Chapter 6: How Cells Grow

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Presentation Outline:

- Introduction
- Batch Growth Characteristics

  Growth Stages, Effects of Environmental Conditions,

  Product Formation, Mathematical Models
- Continuous Growth Characteristics

  Dilution Rate, Optimum Operation

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}), $\mu \equiv \frac{1}{X}\frac{dX}{dt}$

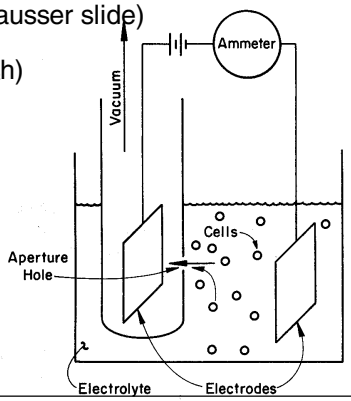
X = cell mass concentration (g / L)

t = time (h)

Product formation is a secondary response.

Determining Cell Concentration

1. Cell number concentration
   a) hemocytometer (Petroff-Hausser slide)
   b) viable cell counts (petri dish)
   c) electronic particle counter



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Determining Cell Concentration (cont.)

2. Cell mass concentration

a) direct methods

→ dry weight (filtration or centrifugation)

→ packed cell volume (centrifugation)

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

Determining Cell Concentration (cont.)

2. Cell mass concentration (cont.)

a) indirect methods

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

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

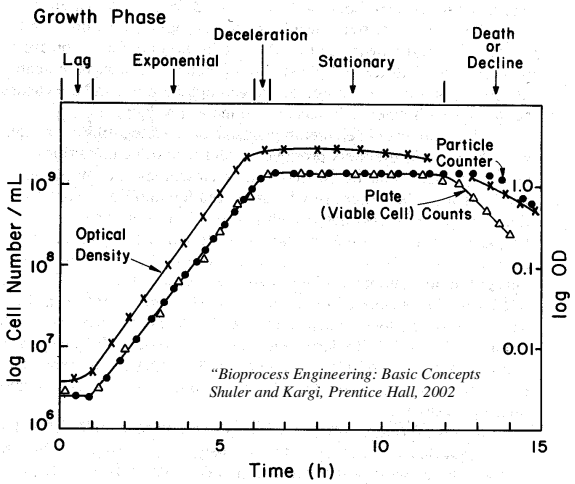
Example 1. NH_4^+ utilization during growth releases H^+, amount of OH^- added is proportional to growth.

Example 2. Luciferin + O_2 + ATP $\xrightarrow[\textit{luciferase}]{}$ light

Batch Growth Curve

Inoculum $[X_o]$ $[S_o]$

growth medium (substrate + nutrients)



Batch Reactor

X = cell Concentration (cells/mL)

Lag Phase

→ no increase in cell numbers

→ induction of enzymes to utilized substrate(s)

→ very important to decrease lag period to ↑ productivity

i. Inoculate with exponential phase cells

ii. Pre-acclimate inoculum in growth media

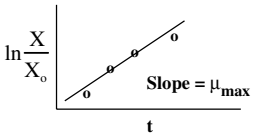
iii. Use high cell inoculum size (5-10% by volume)

Exponential Growth Phase

1. Nutrient and substrate concentrations are large
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), $\frac{X}{X_o} = 2 \Rightarrow \ln(2) = \mu_{max} t_d$

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

4. Balanced growth occurs ⇒ cell composition constant

Deceleration Phase

→ depletion of one or more nutrients

→ accumulation of toxic byproducts of growth

→ unbalanced growth and metabolism shifts for survival

Stationary Phase

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

→ cell growth rate = cell death rate

→ *secondary metabolites* (products) produced

→ *endogenous metabolism* of energy stores can result in maintaining cell viability

