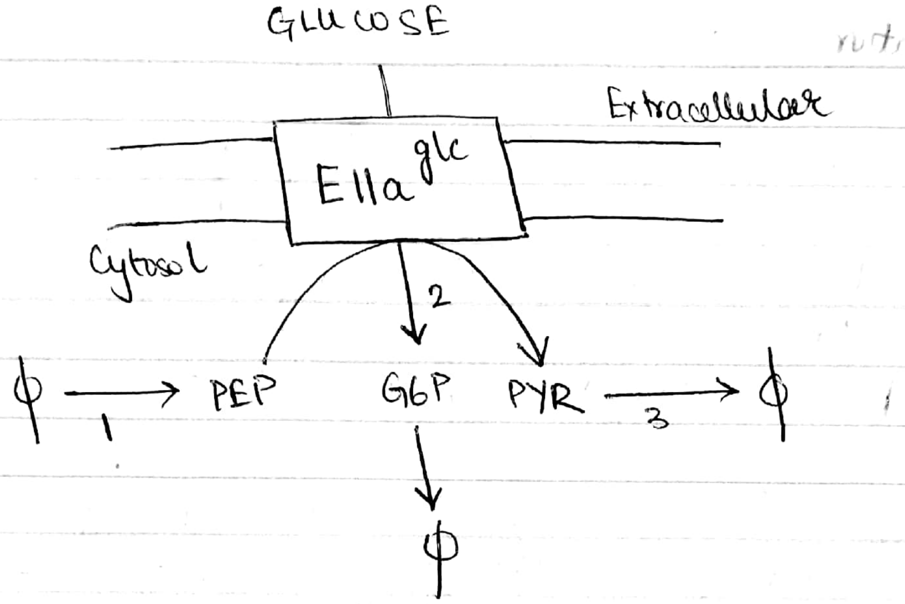
Problem Set 2

Problem 1 a)



Glucose uptake using the PTS in E. coli

Here $r_2 = q_{glc}$ (mmol/B-hr) {specific rate of glucose uptake}

Starting with EIIglc. Lets look at its transcription equations.

$$G_i + R_T \rightleftharpoons G_i - R_T$$

Initiation: $$G_i - R_T \longrightarrow (G_i - R_T)^*$$

Assumption: {Rate Limiting step}

Elongation: $$(G_i - R_T)^* \longrightarrow m_i + G_i + R_p$$

Initiation Rate

$$\bar{r}_{TI} = \bar{k}_{TI} R_T \left(\frac{G_i}{K_T + G_i} \right) \longrightarrow (1)$$

$\bar{k}_{TI}$ = initiation rate constant
R_T = RNA polymerase concentration
G_i = Total amount of gene concentration
K_T = saturation constant

Elongation Rate

$$r_{Ti} = k_{Ti} \; (G_i - R_T)^* \left(\frac{L_T}{L_i}\right) \rightarrow (2)$$

$\frac{L_T}{L_i}$ = characteristic length

k_{ti} = elongation rate constant

Assumptn : T(RNAP) is @ non-zero steady state meaning that the $\frac{d}{dt}(\,)$ = 0. Hence steady state Balance.

$$\frac{d(G_i - R_T)}{dt} = \bar{r}_{Ti} - r_{Ti} - \mu\,(G_i - R_T)^*$$

$$\bar{r}_{Ti} - r_{Ti} - \mu\,(G_i - R_T)^* = 0 \rightarrow (3)$$

Substituting (1) & (2)

$$\bar{k}_{Ti} R_T \left(\frac{G_i}{K_T + G_i}\right) - k_{Ti}\,(G_i - R_T)^* \frac{L_T}{L_i} - \mu\,(G_i - R_T)^* = 0$$

$$\boxed{\frac{\bar{k}_{Ti} R_T \dfrac{G_i}{K_T + G_i}}{\left(k_{Ti}\dfrac{L_T}{L_i} + \mu\right)} = (G_i - R_T)^*} \rightarrow (3)$$

