot [X])}{dt} = -F \cdot [X] + r_X \cdot V$$



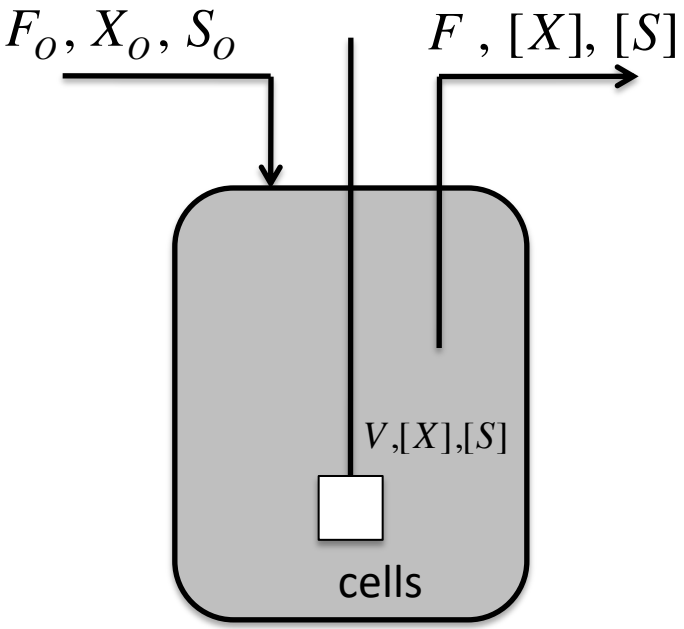
Dimensionality

Dimensions:

$V[=]\ vol$ $\qquad F[=]\ \frac{vol}{time}$

$\rho[=]\ \frac{mass}{vol}$ $\qquad [S][=]\ \frac{mass}{vol}$

$[X][=]\ \frac{mass}{vol}$ $\qquad r_S, r_X[=]\frac{mass}{vol\cdot\ time}$



Rate expressions:

$r_X = \mu[X]$

$\mu = \frac{\mu_m[S]}{K_S + [S]}$

Monod kinetics

(other forms of rate equation may equally apply)

$r_S = \frac{-r_X}{Y_{X/S}} - m\cdot\ [X]$

Batch design

- X_O and S_O at t=0
- After lag phase, biomass grows and substrate is consumed.
- As substrate is exhausted, growth rate slows down and becomes zero when substrate is completely depleted.

Total mass balance:

$$\frac{dV}{dt} = 0$$

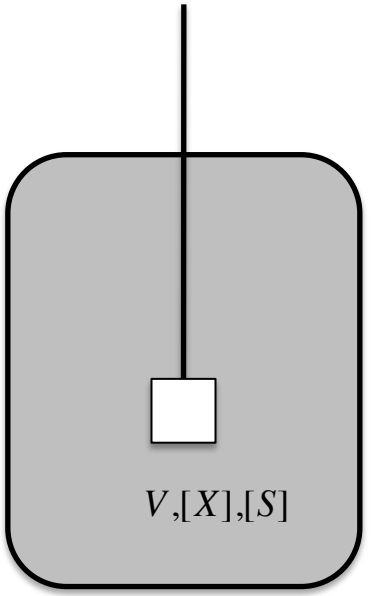
Substrate balance:

$$V\frac{d[S]}{dt} = -r_S V$$

Biomass balance:

$$V\frac{d[X]}{dt} = r_X V$$

+ a model for r_S and r_X



Describes the exponential and limiting growth phases.

Mass yield coefficients

Biomass yield coefficient on substrate

$$Y_{X/S}(t) = \frac{r_X(t)}{r_S(t)}$$

X: Biomass (renewable energy source derived from the cells; mainly C, H, O)
S: substrate

For Batch reactors:

$$\frac{dM_S}{dt} = \cancel{in}^{0} - \cancel{out}^{0} - consume$$

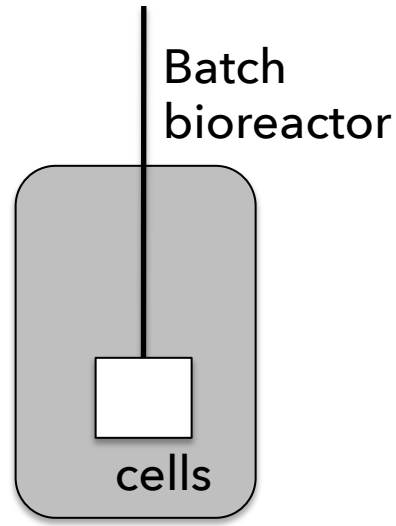
$$V\frac{dC_S}{dt} = -r_S \cdot V$$

$$\frac{dC_X}{dt} = r_X$$

$$Y_{X/S}(t) = -\frac{dC_X(t)}{dC_S(t)}$$

Integrate from time 0 to time t,

$$Y_{X/S} = \frac{C_X(t) - C_X(t=0)}{C_S(t=0) - C_S(t)}$$



Add substrate S @t=0

Mass yield coefficients

Biomass yield coefficient on substrate

$$Y_{X/S}(t) = \frac{r_X(t)}{r_S(t)}$$

X: Biomass (renewable energy source derived from the cells; mainly C, H, O)
S: substrate

For CSTRs:

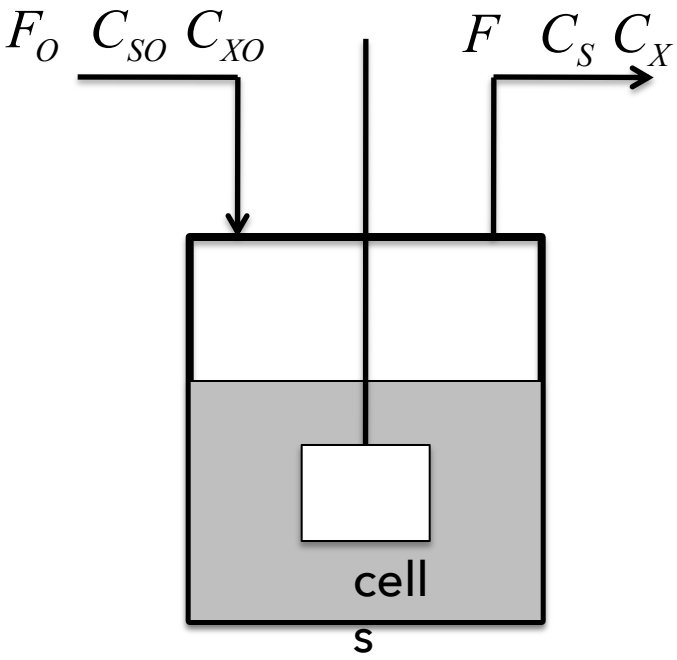
$$\frac{dM_S}{dt} = \dot{\text{in}} - \dot{\text{out}} - \dot{\text{consume}} = 0 \ (\text{steady} - \text{state})$$

$$F_O \cdot C_{SO} - F \cdot C_S - r_S \cdot V = 0$$

$$F_O \cdot C_{XO} - F \cdot C_X + r_X \cdot V = 0$$

$$F_O = F \ (\text{from total mass balance})$$

$$Y_{X/S} = \frac{r_X}{r_S} = \frac{C_X - C_{XO}}{C_{SO} - C_S}$$



Energy yield coefficients

In terms of oxygen uptake :

$$Y_{Q/O2}(t) = \frac{r_Q(t)}{r_{O2}(t)} = \frac{\text{amount of heat released}}{\text{amount of oxygen consumed}}$$

In terms of carbon substrate consumed :

$$Y_{Q/S}(t) = \frac{r_Q(t)}{r_S(t)} = \frac{\text{amount of heat released}}{\text{amount of carbon source consumed}}$$

ATP + H2O → ADP + Pi ΔG° = −30.5 kJ/mol (−7.3 kcal/mol)
ATP + H2O → AMP + PPi ΔG° = −45.6 kJ/mol (−10.9 kcal/mol)

Table 1.1. Typical mass and energy yield values (Roels, 1983; Atkinson and Mavituna, 1991).

Type of yield coefficient	Dimension	Value
$Y_{X/S,aer}$	c-mol / c-mol	0.4-0.7
$Y_{X/S,anaer}$	c-mol / c-mol	0.1-0.2
$Y_{X/O2}$ (Glucose)	c-mol / mol	1-2
$Y_{X/ATP}$	c-mol / mol	0.35
$Y_{Q/O2}$	kJ / mol	380-490
$Y_{Q/CO2}$	kJ / mol	460
$Y_{Q/X,aer}$ (Glucose)	kJ / c-mol	325-500
$Y_{Q/X,anaer}$	kJ / c-mol	120-190