→ 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

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

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

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

Effects of Temperature on Cell Growth

μ_{max} doubles for each 10°C increase near T_{opt}

$\mu_{max} = A\, e^{-E_a / RT}$ and

$k_d' = A' e^{-E_d / RT}$

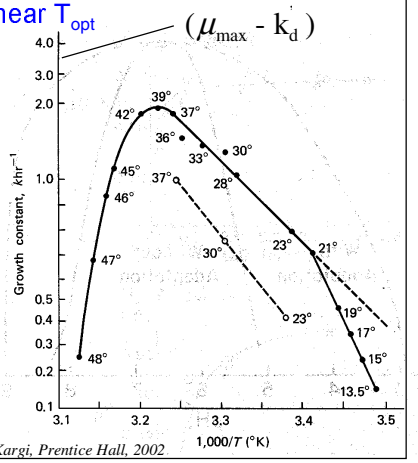
E_a = activation energy for growth

$\approx$ 10 to 20 kcal / mole

E_d = activation energy for death

$\approx$ 60 to 80 kcal / mole

As T ↑, k_d' ↑ faster than μ_{max}, (μ_{max} - k_d') ↓



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pH Effects

→ acceptable pH is ± 1 to 2 pH units

→ pH range varies by organism

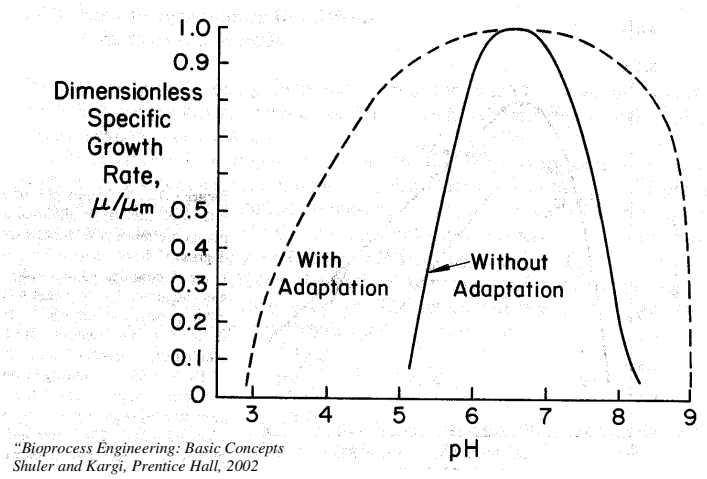
bacteria (most)	pH = 3 to 8
yeast	pH = 3 to 6
plants	pH = 5 to 6
animals	pH = 6.5 to 7.5

→ 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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Dissolved O_2 Effects

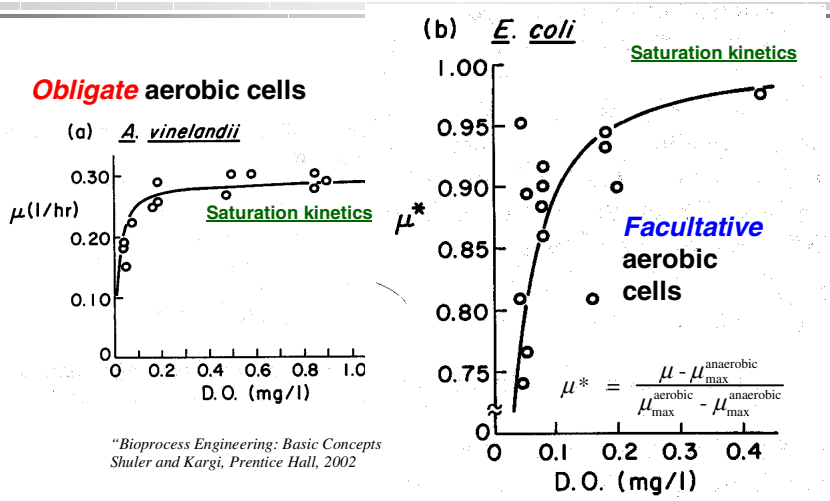
→ O_2 may be a limiting substrate for aerobic fermentation, since O_2 is sparingly soluble in water

→ 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)

Dissolved O_2 Effects (cont.)



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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), ..

