Comments on Cell Growth (Chapter 6)

Overview of cell growth & models models

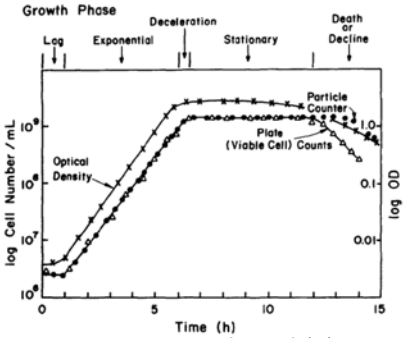
Harvard Law:

- Under controlled conditions of light, temperature, humidity, and nutrition, the organism will do as it damn well pleases.

Growth patterns & kinetics in batch cultures

- Lag
- Exponential
- Deceleration
- Stationary
- Decline/Deat

Quantifying growth & death kinetics



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Updated: October 9, 2017

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Quantifying cell concentration

Determine cell number density

- Direct counting – Petroff-Hausser slide or a *hemocytometer*
- Growth on agar for viable cell count
- Particle count from electrical resistance measurements
- Scattered light intensity

Determine cell mass concentration

- Direct measurements
  - Sample centrifuged/filtered, washed, dried, then weighed
  - Optical scattering / turbidity
- Indirect
  - Based on substrate usage and/or product formation

Growth patterns in batch cultures

Lag

- Microorganisms reorganize to get acclimated to their new environment
- Cell mass may increase but little increase in cell density

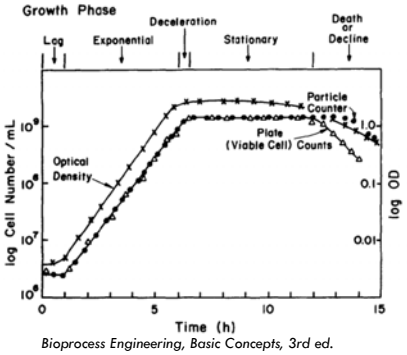
Exponential growth phase

- Period of balanced growth – all components if a cell grow at the same rate

$$\frac{dX}{dt} = \mu_{net} X \quad \Rightarrow \quad X = X_0 \, e^{\mu_{net} t}$$

- Doubling time for cell mass, τ_d

$$\tau_d = \frac{\ln 2}{\mu_{net}}$$



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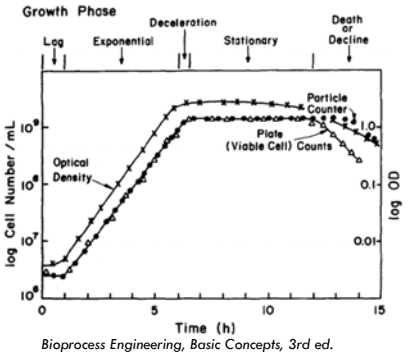
Growth patterns in batch cultures

Deceleration

- Unbalanced growth, possibly due to depletion of nutrients and/or build up of toxins
- Usually a very short period

Stationary

- Growth rate matched by death rate
- What may be happening?
  - Total cell mass concentration constant but number of viable cells decrease
  - Viable cell mass may decrease
  - Cells may not grow but may produce secondary metabolites



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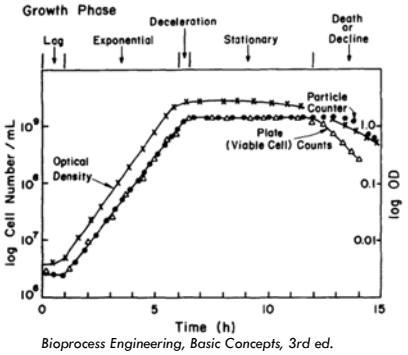
Growth patterns in batch cultures

Decline/Death

- Death of cells occur even before the start of this phase
- Rate of death usually follows first order kinetics

$$\frac{dN}{dt} = -k_d' N \quad \Rightarrow \quad N = N_S \, e^{k_d' t}$$



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Yield expressions

Have already looked at defining yield expressions based on stoichiometry of reactions

The apparent yield relates the creation of a product (cell mass included) to all reactant consumed (possibly through other side reactions)

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

May also define other yield relationships

$$Y_{X/O_2} = -\frac{\Delta X}{\Delta O_2} \qquad Y_{P/S} = -\frac{\Delta P}{\Delta S}$$

