1 Introduction

Coupled differential equations describing metabolite concentration in culture media, cytosol and bacterial micro-compartment through time are presented.

2 Concentrations in time

2.1 Culture media

☐ Vanillate concentration in culture media, assumed constant to analyze steady state.

$$\frac{d[V_m]}{dt} = 0$$

☐ Protocatehuate concentration in culture media, assumed constant to analyze steady state.

$$\frac{d[P_m]}{dt} = 0$$

☐ Catechol concentration in culture media, assumed constant to analyze steady state.

$$\frac{d[C_m]}{dt} = 0$$

☐ Muconic acid concentration in culture media, assumed constant to analyze steady state.

$$\frac{d[M_m]}{dt} = 0$$

2.2 Cytosol

☐ Vanillate concentration in interbacterial media

$$\frac{d[V_c]}{dt} = j_{b_{V_i}} \cdot ([V_b] - [V_c]) + j_{b_{[V_m]}} \cdot ([V_b] - [V_c]) - j_{m_V} \cdot ([V_c] - [V_m]) - \frac{K_{cat}^{ligM} \cdot [ligM]_c \cdot [V_c]}{[V_c] + K_m^{ligM}}$$

- $j_{b_{V_i}}$ is the flux constant of Vanillate introduction to BMC through shell porus. Sub-sub-sub index ”m” stands for ”media”,
- K_{cat}^{ligM} and K_m^{ligM} are catallytic and Michaelis constant of $ligM$ enzyme, respectively.
- $[ligM]_c$ is $ligM$ enzyme concentration in cytosol.
- j_{m_V} is Vanilllate flux constant through membrane, assumed equal for in and out fluxes.

Same analysis applies for $j_{b_{P_i,C_i,M_i}}$ ☐ Protocatehuate concentration in interbacterial media

$$\frac{d[P_c]}{dt} = \cdot j_{b_{P_i}} \cdot ([P_b] - [P_c]) + \cdot j_{b_{[P_m]}} \cdot ([P_b] - [P_c]) - \cdot j_{m_P} \cdot ([P_c] - [P_m])$$

$$+\frac{K_{cat}^{ligM} \cdot [ligM]_c \cdot [V_c]}{[V_c] + K_m^{ligM}} - \frac{K_{cat}^{aroY} \cdot [aroY]_c \cdot [P_c]}{[P_c] + K_m^{aroY}}$$

☐ Catechol concentration in interbacterial media

$$\frac{d[C_c]}{dt} = \cdot j_{b_{C_i}} \cdot ([C_b] - [C_c]) + \cdot j_{b_{C_m}} \cdot ([C_b] - [C_c]) - \cdot j_{M_c} \cdot ([C_c] - C_m)$$

$$+\frac{(K_{cat}^{aroY} \cdot [aroY]_c \cdot [P_c])}{([P_c] + K_m^{aroY})} - \frac{K_{cat}^{catA} \cdot [catA]_c \cdot [C_c]}{[C_c] + K_m^{catA}}$$

☐ Muconic acid concentration in interbacterial media

$$\frac{d[M_c]}{dt} = j_{b_{M_i}} \cdot ([M_b] - [M_c]) + \cdot j_{b_{[M_m]}} \cdot ([M_b] - [M_c]) - \cdot j_{m_M} \cdot ([M_c] - [M_m]) + \frac{K_{cat}^{catA} \cdot [catA]_c \cdot [C_c]}{[C_c] + K_m^{catA}}$$

2.3 BMC

☐ Vanillate concentration inside BMC.

$$\frac{d[V_b]}{dt} = j_{b_{V_i}} \cdot ([V_b] - [V_c]) - j_{b_{V_m}} \cdot ([V_b] - [V_c]) - \frac{K_{cat}^{ligM} \cdot [ligM]_b \cdot [V_b]}{[V_b] + K_m^{ligM}}$$

☐ Protocatehuate concentration inside BMC.

