Modeling Cell Growth

How much time passes between cellular divisions?

Doubling time, t_d

- $G = 2^{\frac{t}{t_d}}$
  - G = number of generations
  - T = time that has passed
  - T_d = doubling time
- Assumption: all cells are the same and have the same doubling time and grow at the same rate etc.
  - This assumption is not always true!!!

Modeling Binary Division

- $X = X_o 2^{\frac{t}{t_d}}$
  - X = number of cells
  - X_o = cells at initial conditions
- $\mu_d = \frac{1}{t_d}$
- $X = X_o 2^{\mu_d t}$
- $\mu = \ln 2 \mu_d = \frac{\ln 2}{t_d}$
- $X = X_o e^{\mu t}$
- $\ln X = \ln X_o e^{\mu t}$
- $\ln X = \mu t + \ln X_o$
- $\frac{1}{X} dX = \mu dt + 0$
- $\frac{1}{X}\frac{dX}{dt} = \mu$
- u = specific growth rate

Coupling Cell Growth to Substrate Use

Yield coefficient: Yx/s = delX/delS = dX/dS (* dS/dt = dX/dt)

- "Yield of cells (X) per utilized substrate (S)"
- Units = g (cells)/g (substrate) [dimensionless]

Assumptions that go into saying that the yield coefficient × dS/dt is part of Monod equation (part in parentheses above):

- Accumulation = in - out + generation - consumption
- $\frac{dX}{dt}$ = generation
  - No consumption == no death (not valid... Slow down)
  - for now, only assuming closed system, cells don't die, but this won't always be the case

Y subscript is a hint to which is the numerator vs denominator

- $Y_{X/S}\frac{dS}{dt} = \frac{dX}{dS}\frac{dS}{dt} = \frac{dX}{dt}$
- $\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} = Y_{S/X}\frac{dX}{dt}$

Derivation of Growth Expression

- Continuity equation: Accumulation = in – out + generation – consumption
  - Mass is conserved
  - Accumulation = VdX
  - In = FXodt
  - Out = FXdt
  - Generation = VμXdt
  - Consumption = 0 (assume no appreciable death)
- $VdX = FX_o dt + V\mu X dt - FX dt$
  - Divide by Vdt
- $\frac{dX}{dt} = DX_o + (\mu - D)X$
  - $D = \frac{F}{V}$
    - Dilution rate: how quickly are you replacing the volume of the reactor?
  - $D = \frac{1}{t_{residence}}$
  - Assume that feed is sterile—no new cells in
- $\frac{dX}{dt} = (\mu - D)X$
  - "Net difference between μ and D, times X)
  - Cell growth – cells leaving
  - At steady state, dX/dt = 0
    - cell growth = cells leaving

Derivation of Substrate-Growth Coupling Expression

- $VdS = FS_o dt - FSdt + 0 - Y_{S/X}\mu XVdt$
  - Assume that generation = 0
    - Not generating substrate, but not always the case
    - Could feed the reactor a different substrate to generate the substrate to make the final product
    - Think about which terms are useful for each situation
  - Consumption: rate of cells growing multiplied by yield coefficient
    - Don't include dilution because it doesn't affect substrate
  - Divide by volume, dt; Dilution factor stays the same as growth expression
- $\frac{dS}{dt} = D(S_o - S) - Y_{S/X}\mu X$
  - Net flux of substrate
  - Assume steady state
- $\frac{dS}{dt} = 0 = D(S_o - S) - Y_{S/X}\mu X$
  - Use what was found above; know that μ = D
- $\mu = D = \frac{\mu_{max} S}{K_m + S}$
  - Ideally get equations into this form: $y = mx + b$
    - Y = dependent variable – things we don't control, result of choices we make (i.e. S)
    - X = independent variable – things we have control over (i.e. S_o)
- $S = \frac{K_m D}{\mu_{max} - D}$
  - Independent variable = S
  - Dependent variable = D
  - Not exactly y = mx + b, but can segregate knowns from parameters to discover
- $\frac{dS}{dt} = 0 = D(S_o - S) - Y_{S/X}\mu X$
- $\mu(S_o - S) = Y_{S/X}\mu X$
- $\mu(S_o - S) = Y_{S/X}\mu X$
- $X = \frac{S_o - S}{Y_{S/X}}$
  - Substitute in S from above
  - $Y_{X/S} = 1/Y_{S/X}$
- $X = Y_{X/S}(S_o - \frac{\mu_{max} D}{K_m D})$
  - Fix one = D, vary other = S_o to look at response
- $X = Y_{X/S}S_o - \frac{Y_{X/S} K_m D}{\mu_{max} - D}$
  - $Y = mx + b$ format!
  - Slope = $Y_{X/S}$
  - Independent variable = S_o
  - B = last term

Cells consume substrate because they are growing *and* because they are alive – apparent yield coefficient in data, not actual yield coefficient!!!