Substituting this in (2) we get,

control term

$$\boxed{r_{Ti} = k_{Ti} \cdot \frac{\bar{k}_{Ti} R_T \dfrac{G_i}{K_T + G_i}}{\left(k_{Ti}\dfrac{L_T}{L_i} + \mu\right)} \cdot \frac{L_T}{L_i} \; u_i} \rightarrow (4)$$

Now lets do Translation of Ellgle

$$mi + Rx \rightleftharpoons mi - Rx$$

Initiation: $mi - Rx \longrightarrow (mi - Rx)^*$

Elongation: $(mi - Rx)^* \longrightarrow Pj + Rx + mi$ { Assumption: Rate limiting step }

Initiation Rate

$$\bar{r}_{xi} = \bar{k}_{xi} R_x \left(\frac{mi}{K_x + mi} \right) \longrightarrow (5)$$

$\bar{k}_{xi}$ = initiation rate constant
R_x = Ribosome concentration
mi = Total amount of mRNA
K_x = Saturation constant

Elongation Rate

$$r_{xi} = k_{xi} (mi - R_x)^* \left(\frac{L_x}{L_i} \right) \longrightarrow (6)$$

k_{xi} = elongation rate constant
$\frac{L_x}{L_i}$ = characteristic length

Assumption: X (Ribosome) is @ non-zero steady state meaning that the $\frac{d}{dt}() = 0$. Hence steady state Balance.

$$\frac{d (mi - R_x)^*}{dt} = \bar{r}_{xi} - r_{xi} - \mu (mi - R_x)^*$$

$$\bar{r}_{xi} - r_{xi} - \mu (m_i - R_x)^* = 0 \quad \rightarrow (7)$$

Substituting (5) & (6)

$$\bar{k}_{xi} R_x \left(\frac{m_i}{K_x + m_i}\right) - k_{xi} (m_i - R_x)^* \frac{L_x}{L_i} - \mu (m_i - R_x)^* = 0$$

$$\boxed{\frac{\bar{k}_{xi} R_x \dfrac{m_i}{(K_x + m_i)}}{\left(k_{xi} \dfrac{L_x}{L_i} + \mu\right)} = (m_i - R_x)^*} \quad \rightarrow (8)$$

Substituting this in (6).

$$\boxed{r_{xi} = k_{xi} \cdot \frac{\bar{k}_{xi} R_x \dfrac{m_i}{K_x + m_i}}{\left(k_x \dfrac{L_x}{L_i} + \mu\right)} \cdot \frac{L_x}{L_i} \, w_x} \quad \rightarrow (9)$$

<u>General Intracellular Balance</u>

$$\frac{d}{dt}(\beta_j x) = \left(\sum_{i=1}^{R} \sigma_{xi} v_i\right) \beta_j$$

x = concentration of metabolite

σ = stoichiometric coefficient

v_i = reaction flux

β_2 = Volume basis for intracellular

$= \langle m \rangle \hat{N}_c V$

$\langle m \rangle$ = average mass per cell (g)

N_c = no. of cells per culture volume (L)

$V =$ Volume (L)

$$\beta_2 \dot{x} + x \dot{\beta}_2 = \left\{ \sum_{i=1}^{R} \sigma x_i v_i \right\} \beta_2$$

Solving for $\dot{x}$

$$\dot{x} = \left\{ \sum_{i=1}^{R} \sigma x_i v_i \right\} - x \dot{\beta}_2 \beta_2^{-1} \longrightarrow 10$$

Finding $\beta_2^{-1} \dot{\beta}_2$

$$\beta_2^{-1} \dot{\beta}_2 = \frac{d}{dt} \left(\langle m \rangle \hat{N}_c V \right) \times \frac{1}{\langle m \rangle \hat{N}_c V}$$

