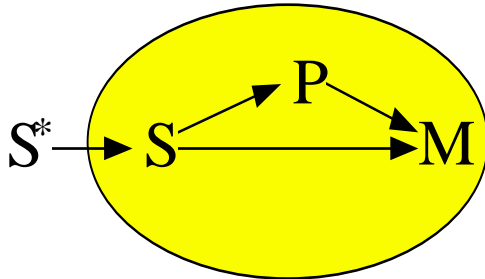


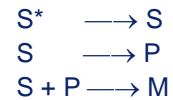
Structured Model of Cell Growth with Membrane Transport, Precursor, and Macromolecules.

Instructor: Nam Sun Wang

**Model Description In English.**

This structured model considers three events:

1. Transport of substrate S across cell membrane
2. Conversion of S into precursor P
3. Conversion of P into macromolecule M, with energy supplied from S



And M is the catalyst of each of the three events.

From the description above and the schematic diagram, we find that the **extrinsic** state variables are:

- S* ... extracellular substrate concentration (g/L reactor)
- S ... intracellular substrate concentration (g/L reactor)
- P ... intracellular precursor concentration (g/L reactor)
- M ... intracellular macromolecule concentration (g/L reactor)

Once the substrate is transported into the cell, it is considered to be part of the cell. In other words, S is a cellular component. In addition, P and M are other cellular components. We define another useful, more readily observable extrinsic state variable for cells.

$$T = S + P + M \quad \dots \text{dry weight of cells or biomass concentration (g cells/L reactor)}$$

It is related to the cell volume V by the cell density ρ (specific gravity), which we assume to be constant. It is also related to cell surface area A by a constant factor a (specific surface area), which we also assume to be constant.

$$V = \frac{T}{\rho} \quad \dots \text{cell volume (cm}^3 \text{ cells/L reactor)}$$

$$A = a \cdot T \quad \dots \text{cell surface area (cm}^2 \text{ cells/L reactor)}$$

Note that everything hereafter is a direct consequence of this model description.

Model Derivation.

Step 1. Prepare/define variables to be used.

Since we should write kinetic equations in terms of concentrations that exist at the reaction site, we define the following **intrinsic** concentrations which have units of (g component/cm³ cells).

$$s' = \frac{S}{V} = \frac{S}{T} \cdot \rho \quad p' = \frac{P}{V} = \frac{P}{T} \cdot \rho \quad m' = \frac{M}{V} = \frac{M}{T} \cdot \rho \quad \dots \text{ cell volume based intrinsic concentration variables}$$

All intrinsic concentrations sum up to cell density. $s' + p' + m' = \rho$

If we assume the cell density ρ is constant, we can simply use mass fractions, which have units of (g cell component/g cells), as the intrinsic concentrations.

$$s = \frac{S}{T} \quad p = \frac{P}{T} \quad m = \frac{M}{T} \quad \dots \text{ cell mass based intrinsic concentration variables.}$$

Naturally, all fractions sum up to unity. $s + p + m = 1$

In an analogous manner, we can also define intrinsic surface area.

$$a = \frac{A}{T} \quad \dots \text{ specific surface area (cm}^2\text{/g cells), which we assume to be constant.}$$

Assuming spherical cells with radius R , the specific surface area typically has the following value.

$$a = \frac{4 \cdot \pi \cdot R^2}{\frac{4}{3} \cdot \pi \cdot R^3 \cdot \rho} = \frac{3}{R \cdot \rho}$$

Example: 10 μ m neutrally buoyant cell: $R := 2.5 \cdot 10^{-6} \cdot \text{m}$ $\rho := 1 \cdot \frac{\text{gm}}{\text{cm}^3}$ $a := \frac{3}{R \cdot \rho}$ $a = 1.2 \cdot 10^4 \cdot \frac{\text{cm}^2}{\text{gm}}$

Step 2. Specify/Derive kinetic expression for each of the events in the model. This amounts to translating the English description into a mathematical form. We approach this task one event at a time.

