General intracellular, extracellular and cellmass balances:

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

where $x \rightarrow$ concentration of metabolite
$\sigma \rightarrow$ stoichiometric co-efficient
$\nu_i \rightarrow$ reaction flux
$\beta \rightarrow$ volume basis

Let β_1 = extracellular volume basis V_R
β_2 = intracellular volume basis $\langle m \rangle \hat{N}_c V_R$

$V_R \rightarrow$ reactor volume
$\langle m \rangle \rightarrow$ avg. mass per cell
$\hat{N}_c \rightarrow$ no. of cells per culture volume

i) Intracellular balance:

$$\frac{d}{dt}(\beta_2 x) = \left(\sum_{i=1}^{IR} \sigma_{xi}\, \nu_i\right) \beta_2$$

$$\Rightarrow x\frac{d\beta_2}{dt} + \beta_2 \frac{dx}{dt} = \left(\sum_{i=1}^{IR} \sigma_{xi}\, \nu_i\right) \beta_2$$

$$\Rightarrow \frac{dx}{dt} = \frac{\left(\sum_{i=1}^{IR} \sigma_{xi}\, \nu_i\right) \beta_2 - x\frac{d\beta_2}{dt}}{\beta_2}$$

$$\frac{dx}{dt} = \dot{x} = \sum_{i=1}^{IR} \sigma_{xi} v_i - x \beta_2^{-1} \dot{\beta}_2 \longrightarrow (1)$$

$$\rightarrow \frac{d\beta_2}{dt} = \frac{d}{dt}\left(\langle m \rangle \hat{N}_c V_R\right)$$

$$= \langle m \rangle V_R \frac{d\hat{N}_c}{dt} + \langle m \rangle \hat{N}_c \frac{dV_R}{dt}$$

★ Assumption : Avg. mass per cell $\langle m \rangle$ is constant

$$\Rightarrow \dot{\beta}_2 = \langle m \rangle V_R \dot{\hat{N}}_c + \langle m \rangle \hat{N}_c \dot{V}_R$$

$$\rightarrow \beta_2^{-1} \dot{\beta}_2 = \frac{\langle m \rangle V_R \dot{\hat{N}}_c}{\langle m \rangle V_R \hat{N}_c} + \frac{\langle m \rangle \hat{N}_c \dot{V}_R}{\langle m \rangle V_R \hat{N}_c}$$

$$\beta_2^{-1} \dot{\beta}_2 = \dot{\hat{N}}_c \hat{N}_c^{-1} + \dot{V}_R V_R^{-1}$$

Substitute in (1),

$$\dot{x} = \sum_{i=1}^{IR} \sigma_{xi} v_i - x\left(\dot{\hat{N}}_c \hat{N}_c^{-1} + \dot{V}_R V_R^{-1}\right)$$

Extracellular mass balance:

$$\frac{d(\beta_1 x_e)}{dt} = \left(\sum_{i=1}^{IR} \sigma_{x_e,i} v_i\right) \beta_1$$

To convert v_i to a β_2 basis:

$$\frac{\beta_2}{\beta_1} = \frac{\langle m \rangle \hat{N}_c V_R}{V_R} = \langle m \rangle N_c \quad \left(\frac{gdW}{L}\right)$$

$\langle m \rangle$: gdW; N_c: no. of cells / L

$$= C \quad (\text{cellmass} \rightarrow gdW/L)$$

$$q_i \frac{\beta_2}{\beta_1} = v_i$$

$$\Rightarrow \frac{d(\beta_1 x_e)}{dt} = \left(\sum_{i=1}^{IR} \sigma_{x_e,i} q_i \frac{\beta_2}{\beta_1}\right) \beta_1$$

$$\Rightarrow \beta_1 \frac{dx_e}{dt} + \frac{x_e}{\beta_1} \frac{d\beta_1}{dt} = \left(\sum_{i=1}^{IR} \sigma_{x_e,i} q_i \frac{\beta_2}{\beta_1}\right) \beta_1$$

$$\Rightarrow \dot{x}_e + x_e \beta_1^{-1} \dot{\beta}_1 = \sum_{i=1}^{IR} \sigma_{x_e,i} q_i \beta_2 \beta_1^{-1}$$

$$\Rightarrow \dot{x}_e = \sum_{i=1}^{IR} \sigma_{x_e,i} q_i \beta_2 \beta_1^{-1} - x_e \beta_1^{-1} \dot{\beta}_1$$

$$\Rightarrow \dot{x}_e = C \sum_{i=1}^{IR} \sigma_{x_e,i} q_i - x_e \beta_1^{-1} \dot{\beta}_1$$

$$\longrightarrow \beta_1^{-1}\,\dot{\beta}_1 = V_R^{-1}\,\dot{V}_R$$

$$\Rightarrow \dot{x}_e = C \sum_{i=1}^{IR} \sigma_{x_e,i}\, q_i - x_e V_R^{-1}\,\dot{V}_R$$