Stoichiometric Coefficients for Growth

Yield coefficients, Y, are defined based on the amount of consumption of another material

Growth Yield $$Y_{X/S} = -\frac{\Delta X}{\Delta S}$$

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

Because ΔS changes with growth condition, $Y_{X/S}$ is not a constant

Stoichiometric Coefficients for Growth (cont.)

Typical range of yield coefficients

Growth Yield $$Y_{X/S} = -\frac{\Delta X}{\Delta S}$$

$Y_{X/S} \approx$ 0.4 to 0.6 g dry cells/g substrate consumed

$Y_{X/S,\ oxidized\ S}$ (0.4 to 0.6) < $Y_{X/S,\ reduced\ S}$ (0.6 to 1.0)

Other Yield Coefficients: $$Y_{X/O_2} = -\frac{\Delta X}{\Delta O_2}$$

$Y_{X/O_2,\ reduced\ S}$ (0.15 to 0.3) < $Y_{X/O_2,\ oxidized\ S}$ (0.3 to 1.5)

Stoichiometric Coefficients for Growth (cont.)

Other Yield Coefficients: $Y_{X/ATP} = \frac{\Delta X}{\Delta ATP} \left(\frac{\text{g dry cells}}{\text{mole ATP generated}}\right)$

$Y_{X/ATP}$ > complex medium (a.a.s and nucleic acids available)

$Y_{X/ATP}$ < minimal medium (only inorganic salts and substrate)

Anaerobic Fermentations:

$Y_{X/ATP} \approx 10.5 \pm 2$ (g dry cell wt./mole ATP)

$C_6H_6O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$: 2 moles ATP/mole glucose

$Y_{X/S} \approx Y_{X/ATP}\,(2)/MW_{glu} = (10.5)(2)/180 = 0.12$ g dcw/g glucose

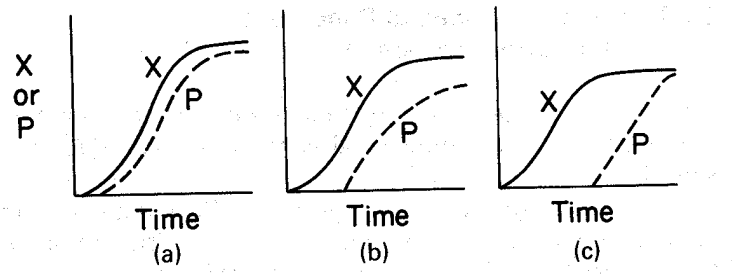
Product Yield Coefficients

1. Growth associated products: products appear simultaneously with cells in culture $q_P = \frac{1}{X}\frac{dP}{dt} = Y_{P/X}\,\mu$

2. Non-growth associated products: products appear during stationary phase of batch growth $q_P = \beta$

3. Mixed-growth associated products: products appear during slow growth and stationary phase $q_P = \alpha\mu + \beta$

Product Yield Coefficients (cont.)



1. $q_P = \frac{1}{X}\frac{dP}{dt} = Y_{P/X}\,\mu$ 3. $q_P = \alpha\mu + \beta$ 2. $q_P = \beta$

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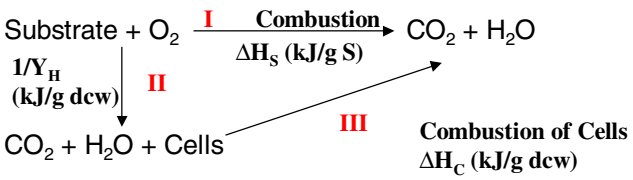
Heat Generation by Growth

Only 40 to 50% of the energy stored in a carbon substrate is converted to biological energy (ATP) during aerobic metabolism. The remainder is released as heat upon conversion to CO_2 and H_2O

Energy Balance: $\left[\begin{matrix}\text{Total Available}\\ \text{Energy of Substrate}\end{matrix}\right]^{I} = \left[\begin{matrix}\text{Energy Released}\\ \text{by Growth}\end{matrix}\right]^{II} + \left[\begin{matrix}\text{Energy Available}\\ \text{in Biomass}\end{matrix}\right]^{III}$



Heat Generation by Growth (cont.)