- Event #1. Transport across the cell membrane is catalyzed by the enzyme permease, which is inhibited by the product (i.e., intracellular substrate in this case). In the absence of additional information, pick any appropriate product inhibition kinetic expression (e.g., competitive, uncompetitive, or noncompetitive). The following flux rate expression is a product of three terms: the specific surface area, the Michaelis-Menten saturation kinetics, and the product inhibition term.

$$r_1 = a \cdot \frac{k_1 \cdot S_e}{K_1 + S_e} \cdot \frac{K_{li}}{K_{li} + s} \quad \text{in (g substrate/g cells/hr)}$$

- Event #2. Formation of P is catalyzed by M and follows Michaelis-Menten saturation kinetics.

$$r_2 = \frac{k_2 \cdot m \cdot s}{K_2 + s} \quad \text{in (g precursor/g cells/hr)}$$

- Event #3: Formation of M is catalyzed by M and follows Michaelis-Menten saturation kinetics. For a multiple reactant system, we employ the multiplicative form.

$$r_3 = \frac{k_3 \cdot m \cdot s}{K_{3s} + s} \cdot \frac{p}{K_{3p} + p} \quad \text{in (g macromolecules/g cells/hr)}$$

Step 3. Derive the dynamic equations for the state variables based on the concept of material balance and the rate expressions from the last step.

Dynamic equations for the extrinsic variables.

$$\frac{d}{dt}S_e = -r_1 \cdot T$$

$$\frac{d}{dt}S = r_1 \cdot T - r_2 \cdot T - \alpha \cdot r_3 \cdot T \quad \leftarrow \text{Here, we assume each gram of S goes into one gram of P, but } \alpha \text{ gram of S is needed to assimilate one gram of M.}$$

$$\frac{d}{dt}P = r_2 \cdot T - r_3 \cdot T \quad \leftarrow \text{Here, we assume one gram of P goes into one gram of M. Thus, a total of } 1 + \alpha \text{ gram of S is needed for each gram of M.}$$

$$\frac{d}{dt}M = r_3 \cdot T$$

Based on the definition of total cell mass T, we have:

$$\frac{d}{dt}T = \frac{d}{dt}S + \frac{d}{dt}P + \frac{d}{dt}M = (r_1 - \alpha \cdot r_3) \cdot T$$

Thus, the **specific growth rate** μ is:

$$\mu = \frac{1}{T} \cdot \frac{d}{dt}T = r_1 - \alpha \cdot r_3 = a \cdot \frac{k_1 \cdot S_e}{K_1 + S_e} \cdot \frac{K_{1i}}{K_{1i} + s} - \alpha \cdot \frac{k_3 \cdot m \cdot s}{K_{3s} + s} \cdot \frac{p}{K_{3p} + p}$$

Dynamic equations for intrinsic state variables.

$$\frac{d}{dt}(S) = \frac{d}{dt}(T \cdot s) = \left(\frac{d}{dt}T\right) \cdot s + T \cdot \left(\frac{d}{dt}s\right) \Rightarrow \frac{d}{dt}s = \frac{1}{T} \cdot \left(\frac{d}{dt}S\right) - \frac{1}{T} \cdot \left(\frac{d}{dt}T\right) \cdot s = r_1 - r_2 - \alpha \cdot r_3 - \mu \cdot s$$

$$\frac{d}{dt}(P) = \frac{d}{dt}(T \cdot p) = \left(\frac{d}{dt}T\right) \cdot p + T \cdot \left(\frac{d}{dt}p\right) \Rightarrow \frac{d}{dt}p = \frac{1}{T} \cdot \left(\frac{d}{dt}P\right) - \frac{1}{T} \cdot \left(\frac{d}{dt}T\right) \cdot p = r_2 - r_3 - \mu \cdot p$$

$$\frac{d}{dt}(M) = \frac{d}{dt}(T \cdot m) = \left(\frac{d}{dt}T\right) \cdot m + T \cdot \left(\frac{d}{dt}m\right) \Rightarrow \frac{d}{dt}m = \frac{1}{T} \cdot \left(\frac{d}{dt}M\right) - \frac{1}{T} \cdot \left(\frac{d}{dt}T\right) \cdot m = r_3 - \mu \cdot m$$

$$\text{Check: } \frac{d}{dt}s + \frac{d}{dt}p + \frac{d}{dt}m = \frac{d}{dt}1 = 0$$

Play with the model and investigate its behavior.