$\longrightarrow$ Cellmass balance : (is extracellular)

$$\dot{C} = C \sum_{i=1}^{IR} \sigma_{\text{Hefe}\,c,i}\, q_i - C\,V_R^{-1}\,\dot{V}_R$$

q_i includes growth and death rates

Growth rate $q_C \rightarrow \mu$ $\left(\frac{gdW}{gdW \cdot time}\right)$

Death rate $q_D \rightarrow k_{d,C}$ $\left(\frac{gdW}{gdW \cdot time}\right)$

$$\Rightarrow \dot{C} = C(q_C - q_D) - C\,V_R^{-1}\,\dot{V}_R$$

$$\dot{C} = C(\mu - k_{d,C}) - C\,V_R^{-1}\,\dot{V}_R$$

★ Assumption : In a batch culture V_R is constant.

$$\Rightarrow \dot{V}_R = 0$$

$$\Rightarrow \dot{C} = C(\mu - k_{d,C}) \quad \text{or} \quad \dot{C}C^{-1} = \bar{\mu}$$

$\bar{\mu}$ → specific growth rate

Also, for batch cultures,

As $\langle m \rangle \hat{N}_c = C$, $\hat{N}_c^{-1} = \frac{\langle m \rangle}{C}$ and $\hat{N}_c = \frac{\dot{C}}{\langle m \rangle}$

$$\hat{N}_c^{-1} \dot{\hat{N}}_c = \frac{\langle m \rangle}{C} \frac{\dot{C}}{\langle m \rangle} = C^{-1} \dot{C} = \bar{\mu}$$

⇒ Intracellular balance for batch cultures:

$$\dot{x} = \sum_{i=1}^{IR} \sigma_{xi} \nu_i - x \bar{\mu}$$

Summary: (Batch cultures, const. $\langle m \rangle$)

Intracellular balance:

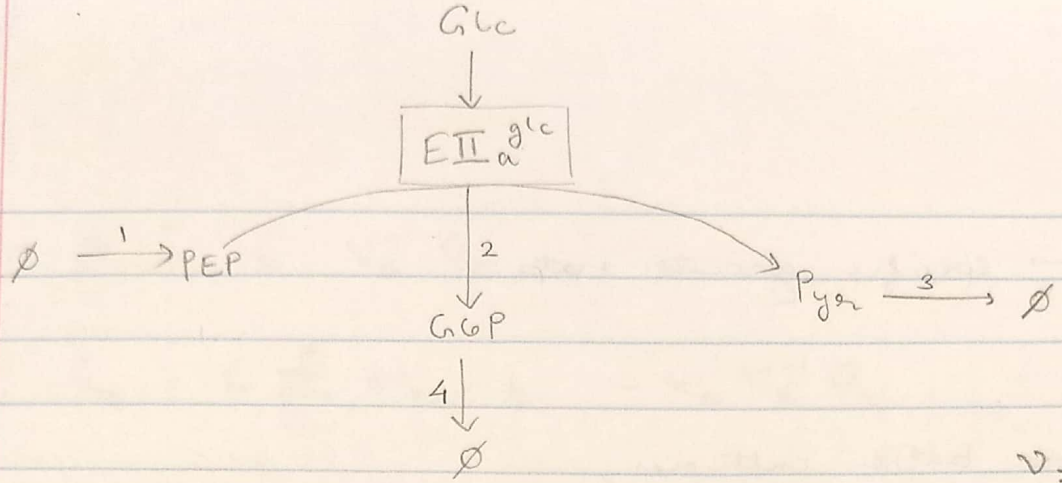
$$\dot{x} = \sum_{i=1}^{IR} \sigma_{xi} \nu_i - x \bar{\mu}$$

Extracellular balance:

$$\dot{x}_e = \left(\sum_{i=1}^{R} \sigma_{x_e,i} q_i \right) C$$

Cell mass:

$$\dot{C} = (\mu - k_{d,c}) C = \bar{\mu} C$$



$v_2 = q_{Glc}$

$$Glc + PEP \xrightarrow{EII a^{glc}} G6P + Pyr$$

Mass balance equations :