Microbial products classifications

Growth-associated products formed simultaneously with microbial growth

$$q_p = \frac{1}{X}\frac{dP}{dt} = Y_{P/X}\mu_g$$

Nongrowth-associated products formed during stationary phase (with no apparent cell mass growth)

$$q_p = \beta = \text{constant}$$

Mixed-growth-associated products formed during slow growth & stationary phases

$$q_p = \alpha\mu_g + \beta$$

Example 6.1
Yield & net growth rate

Plotting the log of cell concentration vs. time shows that the growth period is about 16 to 34 hours (or so)

- Using the end points

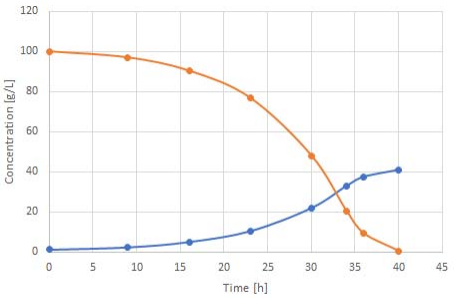
$$\mu_{net}\frac{\ln(X_2/X_1)}{t_2-t_1}=\frac{\ln(37.5/5.1)}{36-16}=0.0998$$

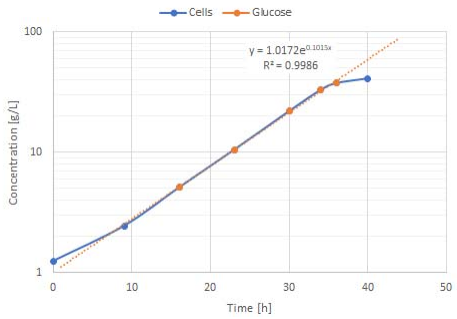
- From linear regression's exponential term

$$\mu_{net}=0.1015$$

- Yield from overall values

$$Y_{X/S}=-\frac{41-1.25}{100-0.63}=0.4$$





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Environmental conditions can affect growth kinetics

Temperature

- Classifications
  - Psychrophiles (less than 20°C)
  - Mesophiles (20 to 50°C)
  - Thermophiles (greater than 50°C)
- Rate
  - Growth rates doubles about every 10°C (when in range). Too high & thermal death may occur
- Product formation
  - Yield may increase but so too energy maintenance requirements

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Environmental conditions can affect growth kinetics

Hydrogen-ion concentration (pH)

- Optimal pH for growth may be different from that for product formation
- Typical optimal ranges
  - Bacteria: 3 to 8
  - Yeast: 3 to 6
  - Molds: 3 to 7
  - Plant cells: 5 to 6
  - Animal cells: 6.5 to 7.4
- During fermentation pH can vary
  - NH_4^+ as nitrogen source, H^+ released, & pH decreases
  - Nitrate as nitrogen source, H^+ removed to form NH_3, & pH increases
  - Acids may be created or consumed

Environmental conditions can affect growth kinetics

Dissolved oxygen (DO)

- May be a limiting substrate in aerobic fermentations since O_2 is not very soluble in water
- May also limit the growth rate since anaerobic growth is slower than aerobic
- When oxygen transfer is the rate-limiting step then the oxygen consumption is equal to the rate of oxygen transfer to the water. If maintenance requirement is negligible…

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

Environmental conditions can affect growth kinetics

Redox potential
- Complex function of dissolved oxygen, pH, & other ion concentrations (that act as reducing and oxidizing agents)

Dissolved CO_2
- Require some CO_2 for metabolic functions, but…
- Too high a concentration may be toxic

Ionic strength
- Affects transport of certain nutrients in & out of cells…
- Metabolic functions of cell
- Solubility of certain nutrients (e.g., dissolved oxygen)

Substrate concentration
- Too high of a concentration can lead to substrate inhibition

Quantifying growth kinetics

"… complete description of the growth kinetics of a culture would involve recognition of the structured nature of each cell and the segregation of the culture into individual units (cells) that may differ from each other."