Balanced Growth. Balanced growth is a special condition such that the cell composition remains constant. In mathematical terms, this amounts to **setting $d/dt=0$** for all the cell fractions.

Find the specific growth rate $\mu(S_e)$ and cell fractions.

Numerical Constants		Rate Expressions
$\alpha := 1$		
$a := 10^4 \text{ (cm}^2/\text{g cell)}$		
$k_1 := 10^{-5} \text{ (g/cm}^2/\text{hr)}$	$K_1 := 1 \text{ (g/L)}$	$K_{1i} := 1 \Rightarrow r_1(S_e, s) := a \cdot \frac{k_1 \cdot S_e}{K_1 + S_e} \cdot \frac{K_{1i}}{K_{1i} + s}$
$k_2 := 1 \text{ (hr}^{-1}\text{)}$	$K_2 := 1$	$\Rightarrow r_2(s, m) := \frac{k_2 \cdot m \cdot s}{K_2 + s}$
$k_3 := 1 \text{ (hr}^{-1}\text{)}$	$K_{3s} := 1$	$K_{3p} := 1 \Rightarrow r_3(s, p, m) := \frac{k_3 \cdot m \cdot s}{K_{3s} + s} \cdot \frac{p}{K_{3p} + p}$

When the diffusion coefficient is high (e.g., $k_1=10^{-4}$ for the above set of model parameters) and when the substrate concentration is of order 1 (e.g., 1g/L), cell growth becomes reaction limited, and the cells become filled with nothing but substrate, i.e., $s=1, m=0, p=0$. Consequently, we have $r_2=0, r_3=0$, and $\mu=r_1$.

Initial guesses: $\mu := 1$ $s := \frac{1}{3}$ $p := \frac{1}{3}$ $m := \frac{1}{3}$

Given

Balanced growth: $r_1(S_e, s) - r_2(s, m) - \alpha \cdot r_3(s, p, m) - \mu \cdot s = 0 \quad \dots ds/dt = 0$
 $r_2(s, m) - r_3(s, p, m) - \mu \cdot p = 0 \quad \dots dp/dt = 0$
 $r_3(s, p, m) - \mu \cdot m = 0 \quad \dots dm/dt = 0$

Mass fraction: $s + p + m = 1$

$\text{ans}(S_e) := \text{Find}(\mu, s, p, m)$

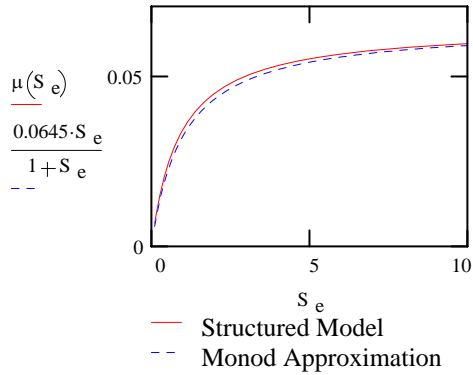
An example: $\text{ans}(1) = \begin{bmatrix} 0.0342 \\ 0.1038 \\ 0.5706 \\ 0.3256 \end{bmatrix}$

Balanced Growth Plots.

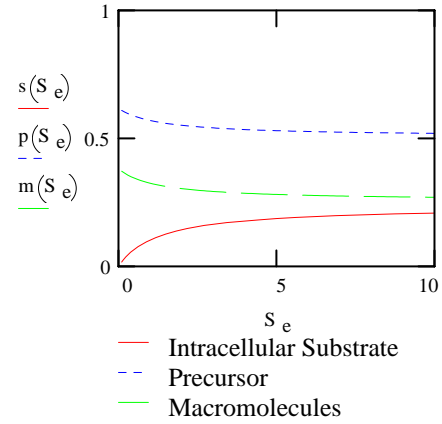
$$\mu(S_e) := \text{ans}(S_e)_0 \quad s(S_e) := \text{ans}(S_e)_1 \quad p(S_e) := \text{ans}(S_e)_2 \quad m(S_e) := \text{ans}(S_e)_3$$

