Equations and model

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Multi substrate enzyme kinetics

Type 1a - Linear

$$v = \frac{V_{\max}\,[S_1][S_2]}{K_{is1}K_{m1} + K_{m1}[S_1] + [S_1][S_2]}, \tag{1}$$

Type 1b - Non-linear

$$v = \frac{V_{\max}\,[S_1][S_2]}{K_{is1}K_{m1} + K_{m1}[S_1] + K_{m2}[S_2] + [S_1][S_2]}, \tag{2}$$

Type 2 - Ping-Pong

$$v = \frac{V_{\max}\,[S_1][S_2]}{K_{m2}[S_1] + K_{m1}[S_2] + [S_1][S_2]}, \tag{3}$$

where: V_{max} is the maximum velocity, and:

$$K_{is1} = \frac{[E][S_1]}{[ES_1]}, K_{m1} = \frac{[ES_1][S_2]}{[ES_1S_2]}, K_{m2} = \frac{[ES_2][S_1]}{[ES_1S_2]}. \tag{4}$$

Statistics

Assumed measurement uncertainty for χ^2 scaling. Because replicate measurements (and thus empirical standard errors) were not available, we assumed a constant measurement uncertainty across all observations. Specifically, for each data point $i = 1, \ldots, n$,

$$\sigma_i \equiv \sigma = 0.02\,\mathrm{SD}(v),$$

where $\mathrm{SD}(v)$ denotes the sample standard deviation of the observed rates $\{v_i\}_{i=1}^{n}$.

To avoid division by zero (or nonpositive values), we enforced a lower bound:

$$\sigma \leftarrow \max(\sigma,\, 1.0).$$

Chi-square statistic. Given observations v_i and model predictions $\hat{v}_i = f(\hat{\theta}; s_{1,i}, s_{2,i})$ with measurement uncertainty σ_i, the weighted residual is

$$r_i(\theta) \;=\; \frac{v_i - f(\theta; s_{1,i}, s_{2,i})}{\sigma_i}. \tag{5}$$

The parameter estimate $\hat{\theta}$ is obtained by minimizing the weighted sum of squares

$$\hat{\theta} \;=\; \arg\min_{\theta} \sum_{i=1}^{n} r_i(\theta)^2 \;=\; \arg\min_{\theta} \sum_{i=1}^{n} \left(\frac{v_i - \hat{v}_i(\theta)}{\sigma_i} \right)^2. \tag{6}$$

After optimization, the chi-square goodness-of-fit statistic is computed as

$$\chi^2 \;=\; \sum_{i=1}^{n} \left(\frac{v_i - \hat{v}_i}{\sigma_i} \right)^2. \tag{7}$$

Degrees of freedom and reduced chi-square. Let p be the number of fitted parameters and n the number of observations. The degrees of freedom are

$$\mathrm{dof} \;=\; \max(n - p,\, 1), \tag{8}$$

and the reduced chi-square is

$$\chi^2_{\mathrm{red}} \;=\; \frac{\chi^2}{\mathrm{dof}}. \tag{9}$$

R^2 **(coefficient of determination).** Define the residual sum of squares and total sum of squares by

$$SS_{\mathrm{res}} \;=\; \sum_{i=1}^{n} (v_i - \hat{v}_i)^2, \qquad SS_{\mathrm{tot}} \;=\; \sum_{i=1}^{n} (v_i - \bar{v})^2, \qquad \bar{v} \;=\; \frac{1}{n} \sum_{i=1}^{n} v_i. \tag{10}$$

Then

$$R^2 \;=\; 1 - \frac{SS_{\mathrm{res}}}{SS_{\mathrm{tot}}} \quad (\text{if } SS_{\mathrm{tot}} > 0;\ \text{otherwise } R^2 \text{ undefined}). \tag{11}$$

Time Calculation

Given. Cofactor S_2 is in excess, so the rate depends effectively only on S_1 via Michaelis–Menten kinetics:

$$v(S_1) = \frac{V_{\max} S_1}{K_m + S_1}, \qquad \frac{dS_1}{dt} = -v(S_1). \tag{12}$$

Molecular weight of S_1: $MW = 150$ g/mol. Initial and final concentrations:

$$S_{1,0} = 100 \text{ g/L}, \qquad S_{1,f} = 1 \text{ g/L}. \tag{13}$$

Unit conversion (g/L to mM). Using 1 mol/L = 1000 mM,

$$S_0 = \frac{100}{150} \text{ mol/L} \times 1000 = 666.67 \text{ mM}, \qquad S_f = \frac{1}{150} \text{ mol/L} \times 1000 = 6.667 \text{ mM}. \tag{14}$$

Integrated batch MM equation. Solve

$$\frac{dS}{dt} = -\frac{V_{\max} S}{K_m + S} \quad \Longrightarrow \quad \left(1 + \frac{K_m}{S}\right) dS = -V_{\max}\, dt. \tag{15}$$