__Assumption:__ Average mass per cell $\langle m \rangle$ is constant

$$\frac{\cancel{\langle m \rangle}}{\cancel{\langle m \rangle} \hat{N}_c V} \left[V \dot{\hat{N}}_c + \hat{N}_c \dot{V} \right] = \beta_2^{-1} \dot{\beta}_2$$

$$\beta_2^{-1} \beta_2 = \frac{\dot{\hat{N}}_c}{\hat{N}_c} + \frac{\dot{V}}{V} = \dot{\hat{N}}_c \hat{N}_c^{-1} + \dot{V} V^{-1}$$

Substitute in 10

$$\dot{x} = \sum_{i=1}^{R} \sigma x_i v_i - x \left(\hat{N}_c^{-1} \dot{\hat{N}}_c + V^{-1} \dot{V} \right)$$

__Assumption__ In batch cultures $\dot{V} = 0$

$$\boxed{\dot{x} = \sum_{i=1}^{R} \sigma x_i v_i - x \hat{N}_c^{-1} \dot{\hat{N}}_c} \longrightarrow 11$$

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Extracellular Mass Balance

$$\frac{d}{dt}(\beta_1 x_e) = \left[\sum_{i=1}^{R} \sigma x_i \cdot V_i\right] \beta_1$$

β_1 : Volume basis for extracellular
$= V$

$$V_i = q_i \times \frac{\beta_2}{\beta_1} = q_i \times \frac{\langle M \rangle \hat{N_c} \cancel{V}}{\cancel{V}}$$

$$\frac{d}{dt}(\beta_1 x_e) = \left[\sum_{i=1}^{R} \sigma x_i \, q_i \frac{\beta_2}{\beta_1}\right] \beta_1$$

$$\beta_1 \dot{x}_e + x_e \dot{\beta}_1 = \left[\sum_{i=1}^{R} \sigma x_i \, q_i \frac{\beta_2}{\beta_1}\right] \beta_1$$

Solving for $\dot{x}_e$

$$\dot{x}_e = \frac{\beta_2}{\beta_1} \sum_{i=1}^{R} \sigma x_i q_i - x_e \dot{\beta}_1 \beta_1^{-1}$$

$$\dot{\beta}_1 \beta_1^{-1} = \dot{V} V^{-1}$$

Assumption: Batch culture

$$\frac{\beta_2}{\beta_1} = \underset{(gdw)}{\langle m \rangle} \; \underset{(\text{no. of cells}/L)}{N_c} = C = \text{Cell mass}$$

$$\boxed{\dot{x}_e = C\left(\sum_{i=1}^{R} \sigma x_i q_i\right) - x_e \dot{V} V^{-1}} \rightarrow (12)$$

Cell Balance (an extracellular variable)

From general extracellular balance,

$$\dot{c} = c \sum_{i=1}^{B} \sigma_{ci} q_i - c V^{-1} \dot{V}$$

q_i leads to the cell growth & cell death

$$q_c = \mu = \text{Growth rate}$$

$$q_d = k_{dc} = \text{death rate}$$

$$\dot{c} = c (q_c - q_d) - c V^{-1} \dot{V}$$

$$\dot{c} = c (\mu - k_{dc}) - c V^{-1} \dot{V}$$

Assumption : we are solving for batch culture.

Hence $\dot{V} V^{-1} = 0$

Assumption : Cell death is negligible $\therefore k_{dc} = 0$

$$\boxed{\dot{c} = c\mu}$$

$$\boxed{\dot{c} c^{-1} = \mu} \longrightarrow 13$$

μ = specific growth rate of the culture

We can modify our Intracellular Balance in eq 11.

$$\hat{N_c}^{-1}\dot{\hat{N_c}} = \frac{\cancel{\langle m \rangle}}{C} \times \frac{\dot{C}}{\cancel{\langle m \rangle}} \quad \left[\because \langle m \rangle \hat{N_c} = C \text{ for batch culture} \right]$$

Assumption

From equation 13 we know $\dot{C}C^{-1} = \mu$

$$\boxed{\hat{N_c}^{-1}\dot{\hat{N_c}} = \mu}$$

Hence equation 11 becomes.