$$\frac{d[P_b]}{dt} = j_{b_{P_i}} \cdot ([P_b] - [P_c]) - j_{b_{P_m}} \cdot ([P_b] - [P_c]) + \frac{K_{cat}^{ligM} \cdot [ligM]_b \cdot [V_b]}{[V_b] + K_m^{ligM}} - \frac{K_{cat}^{aroY} \cdot [aroY]_b \cdot [P_b]}{[P_b] + K_m^{aroY}}$$

☐ Cathecol concentration inside BMC.

$$\frac{d[C_b]}{dt} = j_{b_{C_i}} \cdot ([C_b] - [C_c]) - j_{b_{C_o}} \cdot ([C_b] - [C_c]) + \frac{K_{cat}^{aroY} \cdot [aroY]_b \cdot [P_b]}{[P_b] + K_m^{aroY}} - \frac{K_{cat}^{catA} \cdot [catA]_b \cdot [C_b]}{[C_b] + K_m^{catA}}$$

☐ Muconic acid concentration inside BMC.

$$\frac{d[[M_b]]}{dt} = j_{b_{M_i}} \cdot ([M_b] - [M_c]) - \cdot j_{b_{M_m}} \cdot ([M_b] - [M_c]) + \frac{K_{cat}^{catA} \cdot [catA]_b \cdot [C_b]}{[C_b] + K_m^{catA}}$$

2.4 BMC without ligM

We make a second model where ligM were not introduced to BMC, that is because tetrahydrofolate may not be able to get into a BMC for his big size.

☐ Vanillate concentration inside BMC.

$$\frac{d[V_b]}{dt} = j_{b_{V_i}} \cdot ([V_b] - [V_c]) - j_{b_{V_m}} \cdot ([V_b] - [V_c])$$

☐ Protocatehuate concentration inside BMC.

$$\frac{d[P_b]}{dt} = j_{b_{P_i}} \cdot ([P_b] - [P_c]) - j_{b_{P_m}} \cdot ([P_b] - [P_c]) - \frac{K_{cat}^{aroY} \cdot [aroY]_b \cdot [P_b]}{[P_b] + K_m^{aroY}}$$

☐ Cathecol concentration inside BMC.

$$\frac{d[C_b]}{dt} = j_{b_{C_i}} \cdot ([C_b] - [C_c]) - j_{b_{C_o}} \cdot ([C_b] - [C_c]) + \frac{K_{cat}^{aroY} \cdot [aroY]_b \cdot [P_b]}{[P_b] + K_m^{aroY}} - \frac{K_{cat}^{catA} \cdot [catA]_b \cdot [C_b]}{[C_b] + K_m^{catA}}$$

☐ Muconic acid concentration inside BMC.

$$\frac{d[[M_b]]}{dt} = j_{b_{M_i}} \cdot ([M_b] - [M_c]) - \cdot j_{b_{M_m}} \cdot ([M_b] - [M_c]) + \frac{K_{cat}^{catA} \cdot [catA]_b \cdot [C_b]}{[C_b] + K_m^{catA}}$$

2.5 Expression

☐ Transcription rate from Meyer, A.J. et al. adjustment.

$$\frac{d\,[mRNA]}{dt} = \frac{c * 0.03 \frac{PoPS}{RPU} * \left(Ymin + (Ymax - Ymin)\,\frac{X^n}{K^n + X^n}\right)}{V} - \gamma_{mRNA}\,[mRNA]$$

☐ ligM expression.

$$\frac{d\,[ligM]}{dt} = a\,[mRNA] - (\gamma_{ligM})\,[ligM]$$

☐ aroY expression.

$$\frac{d\,[aroY]}{dt} = a\,[mRNA] - (\gamma_{aroY})\,[aroY]$$

☐ catA expression.

$$\frac{d\,[catA]}{dt} = a\,[mRNA] - (\gamma_{catA})\,[catA]$$

3 Integrating ODE's

We use python to integrate all this ODE's, all models can be found at: https://github.com/uanl-igem/igem-2019