On a per gram of substrate basis

(1 g S) ΔH_S = (1 g S) $Y_{X/S}/Y_H$ + (1 g S) $Y_{X/S}$ ΔH_C

Solving for Y_H

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

Typical ΔH_C = 20 to 25 kJ/g dcw

Heat Generation by Growth (cont.)

For Substrates:

S	ΔH_S (kJ/g S)	Y_H (g dcw/kJ)
Glucose	15.64	0.072
Methanol	22.68	0.029
Ethanol	29.67	0.043
n-Decane	47.64	0.038
Methane	55.51	0.015

The oxidation state of S has a large effect on 1/ Y_H

Rate of Heat Generation by Growth, Q_{Gr}

$$Q_{Gr} = V_L \mu X \frac{1}{Y_H} \left(\frac{kJ}{hr}\right)$$

Liquid Volume

Cell Mass Concentration

Specific Growth Rate of Cells

Heat can be removed by circulating cooling water through an external jacket around the reactor vessel or through a coiled tube within the reactor.

Modeling Cell Growth

Structured Models:

Model divides cell mass into components (by molecule or by element) and predicts how these components change as a result of growth. These models are very complex and not used very often.

Unstructured Models:

Model assumes balanced growth where cell components do not change with time. Much less complex and much more commonly used. Valid for batch growth during exponential growth stage and also for continuous culture during steady-state operation.

Monod Equation

Similar to Michaelis-Menten Kinetics

Assumes that a single enzyme system is responsible for the uptake of substrate (S), and that S is limited (growth-dependent variable). This is the most common kinetic model for cell growth.

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

μ = specific cell growth rate (hr^{-1})

μ_m = maximum specific cell growth rate (hr^{-1})

S = substrate concentration (g/L)

K_S = Saturation constant (g/L) = S when $\mu = 1/2\ \mu_m$.

Batch Culture Growth Model

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_m S}{K_S + S}$$

We relate changes in S to changes in X through $Y_{X/S}$

$X - X_o = Y_{X/S}(S_o - S)$, or

$S = S_o + X_o/Y_{X/S} - X/Y_{X/S}$

$Y_{X/S}$ = cell mass yield (g dcw/g S consumed)

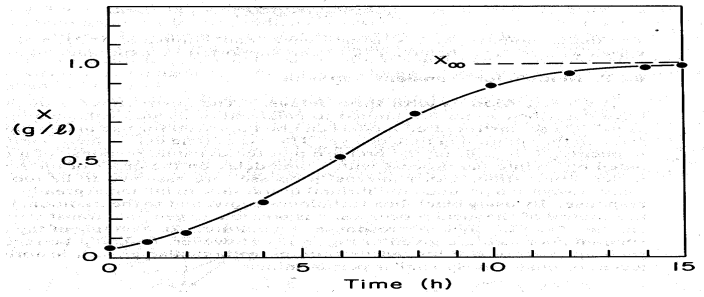
X_o, S_o = initial concentrations of cells and substrate

$$\frac{dX}{dt} = \frac{\mu_m (S_o Y_{X/S} + X_o - X)}{(K_S Y_{X/S} + S_o Y_{X/S} + X_o - X)} X \quad ; \quad \text{at } t = 0,\ X = X_o$$

Batch Culture Growth Model (cont.)

Logistic Equation

$$\frac{(K_S Y_{X/S} + S_o Y_{X/S} + X_o)}{(S_o Y_{X/S} + X_o)} \ln\left(\frac{X}{X_o}\right) - \frac{K_S Y_{X/S}}{(S_o Y_{X/S} + X_o)} \ln\{(S_o Y_{X/S} + X_o - X) S_o Y_{X/S}\} = \mu_m t$$



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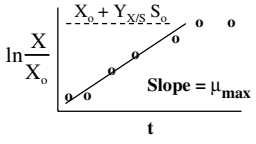
Batch Growth Data and Monod Parameters

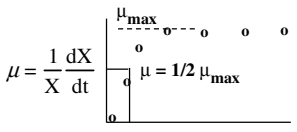
Though the Logistic Equation qualitatively predicts the shape of batch growth, it is not very useful when attempting to determine K_S and μ_{max} from X versus time data.