Intracellular Balance for Batch culture

$$\boxed{\dot{x} = \sum_{i=1}^{R} \sigma_{xi} v_i - x\mu} \rightarrow (14)$$

Slly equation 12 can be rewritten for

Assumption: batch culture as

Extracellular Balance for Batch Culture

$$\boxed{\dot{x_e} = C \sum_{i=1}^{R} \sigma_{xi} q_i} \rightarrow (15)$$

$\boxed{\text{because } W^{-1} = 0}$

Mass Balance for Extracellular Glucose

~~[illegible]~~ from eq 15 we get

$$\frac{d(glc)}{dt} = c\sum_{i=1}^{R} \sigma_{glc,i}\, q_i$$

$$\boxed{\frac{d(glc)}{dt} = -c\, q_{glc}} \rightarrow 16$$

q_{glc} = rate of glucose uptake

$\sigma_{glc,i} = -1$ {being used ups in the reaction}

Mass Balance for Intracellular Species (using eq 14)

i) G6P

$$\frac{d(G6P)}{dt} = \sum_{i=1}^{R} \sigma_{G6P,i}\, V_i - x_{G6P}\, \mu$$

$$\boxed{\frac{d(G6P)}{dt} = V_2 - V_4 - x_{G6P}\, \mu} \rightarrow 17$$

$\sigma_{G6P,2} = 1$ (G6P formed)

$\sigma_{G6P,4} = -1$ (" consumed)

ii) $\frac{d(PEP)}{dt} = \sum_{i=1}^{R} \sigma_{PEP,i}\, V_i - x_{PEP}\, \mu$

$$\boxed{\frac{d(PEP)}{dt} = V_1 - V_2 - x_{PEP}\, \mu = V_1 - q_{glc} - x_{PEP}\, \mu} \rightarrow 18$$

$\sigma_{PEP,1} = 1$

$\sigma_{PEP,2} = 2$

iii) $$\frac{d}{dt}(Pyr) = \sigma_{Pyr,i} v_i - x_{pyr}\mu$$

$$\boxed{\frac{d(Pyr)}{dt} = v_2 - v_3 - x_{pyr}\mu} \longrightarrow (19)$$

<u>Cell Mass Balance</u> (using eq (13))

$$\boxed{\dot{C} = \mu C} \longrightarrow (20)$$

<u>Other Assumptions</u>

- v_1 is a 0 order reaction
- v_3, v_4 are at non zero steady state.
- Neglect the dilution terms

c) Assuming all intracellular variables @ steady state:

$$\cancel{V_2 = q_{glc}} \qquad \cancel{\frac{d(G6P)}{dt} = \begin{bmatrix} \; \end{bmatrix}\begin{bmatrix} V_1 \\ V_3 \\ V_4 \end{bmatrix}}$$

$$\boxed{V_2 = q_{glc}}$$

$$\begin{matrix} \frac{d(G6P)}{dt} = \\ \frac{d(PEP)}{dt} = \\ \frac{d(Pyr)}{dt} = \end{matrix} \begin{bmatrix} 0 & 0 & -1 \\ 1 & 0 & 0 \\ 0 & -1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_3 \\ v_4 \end{bmatrix} + \begin{bmatrix} 1 \\ -1 \\ 1 \end{bmatrix} q_{glc}$$

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Now,

$$\begin{bmatrix} 0 & 0 & -1 \\ 1 & 0 & 0 \\ 0 & -1 & 0 \end{bmatrix} \begin{bmatrix} V_1 \\ V_3 \\ V_4 \end{bmatrix} + \begin{bmatrix} 1 \\ -1 \\ 1 \end{bmatrix} q_{glc} = \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}$$

$$A \cdot V + Tq = 0$$

$$A.V = -Tq$$

$$V = A^{-1}(-Tq)$$

Hence if we know our q, T is already known. The only way to find V is to have a matrix A which is invertible

$$\det(A) = 0(0) - 0(0) + (-1)(-1)$$
$$= 1$$

Hence the determinant is non-zero which satisfies the invertible matrix theorem. Thus we will be able to measure V_1, V_3, V_4 if q is known.