- In a batch reactor: $\frac{dS}{dt} = \left(\frac{m_e}{\mu} + Y_{S/X}\right)\mu X = Y_{S/X,app}\mu X$
  - $Y_{S/X,app} = \left(\frac{m_e}{\mu} + Y_{S/X}\right)$
  - $Y_{S/X,app}$ = observed disappearance of S per appearance of X
- In a CSTBR at steady state μ = D
  - $Y_{S/X,app} = \left(\frac{m_e}{\mu} + Y_{S/X}\right) = \left(\frac{m_e}{D} + Y_{S/X}\right)$
  - $Y_{S/X,app} = m_e \frac{1}{D} + Y_{S/X}$
  - $Y = mx + b$ format!
  - Slope is maintenance coefficient (m_e), intercept is true yield coefficient

Using Levenspiel Equation

- $\frac{dX}{dt} = \mu_{max}\left[\frac{S}{S+K_m}\right][1 - \frac{P}{P_{max}}]^n X$

Maiorella Ethanol Model

- μ = Eυ
- $\upsilon = \upsilon_{max}\left[\frac{S}{S+K_m}\right][1 - \frac{P}{P_{max}}]^n$
  - E = efficiency of cell mass production
  - Specific ethanol production rate
  - Substrate concentration
  - υ_{max} = maximum specific production rate
  - K_m = Monod constant
  - n = toxic power constant
  - P_{max} = maximum product concentration
- Productivity of product is primary equation, everything else is linked to that

Luedeking-Piret Model

- $\frac{dS}{dt} = \alpha\frac{dx}{dt} + \beta x$
  - Substrate consumption = growth associated + non-growth associated
- $\frac{dX}{dt} = \mu X = \frac{\mu_{max}[S]}{K_S + [S]}X$, or other function!
  - α = $Y_{S/X}$ = Yield coefficient
  - β = m_e = maintenance coefficient

Monod Equation

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

- Where X = cell concentration (g/L)
- μ = specific growth rate

$X_t = X_0 e^{\mu t}$

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

- X = cell concentration [g/L]
- μ_{max} = maximum specific growth rate
- S = concentration of limiting nutrient
- K_S = Monod coefficient

Homogeneous

Exponential growth

- Cells grow as quickly as possible

Balanced growth

- All cells are equal metabolically/physiologically
- All cells grow at same rate: "synchronization of cell division" – Campbell 1957
- **Because all cells are the same, we can assume that the mass of each cell is the same**
  - X = # cells/L becomes X = g cells/L

Substrate controlled

- Some nutrient is the primary driver of how fast cells can grow
- Assume that it is the carbon source, but could also be oxygen

Saturated vs. limited growth

- Fundamental discovery that Monod pointed out in dissertation work was that cells, like enzymes, have a saturation
  - MM enzyme kinetics: 1917, a couple decades before Monod
  - There is some maximum growth rate for cell division; give more food and don't grow any faster
  - Below that threshold, they will grow, but more slowly because there aren't enough resources for them to grow!

Fermentation types

1. substrate consumption/product formation proportional to rate of growth – product is part of central metabolism
2. substrate and growth proportional; after some time, product formation proportional but not equal – product secondary or intermediate
3. no relationship between product formation and cell growth – things just needed to maintain what you have to survive (antibiotics)

Maximum Cell Concentration

- $X_m = Y_{X/S}[S_o + K_S - \{K_S(S_o + K_S)\}^{\frac{1}{2}}]$
- $\overline{x_m} = Y_{X/S}S_o$ if $S_o >> K_S$
  - Maximum cells = yield coefficient * substrate available
- Cell concentrations rapidly plummet to zero if dilution rate fluctuates over critical
  - Usually want to run just below in case of fluctuations

Steady State to Find Critical Condition

- $X = 0 = Y_{X/S}\left(S_o - \frac{K_S D}{(\mu_{max} - D)}\right)$
  - $S_o = \frac{K_S D_C}{(\mu_{max} - D_C)}$
  - $D_C = \frac{\mu_{max} S_o}{(K - S_o)}$ (critical dilution rate)
  - When $S_o >> K_s$, μ = D_C = μ_{max}
- Graphing biomass output (concentration of cells per hour) vs. D
  - Biomass output = DX
  - Constant S_o
  - Critical point is peak, just before washout when productivity goes to zero
- Zooming in on the roll off point – critical point doesn't quite meet the maximum productivity

Maximum productivity

- Rate of cell output = R = DX
- $R = DY_{X/S}[S_o - \frac{K_S D}{(\mu_{max} - D)}]$
- $\frac{dR}{dD} = 0 = \frac{d}{dD}[DY_{\frac{X}{S}}(S_r - \frac{K_S D}{(\mu_{max} - D)})]$