K_S is determined differently.

K_S is equal to S when μ = 1/2 μ_{max}



μ = 1/X dX/dt needs to be determined from available data, especially data at low S concentrations.



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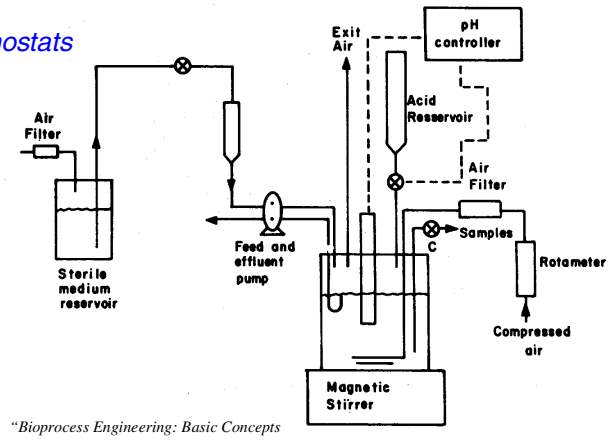
6.4 Cell Growth in Continuous Culture

Automated Chemostats

→ control of pH, temp. agitation, dissolved oxygen

→ sterilization required



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Chemostat as a Tool

→ evaluate K_S, μ_{max}, $Y_{X/S}$ and other system parameters

→ study changes in environment and effects on cell physiology

→ select for cells with desired metabolic capabilities (e.g. selection for cells capable of degrading a toxic compound)

Chemostat Mass Balance

Why derive mass balance equation?

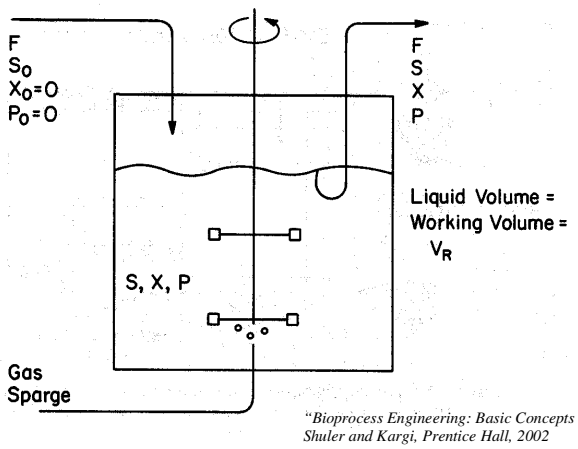
1. Describe dynamics of cell growth, substrate utilization, and product formation.

2. Useful for control of bioreactors.

3. Evaluate kinetic and yield parameters.

4. Determine the optimum values for bioreactor operating parameters.

Ideal Constant-Stirred Tank Reactor Chemostat



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Mass Balance Statement for Cell Mass

$$\begin{bmatrix}\text{mass rate}\\ \text{of cells into}\\ \text{bioreactor}\end{bmatrix} - \begin{bmatrix}\text{mass rate}\\ \text{of cells out}\\ \text{of bioreactor}\end{bmatrix} + \begin{bmatrix}\text{mass rate of cell}\\ \text{growth without}\\ \text{endogenous}\\ \text{metabolism}\end{bmatrix} - \begin{bmatrix}\text{mass rate}\\ \text{of cell loss}\\ \text{by endogenous}\\ \text{metabolism}\end{bmatrix} = \begin{bmatrix}\text{mass rate}\\ \text{of cells}\\ \text{accumulation}\\ \text{in bioreactor}\end{bmatrix}$$

or

$$FX_o - FX + V_R\mu X - V_R k_d X = V_R \frac{dX}{dt}$$

F = in and out volumetric flow rate (L/hr)

X = bioreactor and outlet cell mass concentration (g/L)

X_o = inlet cell mass concentration (g/L)

μ = specific cell growth rate neglecting endogenous metabolism (hr^{-1})

k_d = endogenous cell loss rate constant (hr^{-1})

Steady-State and Sterile Feed

Chemostats are normally operated at steady-state, d/X/dt = 0. Assume a sterile feed (X_o = 0), and k_d is so small that is neglected, k_d = 0.