Extracellular vol. basis $= V_R$ (L) (culture volume)

Intracellular vol. basis $B = \langle m \rangle \hat{N}_c V_R$ (gdw)

gdw × no. of cells × L

~~★ Assumptions : Batch culture, const $\langle m \rangle$,~~

1) Extracellular glucose :

$$\frac{d}{dt}[Glc] = C \sum_{i=1}^{IR} \sigma_{Glc,i}\, q_i$$

$$\frac{d}{dt}[Glc] = -C q_{Glc}$$ (-ve as Glc is being consumed)

→ Intracellular species : ★ $v_2 = q_{Glc}$

2) G6P balance :

$$\frac{d}{dt}[G6P] = \sum_{i=1}^{IR} \sigma_{x,i} v_i - \text{[illegible]}\,[G6P]\,\bar{u}$$

$$\frac{d[G6P]}{dt} = \nu_2 - \nu_4 - [G6P]\,\bar{\mu}$$

ν_2 ↓ ie. q_{Glc}

3) PEP balance:

$$\frac{d[PEP]}{dt} = \nu_1 - \nu_2 - [PEP]\,\bar{\mu}$$

4) Pyr balance:

$$\frac{d[Pyr]}{dt} = \nu_2 - \nu_3 - [Pyr]\,\bar{\mu}$$

Including dilution rates due to cell growth, but assuming negligible degradation rates for metabolites

→ 5) Cellmass balance:

$$\frac{dC}{dt} = \bar{\mu} C$$

→ $EIIa^{glc}$ balance: $\frac{dx_i}{dt} = \sum_{j=1}^{IR} \sigma_{ij} r_j - (\mu + k_{d,i}) x_i$

Protein balance:

$$\frac{d[EIIa^{glc}]}{dt} = r_{x,i} - (\bar{\mu} + k_{d,i})[EIIa^{glc}]$$

where $r_{x,i}$ ⇒ rate of translation of $EIIa^{glc}$

$\bar{\mu}$ → specific growth rate

$k_{d,i}$ → non-specific degradation rate const. of $EIIa^{glc}$

mRNA balance:

$$\frac{d[EIIa^{glc}\ mRNA]}{dt} = r_{T,i} - (\bar{\mu} + \bar{k}_{d,i})[EIIa^{glc}\ mRNA]$$

where $r_{T,i}$ → rate of elongation of mRNA

$\bar{k}_{d,i}$ → non-specific degradation of $EIIa^{glc}$ mRNA

where $\lambda_{T,i} = k_{T,i} (G_i - R_T)^* \left(\frac{L_T}{L_i}\right)$

$k_{T,i}$ → mRNA elongation rate

$(G_i - R_T)^*$ → $E\Pi a^{glc}$ gene complex with RNAP conc.

$\frac{L_T}{L_i} \approx 1$

L_i → length of gene

L_T → avg gene length

Extended $EII a^{glc}$ balance derivations :

Transcription :

$$G_i + R_T \rightleftharpoons G_i\text{-}R_T$$

$$G_i\text{-}R_T \longrightarrow (G_i\text{-}R_T)^* \qquad \text{(initiation)}$$

$$(G_i\text{-}R_T)^* \longrightarrow m_i + G_i + R_T \qquad \text{(elongation)}$$

Rate of initiation $\bar{r}_{T,i} = \bar{k}_{T,i} R_T \left(\frac{G_i}{K_T + G_i} \right) \rightarrow (A)$

where $\bar{k}_{T,i}$ = initiation rate constant
R_T = conc. of RNAP
G_i = gene conc.
K_T = saturation const.

Rate of elongation $r_{T,i} = k_{T,i} (G_i\text{-}R_T)^* \left(\frac{L_T}{L_i} \right) \rightarrow (B)$

where $k_{T,i}$ = elongation rate constant
$L_T/L_i \sim 1$ (characteristic length)

Given T (RNAP) is at steady state

$$\frac{d}{dt}[G_i\text{-}R_T] = 0 = \bar{r}_{T,i} - r_{T,i} - \mu (G_i\text{-}R_T)^* \rightarrow (C)$$

From (A) and (B)

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

Solving for $(G_i - R_T)^*$ and substituting it in (B)

$$r_{T,i} = \frac{k_{T,i}\ \bar{k}_{T,i}\ R_T \left(\frac{G_i}{K_T + G_i}\right) \times \frac{L_T}{L_i}\ u_i}{k_{T,i}\ \frac{L_T}{L_i} + u}$$

where $u_i \rightarrow$ control term

→ Translation of $EIIa^{glc}$

$$m_i + R_x \rightleftharpoons m_i - R_x$$
$$m_i - R_x \longrightarrow (m_i - R_x)^* \quad \text{(initiation)}$$
$$(m_i - R_x)^* \longrightarrow P_j + R_x + m_i \quad \text{(elongation)}$$