$$S_e := 0.1, 0.2 \dots 10$$

Specific growth rate $\mu(s)$ for balanced growth



Cellular fractions for balanced growth



As shown in the last plot, the overall expression of specific growth rate μ versus the external substrate concentration S_e closely follows the Monod model, although the expression for each step may have product inhibition or multiple substrates. A bunch of Michaelis-Menten kinetic steps usually result in a saturation kinetic behavior exemplified by the Monod model.

Rational Choice of Monod model. We can pick μ_m and K_s in a Monod model rationally. When S_e is very large (e.g., $S_e=10^6$), $\mu \rightarrow \mu_m$, and K_s is the value of S_e at which $\mu = \mu_m/2$.

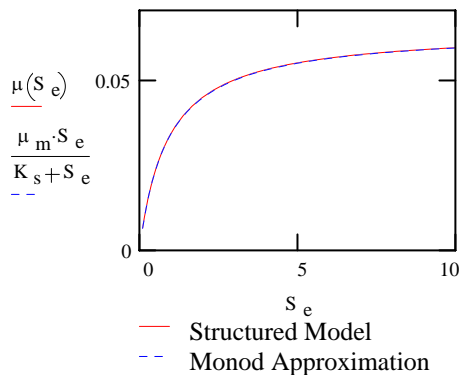
$$\mu_m := \mu(10^6) \quad \mu_m = 0.06435$$

$$K_s := 1 \quad \text{Given} \quad \mu(K_s) = \frac{\mu_m}{2} \quad K_s := \text{Find}(K_s) \quad K_s = 0.884$$

Alternatively, we can match $d\mu(0)/dS_e = \mu_m/K_s$ to obtain K_s in the Monod model.

$$d\mu dS_e := \frac{\mu(0.00001) - \mu(0)}{0.00001} \quad K_s := \frac{\mu_m}{d\mu dS_e} \quad K_s = 0.889$$

Specific growth rate $\mu(s)$ for balanced growth



← There is practically no difference.

Simulate CSTR. Add the dilution rate term to the dynamic equations for the extrinsic variables.

$$\frac{d}{dt}S_e = D(S_f - S_e) - r_1 \cdot T$$

$$\frac{d}{dt}S = r_1 \cdot T - r_2 \cdot T - \alpha \cdot r_3 \cdot T - D \cdot S$$

$$\frac{d}{dt}P = r_2 \cdot T - r_3 \cdot T - D \cdot P$$

$$\frac{d}{dt}M = r_3 \cdot T - D \cdot M$$

$$\frac{d}{dt}T = (r_1 - \alpha \cdot r_3) \cdot T - D \cdot T$$

Numerical expression of the CSTR dynamic equations with $S_f := 10$

$$dSedt(S_e, S, P, M, T, D) := D \cdot (S_f - S_e) - r_1 \left(S_e, \frac{S}{T} \right) \cdot T$$

$$dSdt(S_e, S, P, M, T, D) := r_1 \left(S_e, \frac{S}{T} \right) \cdot T - r_2 \left(\frac{S}{T}, \frac{M}{T} \right) \cdot T - \alpha \cdot r_3 \left(\frac{S}{T}, \frac{P}{T}, \frac{M}{T} \right) \cdot T - D \cdot S$$

$$dPdt(S_e, S, P, M, T, D) := r_2 \left(\frac{S}{T}, \frac{M}{T} \right) \cdot T - r_3 \left(\frac{S}{T}, \frac{P}{T}, \frac{M}{T} \right) \cdot T - D \cdot P$$

$$dMdt(S_e, S, P, M, T, D) := r_3 \left(\frac{S}{T}, \frac{P}{T}, \frac{M}{T} \right) \cdot T - D \cdot M$$

$$dTdt(S_e, S, P, M, T, D) := r_1 \left(S_e, \frac{S}{T} \right) \cdot T - \alpha \cdot r_3 \left(\frac{S}{T}, \frac{P}{T}, \frac{M}{T} \right) \cdot T - D \cdot T$$

CSTR Operated at Steady-State. Calculate the state variables by setting $d/dt=0$.