The cell mass balance equations becomes,

$$\begin{bmatrix}\text{mass rate}\\ \text{of cells out}\\ \text{of bioreactor}\end{bmatrix} = \begin{bmatrix}\text{mass rate of cell}\\ \text{growth without}\\ \text{endogenous}\\ \text{metabolism}\end{bmatrix}$$

or

$$FX = V_R\mu X$$

$$\frac{F}{V_R} = \mu \quad \text{or} \quad \boxed{D = \mu}$$

where $\frac{F}{V_R}$ = D, dilution rate

Substrate Concentration

Using the Monod Equation, we can predict the bioreactor and outlet stream concentration of Substrate.

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

rearranging, $$\boxed{S = \frac{K_S\, D}{\mu_{max} - D}}$$

Bioreactor "Washout" Condition

There is an upper limit on D, or the cells will washed out of the bioreactor.

$$\boxed{D \leq \frac{\mu_{max}\, S_o}{K_S + S_o}}$$

With Endogenous Metabolism

If endogenous metabolism is considered, it is left as an exercise for the students to show that (for k_d ≠ 0)

$$D = \mu - k_d$$

and

$$S = \frac{K_S(D + k_d)}{\mu_{max} - D - k_d}$$

Why is S higher than the case when $k_d = 0$?

Answer: X is lower!

Cell Concentration

How is X affected by D? A similar mass balance equation for S *in the absence* of endogenous metabolism is written to answer this question.

$$FS_o - FS - V_R \mu X \frac{1}{Y^M_{X/S}} - V_R q_p X \frac{1}{Y_{P/S}} = V_R \frac{dS}{dt}$$

S = bioreactor and outlet substrate concentration (g/L)

S_o = inlet substrate concentration (g/L)

$Y^M_{X/S}$ = maximum cell yield coefficient (g cells / g substrate)

$Y_{P/S}$ = product yield coefficient (g product / g substrate)

q_p = specific rate of extracellular product formation $\left(\frac{g\ P}{g\ cells \bullet hr}\right)$

Cell Concentration (cont.)

For the simple case of no product formation ($q_p=0$), steady-state ($dS/dt=0$), and no endogenous metabolism, $k_d=0$.

$$D(S_o - S) = \frac{\mu X}{Y^M_{X/S}}$$

at steady-state, $\mu = D$, and solving for X,

$$X = Y^M_{X/S}(S_o - S)$$

or

$$X = Y^M_{X/S}\left(S_o - \frac{K_S D}{\mu_{max} - D}\right)$$

Effects of Endogenous Metabolism

Thus far, the substrate balance eqn. Has been written assuming that $Y_{X/S}$ is a constant at $Y^M_{X/S}$.

With endogenous metabolism, $\mu = D + k_d$ and with no extracellular product formation, the substrate mass balance is at steady-state,

$$D\frac{(S_o - S)}{X} - \frac{(D+k_d)}{Y^M_{X/S}} = 0$$

rearranging,

$$D\frac{(S_o - S)}{X} - \frac{D}{Y^M_{X/S}} - \frac{k_d}{Y^M_{X/S}} = 0$$

and

$$\frac{D}{Y^{AP}_{X/S}} - \frac{D}{Y^M_{X/S}} - \frac{k_d}{Y^M_{X/S}} = 0$$

$$\frac{1}{Y^{AP}_{X/S}} = \frac{1}{Y^M_{X/S}} + \frac{m_S}{D} = 0$$

where $m_S = \frac{k_d}{Y^M_{X/S}}$ maintenance coefficient based on S.