Rate of initiation :

$$\bar{r}_{x,i} = \bar{k}_{x,i}\ R_x \left(\frac{m_i}{K_x + m_i}\right) \rightarrow (I)$$

where m_i = mRNA conc.
$\bar{k}_{x,i}$ = initiation rate constant
K_x = saturation constant
R_x = ribosome concentration

Rate of elongation :

$$r_{x,i} = k_{x,i}\ (m_i - R_x)^* \left(\frac{L_x}{L_i}\right) \rightarrow (II)$$

where $k_{x,i}$ = elongation rate constant

Given: Ribosome (X) is at steady state.

$$\Rightarrow \frac{d}{dt}(m_i - R_x)^* = \bar{r}_{xi} - r_{xi} - \mu(m_i - R_x)^* = 0$$

Substituting (I) and (II),

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

Solving for $(m_i - R_x)^*$ and substituting in (II)

$$r_{x,i} = \frac{k_{xi} \bar{k}_{xi} R_x \frac{m_i}{k_x + m_i} \frac{L_x}{L_i} w_x}{k_x \frac{L_x}{L_i} + \mu}$$

where w_x is a control term.

The assumptions used were: See next pg for

1) Batch culture (constant V_R)
2) Constant $\langle m \rangle$ for intracellular volume basis B
3) For part (c) dilution / degradations terms for metabolites were neglected ie. $\bar{\mu}[G6P]$, $\bar{\mu}[PEP]$, etc. terms neglected (negligible cell death)
4) Rxns. rates from $\emptyset$ are treated as zero order
5) Specific growth rate $\bar{\mu}$ is at its maximum possible growth rate $\mu \simeq \mu_g^{max}$
6) Enz. for rxns. r_1, r_3, r_4, T and x are at non-zero steady state.

b) Table of parameters: (See attached Excel sheet)

c) Metabolite balances $(v_2 = q_{Glc})$

Neglecting dilution terms

$$\frac{d}{dt}\begin{bmatrix} Glc \\ G6P \\ PEP \\ Pyr \end{bmatrix} = \begin{bmatrix} -C & 0 & 0 & 0 \\ 1 & 0 & 0 & -1 \\ -1 & 1 & 0 & 0 \\ 1 & 0 & -1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ q_{Glc} = v_2 \\ v_3 \\ v_4 \end{bmatrix}$$

Given $\frac{d[G6P]}{dt} = \frac{d[PEP]}{dt} = \frac{d[Pyr]}{dt} = 0$

and q_{Glc} is known, C is known

By knowing cellmass, $\frac{d}{dt}[Glc]$ is known.

We get $v_2 - v_4 = 0$ $\Rightarrow$ $v_1 = v_3 = v_4 = q_{Glc}$

$v_1 - v_2 = 0$

$v_2 - v_3 = 0$

As Glc is consumed to produce G6P, for G6P to be at steady state, it must be consumed at the same rate as its production $\Rightarrow$ fluxes are equal.

Similar explanation for PEP and Pyr also.

OR

Considering intracellular variables,

$$\frac{d}{dt}\begin{bmatrix} G6P \\ PEP \\ P_{gx} \end{bmatrix} = \begin{bmatrix} 0 & 0 & -1 \\ 1 & 0 & 0 \\ 0 & -1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_3 \\ v_4 \end{bmatrix} + \begin{bmatrix} 1 \\ -1 \\ 1 \end{bmatrix} q_{gec}$$

$$\Rightarrow \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix} = \begin{bmatrix} 0 & 0 & -1 \\ 1 & 0 & 0 \\ 0 & -1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_3 \\ v_4 \end{bmatrix} + \begin{bmatrix} 1 \\ -1 \\ 1 \end{bmatrix} q_{gec}$$

$\underline{\underline{A}}$ $\underline{v}$ $\underline{\underline{I}}$

$$\Rightarrow \quad 0 = \underline{\underline{A}}\,\underline{v} + \underline{\underline{I}}\, q_v$$

$$\Rightarrow \quad \underline{v} = A^{-1}(-I q_v)$$

So A^{-1} must exist to solve for fluxes.
ie. $\det A$ must be non-zero.

Here $\det A = \underline{1}$

$\Rightarrow$ A is invertible and fluxes can be calculated.