The degree of realism & complexity required in a model depends on what is being described & what answers are desired

Simple models – *unstructured* & *nonsegregated* models
- Ignore the structured nature of the cell mass
- Ignore different types of cell mass present

Some growth models

Substrate-Limited Growth (*Moser* equation, *Monod* for n=1)

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

Substrate-Limited Growth (*Contois* equation)

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

Noncompetitive Substrate Inhibition

$$\mu_g = \frac{\mu_m}{\left(1 + \frac{K_S}{S}\right)\left(1 + \frac{S}{K_1}\right)}$$

Competitive Substrate Inhibition

$$\mu_g = \frac{\mu_m}{K_S\left(1 + \frac{S}{K_1}\right) + S}$$

Noncompetitive Product Inhibition

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

Competitive Product Inhibition

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

Some more growth models

For rapidly growing dense cultures

$$\mu_g = \frac{\mu_m S}{K_{S0} S_0 + S}$$

$$\mu_g = \frac{\mu_m S}{K_{S1} + K_{S0} S_0 + S}$$

Alternatives to the Monod equation

- Blackman

$$\mu_g = \begin{cases} \mu_m & S \geq 2K_S \\ \frac{\mu_m}{2K_S} S & S < 2K_S \end{cases}$$

- Tessier

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

- Contois

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

Logistic equation

When plotted the growth curve has a sigmoidal shape (S shape)

Starting with the Monod equation for rate of cell mass growth…

$$\frac{dX}{dt} = \mu_g X = \frac{\mu_m S}{K_s + S} X$$

include relationship of substrate consumption to cell mass growth…

$$\frac{dX}{dt} = \mu_g X = \frac{\mu_m \left(Y_{X/S} S_0 + X_0 - X\right)}{K_s + Y_{X/S} S_0 + X_0 - X} X \quad \text{since} \quad Y_{X/S} = \frac{X - X_0}{S_0 - S}$$

And this integrates to…

$$\frac{K_s + Y_{X/S} S_0 + X_0}{Y_{X/S} S_0 + X_0} \ln\left(\frac{X}{X_0}\right) - \frac{K_s}{Y_{X/S} S_0 + X_0} \ln\left(\frac{Y_{X/S} S_0 + X_0 - X}{Y_{X/S} S_0}\right) = \mu_m t$$

Logistic equation

Can be put into a more generalized form

$$\mu_g = k\left(1 - \frac{X}{X_\infty}\right)$$

$$\frac{dX}{dt} = \mu_g X = kX\left(1 - \frac{X}{X_\infty}\right)$$

$$X = \frac{X_0 e^{kt}}{1 - \frac{X_0}{X_\infty}\left(1 - e^{kt}\right)} \quad \Rightarrow \quad -kt = \ln\left(\frac{\frac{X_0}{X} - \frac{X_0}{X_\infty}}{1 - \frac{X_0}{X_\infty}}\right)$$

Chemostat data, cell growth only

Steady state, only growth of cells, substrate only for cell growth:

$$\mu_{net} = \mu_g = D$$

$$\frac{D(S_0 - S)}{X} = \frac{\mu_g}{Y^M_{X/S}} \quad \Rightarrow \quad Y^M_{X/S} = \frac{X}{S_0 - S}$$

With Monod growth kinetics:

$$\mu_g = D = \frac{\mu_m S}{K_S + S} \quad \Rightarrow \quad \frac{1}{D} = \left(\frac{1}{\mu_m}\right) + \left(\frac{K_S}{\mu_m}\right)\frac{1}{S}$$

Chemostat data with cell death

Steady state, growth & death of cells, substrate only for cell growth:

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

$$\frac{D(S_0 - S)}{X} = \frac{\mu_g}{Y^M_{X/S}} \quad \Rightarrow \quad \frac{D}{Y^{app}_{X/S}} = \frac{D + k_d}{Y^M_{X/S}} \quad \text{where} \quad Y^{app}_{X/S} = \frac{X}{S_0 - S}$$

$$\frac{1}{Y^{app}_{X/S}} = \frac{1}{Y^M_{X/S}} + \frac{m_s}{D} \quad \text{where} \quad k_d = m_s Y^M_{X/S}$$

With Monod growth kinetics:

$$\mu_g = D - k_d = \frac{\mu_m S}{K_S + S} \quad \Rightarrow \quad \frac{1}{D - k_d} = \left(\frac{1}{\mu_m}\right) + \left(\frac{K_S}{\mu_m}\right)\frac{1}{S}$$

Summary

Focused on unstructured & nonsegregated models

4 major phases of cell growth in a batch culture

- May need a different model for each phase
- During exponential growth phase the time constant (k) can be determined from the linear portion of the log(concentration) vs time curve
- Cell concentration vs. time is sigmoidal (s-shaped) in nature. Can derive expressions from growth model or just use a general logistics model

Model parameters can be determined from batch results or from steady-state chemostat results

- Batch models require calculation of time derivatives from measured data (potentially with a lot of scatter)
- Chemostat models require difference of inlet & outlet concentrations

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