3.1 Expression model

☐ Pharameters
K1cat = (0.078*9.14) -Kcat LigM
K1m = 0.078 Km LigM
K2cat = 0.00009 Kcat AroY
K2m = 0.59 -Km AroY
K3cat = (0.00185*10485) -Kcat CatA
K3m = 0.00185 Kmm CatA
volo = 1000000 Medium volume (outside)
volc = 1e-15 Citoplasm volume
volb = 0 BMC volume
Ym = 0.0043 mRNA degradation rate
n = 15 Number of plasmid copies
PoPS = 0.03 Transcription rate
Ymin = 2.4e-3 Min transcription rate (RPU's)
Ymax = 3 Max transcription rate (RPU's)
K = 26 Marionette constant (uM)
n2 = 2.3 Another marionette constant
a = 29.97 ligM traduction rate
b = 26.30 aroY traduction rate
c = 41.30 catA traduction rate
Ecr = 0.000385 ligM dilution rate
EEcr = 0.000385 aroY dilution rate
EEEcr = 0.000385 catA dilution rate
Vo = 3e-3 vanilic acid outside
Po = 0 protocatechuic acid outside
Co = 0 catechol outside
Mo = 0 cis,cis-muconic acid outside
flux constants for species trough membrane
$j_{mV} = 1e-13Va$
$j_{mP} = 1e-13PCA$
$j_{mC} = 1e-13Catechol$
$j_{mM} = 1e-13MA$

3.2 Modified BMC

☐ Pharameters K1cat = (0.078*9.14) Kcat LigM
K1m = 0.078 Km LigM
K2cat = 0.00009*1 Kcat AroY
K2m = 0.59 Km AroY
K3cat = (0.00185*10485) Kcat CatA
K3m = 0.00185 Kmm CatA
volo = 1000000 Medium volume (outside)
volc = 1e-15 Citoplasm volume
volb = 0.1e-15 BMC volume

Vo = 3e-3 vanilic acid outside
Po = 0 protocatechuic acid outside
Co = 0 catechol outside
Mo = 0 cis,cis-muconic acid outside
Ec = 0.1479
EEc = 0.04329
EEEc = 0.2039
Eb = 1.2*Ec
EEb = 1.2*EEc
EEEb = 1.2*EEEc
flux constants for species trough membrane
$j_{mV} = 1e-13VAtroughMembrane$
$j_{mP} = 1e-13PCA$
$j_{mC} = 1e-13Catechol$
$j_{mM} = 1e-13MA$
$j_{bV}o = 0.1e-13throughpduU$
$j_{bP}o = 0.1e-13$
$j_{bC}o = 0.1e-13$
$j_{bM}o = 0.1e-13$
$j_{bV}i = 1e-15pduA$
$j_{bP}i = 1e-15$
$j_{bC}i = 1e-15$
$j_{bM}i = 1e-15$

3.3 Modified BMC

☐ Pharameters K1cat = (0.078*9.14) Kcat LigM
K1m = 0.078 Km LigM
K2cat = 0.00009*1 Kcat AroY
K2m = 0.59 Km AroY
K3cat = (0.00185*10485) Kcat CatA
K3m = 0.00185 Kmm CatA
volo = 1000000 Medium volume (outside)
volc = 1e-15 Citoplasm volume
volb = 0.1e-15 BMC volume
Vo = 3e-3 vanilic acid outside
Po = 0 protocatechuic acid outside
Co = 0 catechol outside
Mo = 0 cis,cis-muconic acid outside
Ec = 0.1479
EEc = 0.04329
EEEc = 0.2039
Eb = 1.2*Ec
EEb = 1.2*EEc
EEEb = 1.2*EEEc
flux constants for species trough membrane

$\mathrm{j}_{mV} = 1e - 13VAtroughMembrane$
$\mathrm{j}_{mP} = 1e - 13PCA$
$\mathrm{j}_{mC} = 1e - 13Catechol$
$\mathrm{j}_{mM} = 1e - 13MA$
$\mathrm{j}_{bV}o = 0e - 13throughpduU$
$\mathrm{j}_{bP}o = 0e - 13$
$\mathrm{j}_{bC}o = 0e - 13$
$\mathrm{j}_{bM}o = 0e - 13$
$\mathrm{j}_{bV}i = 1e - 15pduA$
$\mathrm{j}_{bP}i = 1e - 15$
$\mathrm{j}_{bC}i = 1e - 15$
$\mathrm{j}_{bM}i = 1e - 15$