Initial guesses: $S_e := 0$ $S := 1$ $P := 1$ $M := 1$ $T := S + P + M$

Given

$$dS_{edt}(S_e, S, P, M, T, D) = 0$$

$$dS_{dt}(S_e, S, P, M, T, D) = 0$$

$$dP_{dt}(S_e, S, P, M, T, D) = 0$$

$$dM_{dt}(S_e, S, P, M, T, D) = 0$$

$$dT_{dt}(S_e, S, P, M, T, D) = 0$$

$$\text{ANS}(D) := \text{Find}(S_e, S, P, M, T)$$

An example:

$$\text{ANS}(0.05) = \begin{bmatrix} 3.061 \\ 0.891 \\ 2.905 \\ 1.572 \\ 5.367 \end{bmatrix}$$

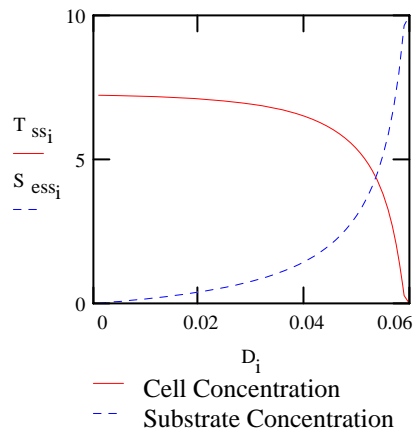
Steady-State Plots.

$$i := 1 \dots 60 \quad D_i := 0.001 \cdot i \quad \text{steady}^{<i>} := \text{ANS}(D_i)$$

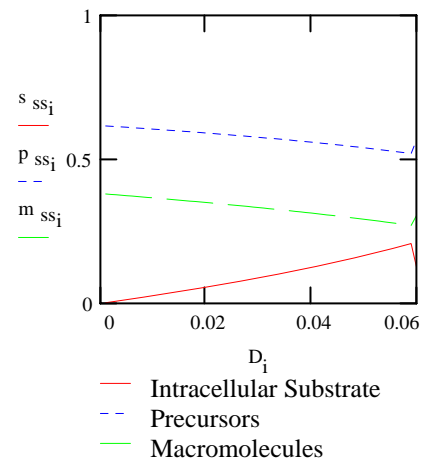
$$S_{\text{ess}_i} := (\text{steady}^{<i>})_0 \quad S_{\text{ss}_i} := (\text{steady}^{<i>})_1 \quad P_{\text{ss}_i} := (\text{steady}^{<i>})_2 \quad M_{\text{ss}_i} := (\text{steady}^{<i>})_3$$

$$T_{\text{ss}_i} := (\text{steady}^{<i>})_4 \quad s_{\text{ss}_i} := \frac{S_{\text{ss}_i}}{T_{\text{ss}_i}} \quad p_{\text{ss}_i} := \frac{P_{\text{ss}_i}}{T_{\text{ss}_i}} \quad m_{\text{ss}_i} := \frac{M_{\text{ss}_i}}{T_{\text{ss}_i}}$$

Steady-State Cell and Substrate Conc. (g/L)



Steady-State Cellular Composition



The ratio of the total biomass to the macromolecules (i.e., the inverse of m_{ss}) is indicative of the mean cell size, if we assume the macromolecule content per cell is approximately constant.

Thus, the last plot shows that the cell size ($1/m$) increases with the dilution rate, which equals cell growth rate at steady-state. In other words, this structured model predicts that faster growing cells are larger.