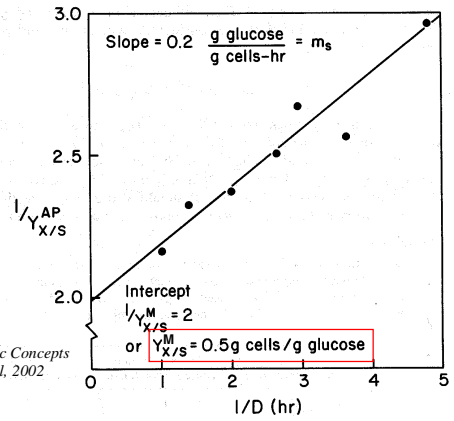
Measurement of Maximum Cell Yield and Maintenance using a Chemostat

From measurements of X, S, S_o, and D in a chemostat experiment at different D values, a double reciprocal plot can be made.

with

$$m_S = \frac{k_d}{Y^M_{X/S}}$$

$$k_d = m_S Y^M_{X/S}$$



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Using a Chemostat to Determine μ_{max} and K_S

From data collected using a chemostat, we can obtain the Monod Equation kinetic parameters.

Data include S at several Dilution Rates (D),

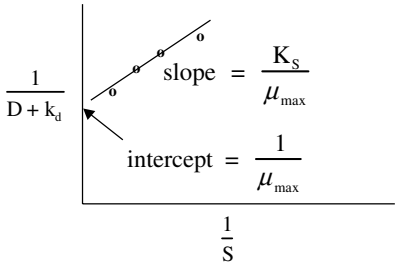
Recall that,

$$D = \mu - k_d$$

$$D = \frac{\mu_{max} S}{K_S + S} - k_d$$

rearranging

$$\frac{1}{D + k_d} = \frac{1}{\mu_{max}} + \frac{K_S}{\mu_{max}} \frac{1}{S}$$



Productivity of a Chemostat

Pr_X = productivity for cell production = DX
Pr_P = productivity for product formation = DP

The dilution rate (D) which maximizes productivity is found by taking dPr/dD = 0 and solving for D ($D_{optimum}$).

For example, $D_{optimum}$ for X with $k_d = 0$ and $q_P = 0$

$$X = Y^M_{X/S}(S_o - \frac{K_S D}{\mu_{max} - D}) \quad \Rightarrow \quad DX = Y^M_{X/S} D(S_o - \frac{K_S D}{\mu_{max} - D})$$

$$\text{take } \frac{d(DX)}{dD} = 0 \text{ and solve for D } (D_{opt})$$

$$D_{opt} = \mu_{max}(1 - \sqrt{\frac{K_S}{K_S + S_o}})$$

K_S is usually << S
so $D_{opt} \sim \mu_{max}$ (washout point)

Product Mass Balance

$$FP_o - FP + V_R q_P X = V_R \frac{dP}{dt}$$

$$\text{at steady - state, } dP/dt = 0 \text{ and for } P_o = 0$$

$$DP = q_P X \text{ or } P = \frac{q_P X}{D}$$

for $k_d = 0$, no endogenous metabolism

$$S = \frac{K_S D}{\mu_{max} - D} \text{ from X mass balance}$$

$$X = Y^M_{X/S}(S_o - S)\frac{D}{(D + q_P \frac{Y^M_{X/S}}{Y_{P/S}})} \text{ from S mass balance}$$

Product Mass Balance

for $k_d \neq 0$, with endogenous metabolism

$$S = \frac{K_S (D + k_d)}{(\mu_{max} - D - k_d)} \quad \text{from X mass balance}$$

$$X = Y^M_{X/S}(S_o - S)\frac{D}{(D + k_d + q_P \frac{Y^M_{X/S}}{Y_{P/S}})} \quad \text{from S mass balance}$$

to determine D for optimum P formation,

$$\frac{d(DP)}{dD} = 0 \quad \Rightarrow \quad \text{solve for } D_{opt}$$

A Summary of Chemostat Response to D