2,3-Butanediol

- Plot xylose, 2,3-butanediol concentration, ln(cell weight) vs hours.
  - Ln of cell dry weight because cells grow exponentially, but ln makes it appear linear!
  - When no 2,3-butane diol, just enough oxygen to respirate
  - When cell dry weight goes off of the linear, oxygen deprived so 2,3-butanediol was formed
  - Rate of aeration constant throughout experiment
  - When rate of oxygen consumption exceeds rate of oxygen supply → fermentation occurs

Modeling Approach

- Cellular function is ATP constrained
  - "Gibbs Free Energy is electron-rich"
- ATP use is prioritized: cell maintenance then cell growth
  - Cells try to stay alive first, then divide
- Metabolism is regulated to maximize ATP production
  - Cells make as much ATP as they can, given environmental constraints
- O_2 is required for maximum ATP generation
  - Respiration is preferred until O_2 deficiency
- When O_2 is limiting, anaerobic metabolism is induced to make up the ATP deficiency
  - Cell growth is scaled to make up for deficiency

hase I – Aerobic Growth

- $\frac{dX}{dt} = \mu_{max} X$
- $\frac{dS}{dt} = -(Q_{SA} + Q_{SR} + Q_{SF})MW_{xylose}$
- $Q_{Si} = [\frac{mol}{L\,hr}]$
- $Q_{Sa} = \frac{1\,mol\,Xylose}{120\,g\,Cells}\frac{dx}{dt} = \frac{1}{120}\frac{dx}{dt}$
- $Q_{Sr} = \frac{3}{70}\left(\frac{dX}{dt}\frac{1}{Y_{ATP,1}} + m_{e,1}\right)X$
- $Q_{Sa} = \frac{1}{120}\frac{dx}{dt}\,[\frac{mol}{L\,hr}]$
- $MW_{xylose} = 150\frac{g}{mol}$
- $Q_{Sa}150\frac{g}{mol} = Y_{\frac{S}{X}}\frac{dX}{dt} = \frac{1}{120}\frac{dX}{dt}$
- $Y_{\frac{S}{X}} = \frac{150}{120} = 1.25$

Phase II – When is O_2 limiting?

- Xylose + 5 O_2 → 5 CO_2 + 70/3 ATP
- Limiting when OUT > Oxygen Transfer Rate
- 5 $Q_{SR} > k_L aC^*$
- $N_A = Hk_L a(p - p^*) = k_L a(C - C^*)$
  - H = Henry's law constant
  - K_L = lumped transfer coefficient
  - A = surface area of bubble
  - P = partial pressure
  - C = concentration of O_2 in the water/bubble
  - High temperatures = low solubility of a gas
  - Concentration decreases as distance from bubble increases
  - At the limit, no oxygen in solution, rate of mass transfer = C*
    - Once molecules go into solution, bacteria uptake them
- Metabolism is balanced so that oxidative phosphorylation (electron transport system, ETS) is saturated
  - NADH + ½ O_2 + H^+ → ATP + NAD^+
    - dNADH/dt = 0
      - generation = consumption
    - consumption = Q_{ETS} = 2 $k_L aC^*$
    - generation = 10 Q_{SR} + 5/6 Q_{SF}
    - Assumption: cell will self-optimize to make the most ATP possible
      - Only do fermentation out of desperation
    - Q_{ETS} = dNADH/dt
    - Q_{ETS} = -2$k_L$ac* + 10 Q_{SR} + 5/6 Q_{SF}
  - Maximize ATP generation!
    - Q_{SR} = 1/10 (Q_{ETS} – 5/6 Q_{SF})
    - Consumption = μ_{max} X/Y_{ATP} + m_eX
    - dATP/dt = 0 → generation = consumption
    - generation = 10/3 Q_{SR} + 5/3 Q_{SF} + 2 Q_{ETS}
    - Q_{SF} = 18/25 (μ_{max}X/Y_{ATP} + m_eX – 7/3 Q_{ETS})

Phase III - Fermentation

- Q_{ETS} = 2 $k_L aC^*$
- Q_{SF} = 18/25 (μ_{max}X/Y_{ATP} + m_eX – 14/3 2 $k_L aC^*$)
- Q_{SR} = 1/10 (2 $k_L aC^*$ - 5/6 Q_{SF})
- Q_{SA} 1/120 μ_{max}X
- dX/dt = μ_{max}X - k_dPX
- dP/dt = 5/6 Q_{SF} $MW_{2,3butanediol}$
- $MW_{2,3butanediol}$ = 90 g/mol
- dS/dt = -(Q_{SR} + Q_{SF} + Q_{SA}) MW_{xylose}

Effect of Dissolved Oxygen on Cell Growth

- $\mu = \frac{\mu_{max} DO}{K_S^{DO} + DO}$
  - $K_S^{DO} \ll DO$
  - μ ~ μ_{max}