Dynamic Simulation of a Continuous Reactor (Shift-up from a steady-state)

Initial Conditions (Assign numbers corresponding to an inoculum at the stationary phase with):

Before shift-up $D := 0.01$ $y_{\text{initial}} := \text{ANS}(D)$

After shift-up: $D := 0.05$

Combine the dynamic equations into a vector form suitable for the "rkfixed" function.

$$\text{dydt}(t, y) := \begin{bmatrix} \text{dSedt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dSdt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dPdt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dMdt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dTdt}(y_0, y_1, y_2, y_3, y_4, D) \end{bmatrix}$$

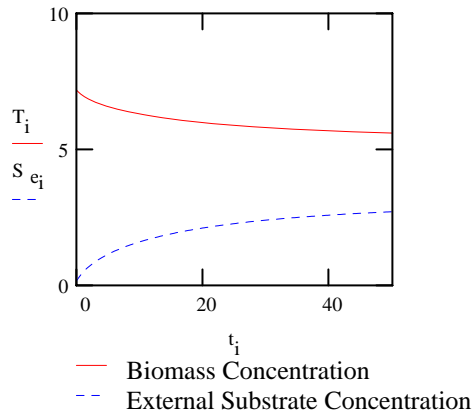
Integrate from $t=0$ to $t_f := 50$ $N := 200$ steps $i := 0..N$

$y_{\text{out}} := \text{rkfixed}(y_{\text{initial}}, 0, t_f, N, \text{dydt})$

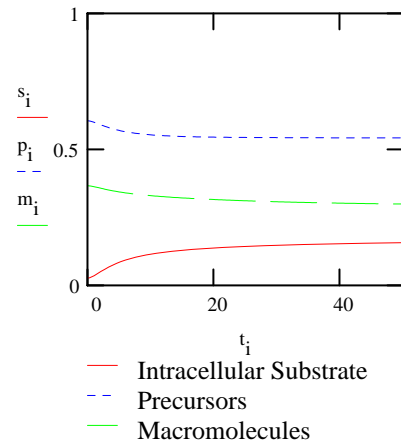
$t := y_{\text{out}}^{<0>}$ $S_e := y_{\text{out}}^{<1>}$ $S := y_{\text{out}}^{<2>}$ $P := y_{\text{out}}^{<3>}$ $M := y_{\text{out}}^{<4>}$ $T := y_{\text{out}}^{<5>}$

$$s_i := \frac{S_i}{T_i} \quad p_i := \frac{P_i}{T_i} \quad m_i := \frac{M_i}{T_i}$$

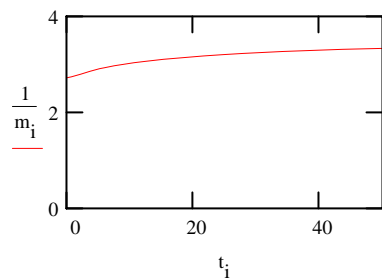
Cell and Substrate Conc. (g/L) for Continuous Culture



Changes in the Cellular Composition



Relative Mean Cell Size



Dynamic Simulation of a Continuous Reactor (Shift-down from a steady-state)

Initial Conditions (Assign numbers corresponding to an inoculum at the stationary phase with):

Before shift-down $D := 0.05$ $y_{\text{initial}} := \text{ANS}(D)$

After shift-down: $D := 0.01$

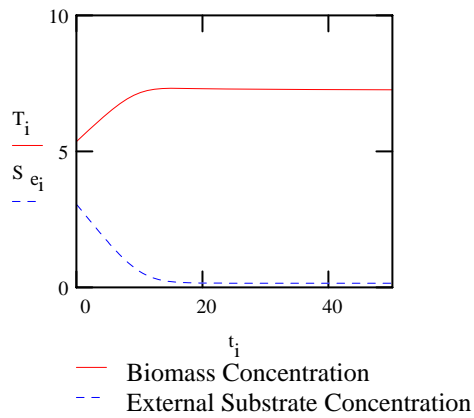
Combine the dynamic equations into a vector form suitable for the "rkfixed" function.

$$\text{dydt}(t, y) := \begin{bmatrix} \text{dSedt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dSdt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dPdt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dMdt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dTdt}(y_0, y_1, y_2, y_3, y_4, D) \end{bmatrix}$$

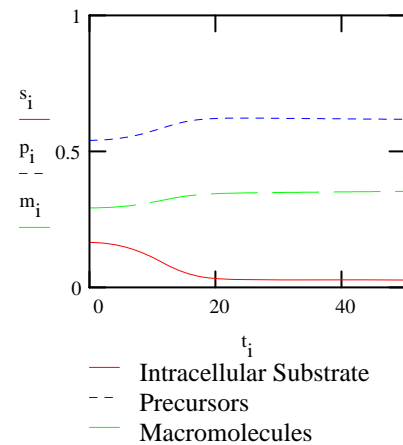
Integrate $y_{\text{out}} := \text{rkfixed}(y_{\text{initial}}, 0, t_f, N, \text{dydt})$

$$\begin{aligned} t &:= y_{\text{out}}^{<0>} & S_e &:= y_{\text{out}}^{<1>} & S &:= y_{\text{out}}^{<2>} & P &:= y_{\text{out}}^{<3>} & M &:= y_{\text{out}}^{<4>} & T &:= y_{\text{out}}^{<5>} \\ & & s_i &:= \frac{S_i}{T_i} & p_i &:= \frac{P_i}{T_i} & m_i &:= \frac{M_i}{T_i} \end{aligned}$$

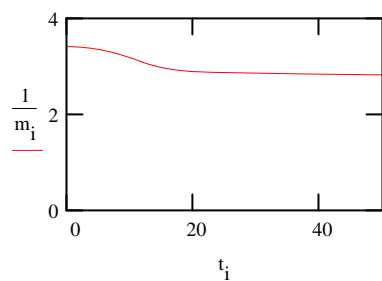
Cell and Substrate Conc. (g/L) for Continuous Culture



Changes in the Cellular Composition



Relative Mean Cell Size



Dynamic Simulation of a Batch Reactor with $D := 0$

Initial Conditions (Assign numbers corresponding to an inoculum at the stationary phase with):

$$S_{e0} := S_f \quad T_0 := 0.1 \quad S_0 := \text{ans}(0.001)_1 \cdot T_0 \quad P_0 := \text{ans}(0.001)_2 \cdot T_0 \quad M_0 := \text{ans}(0.001)_3 \cdot T_0$$

Combine the dynamic equations into a vector form suitable for the "rkfixed" function.

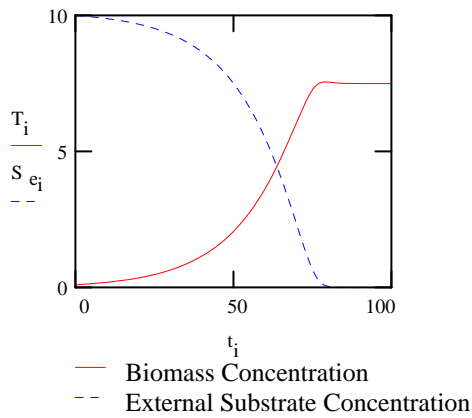
$$\text{dydt}(t, y) := \begin{bmatrix} \text{dSedt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dSdt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dPdt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dMdt}(y_0, y_1, y_2, y_3, y_4, D) \\ \text{dTdt}(y_0, y_1, y_2, y_3, y_4, D) \end{bmatrix} \quad \text{I.C.: } y_{\text{initial}} := \begin{bmatrix} S_{e0} \\ S_0 \\ P_0 \\ M_0 \\ T_0 \end{bmatrix}$$

Integrate from $t=0$ to $t_f := 100$ $N := 200$ steps $i := 0..N$

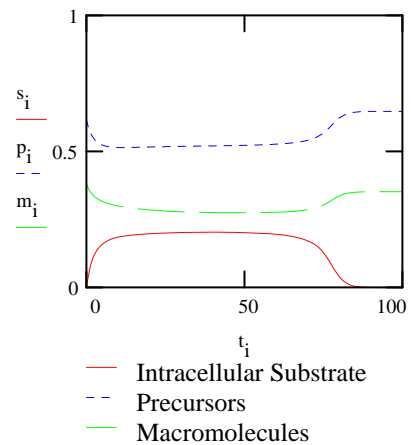
$$yout := \text{rkfixed}(y_{\text{initial}}, 0, t_f, N, \text{dydt})$$

$$t := yout^{<0>} \quad S_e := yout^{<1>} \quad S := yout^{<2>} \quad P := yout^{<3>} \quad M := yout^{<4>} \quad T := yout^{<5>} \\ s_i := \frac{S_i}{T_i} \quad p_i := \frac{P_i}{T_i} \quad m_i := \frac{M_i}{T_i}$$

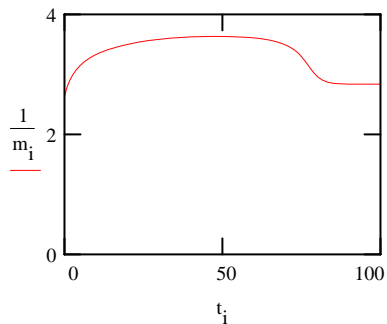
Cell and Substrate Conc. (g/L) during Batch Culture



Changes in the Cellular Composition



Relative Mean Cell Size



Another simulation with a different set of initial conditions (Assign numbers corresponding to an inoculum with only the macromolecules):

$$S_{e0} := S_f \quad S_0 := 0 \quad P_0 := 0 \quad M_0 := 0.1 \quad T_0 := S_0 + P_0 + M_0$$

$$\text{I.C.: } y_{\text{initial}} := (S_{e0} \ S_0 \ P_0 \ M_0 \ T_0)^T$$

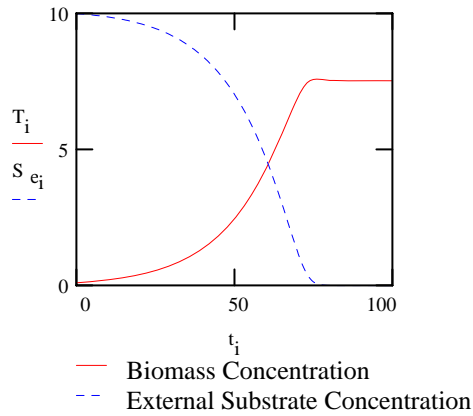
Integrate from $t=0$ to $t_f := 100$ $N := 500$ steps $i := 0..N$

$$y_{\text{out}} := \text{rkfixed}(y_{\text{initial}}, 0, t_f, N, \text{dydt})$$

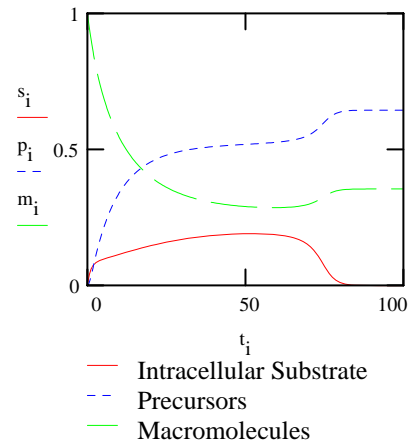
$$t := y_{\text{out}}^{<0>} \quad S_e := y_{\text{out}}^{<1>} \quad S := y_{\text{out}}^{<2>} \quad P := y_{\text{out}}^{<3>} \quad M := y_{\text{out}}^{<4>} \quad T := y_{\text{out}}^{<5>}$$

$$s_i := \frac{S_i}{T_i} \quad p_i := \frac{P_i}{T_i} \quad m_i := \frac{M_i}{T_i}$$

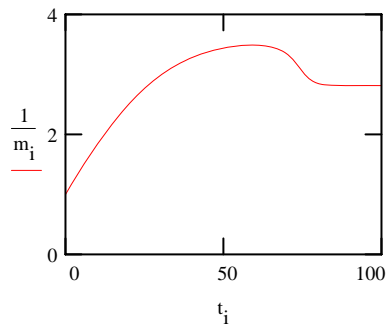
Cell and Substrate Conc. (g/L) during Batch Culture



Changes in the Cellular Composition



Relative Mean Cell Size



Note that the intracellular substrate pool builds up first and is depleted at the end of the batch run. On the other hand, precursor pool builds up toward the end of the batch run because there is no more substrate to provide the energy needed to convert it to macromolecules.