Integrating from S_0 to S_f gives

$$\int_{S_0}^{S_f} \left(1 + \frac{K_m}{S}\right) dS = -\int_0^t V_{\max}\, dt \tag{16}$$

$$(S_f - S_0) + K_m \ln\left(\frac{S_f}{S_0}\right) = -V_{\max} t, \tag{17}$$

hence

$$t = \frac{S_0 - S_f}{V_{\max}} + \frac{K_m}{V_{\max}} \ln\left(\frac{S_0}{S_f}\right). \tag{18}$$

Numerical evaluation. Using the fitted parameters (from the best model fit)

$$V_{\max} = 1.0 \text{ mM/s}, \qquad K_m = 0.1 \text{ mM},$$

and $S_0 = 666.67$ mM, $S_f = 6.667$ mM,

$$t = \frac{666.67 - 6.667}{1.0} + \frac{0.1}{1.0} \ln\left(\frac{666.67}{6.667}\right) = 660.0 + 0.1 \ln(100).$$

$$t \approx 660.0 + 0.4605 = 660.46 \text{ s} \approx 11.01 \text{ min}.$$

$$\boxed{t \approx 6.60 \times 10^2 \text{ s } \approx 11.0 \text{ min.}}$$

Biomass Production

Stoichiometric Matrix

$$\begin{array}{c|ccccccccc}
 & v_1 & v_2 & v_3 & v_4 & v_5 & v_6 & v_7 & v_8 & v_9 \\
\hline
\text{CIT} & +1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\text{ICT} & 0 & +1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
\text{AKG} & 0 & 0 & +1 & -1 & 0 & 0 & 0 & 0 & 0 \\
\text{SCA} & 0 & 0 & 0 & +1 & -1 & 0 & 0 & 0 & 0 \\
\text{SUC} & 0 & 0 & 0 & 0 & +1 & -1 & 0 & 0 & 0 \\
\text{FUM} & 0 & 0 & 0 & 0 & 0 & +1 & -1 & 0 & 0 \\
\text{MAL} & 0 & 0 & 0 & 0 & 0 & 0 & +1 & -1 & 0 \\
\text{OAA} & -1 & 0 & 0 & 0 & 0 & 0 & 0 & +1 & -1 \\
X & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & +1
\end{array} \tag{19}$$

Steady-state mass balance equations

$$\begin{aligned}
\text{CIT}: &\quad v_1 - v_2 = 0, && (20)\\
\text{ICT}: &\quad v_2 - v_3 = 0, && (21)\\
\text{AKG}: &\quad v_3 - v_4 = 0, && (22)\\
\text{SCA}: &\quad v_4 - v_5 = 0, && (23)\\
\text{SUC}: &\quad v_5 - v_6 = 0, && (24)\\
\text{FUM}: &\quad v_6 - v_7 = 0, && (25)\\
\text{MAL}: &\quad v_7 - v_8 = 0, && (26)\\
\text{OAA}: &\quad v_8 - v_1 - v_9 = 0 && (27)\\
& && (28)
\end{aligned}$$

Solving the steady-state equations

Given that:

$$v_9 = D, \tag{29}$$

and

$$\begin{aligned}
v_2 &= v_1, && (30)\\
v_3 &= v_2 = v_1, && (31)\\
v_4 &= v_3 = v_1. && (32)\\
v_5 &= v_4 = v_1, && (33)\\
v_7 &= v_6, && (34)\\
v_8 &= v_7 = v_6, && (35)\\
& && (36)
\end{aligned}$$

we can obtain this equation:

$$v_6 - v_1 - D = 0 \tag{37}$$

giving us:

$$\begin{aligned}
v_1 &= v_6 - D, && (38)\\
v_6 &= v_1 + D, && (39)\\
D &= v_6 - v_1 && (40)
\end{aligned}$$

Constraints

Irreversibility constraints:

$$v_i \geq 0 \quad \text{for } i \in \{1, 2, 3, 4, 5, 7, 8, 9\}. \tag{41}$$

$$\begin{aligned}
v_1 &= v_6 - D \geq 0, && (42)\\
v_6 &\geq D && (43)
\end{aligned}$$

$$v_6 = v_1 + D \geq 0, \tag{44}$$
$$v_1 \geq -D \tag{45}$$

$$D = v_6 - v_1 \geq 0, \tag{46}$$
$$v_6 \geq v_1 \tag{47}$$

$$v_1 \geq 0, \tag{48}$$
$$v_6 \geq 0, \tag{49}$$
$$D \geq 0 \tag{50}$$

Maximal enzyme capacity constraint:

$$v_6 \leq v_{6,max} \tag{51}$$

MM constraints:

$$v_6 = \frac{V_{\max}\,[\text{SUC}]}{K_m + [\text{SUC}]} - \frac{V_{\max}\,[\text{FUM}]}{K_m + [\text{FUM}]}, \tag{52}$$

$$|v_6| \leq V_{max} \tag{53}$$

Since $v_6 \geq D$, $v_6 \geq v_1$ and $D \geq 0$,

$$max(v_1, D) \leq v_6 \leq U, \qquad U := \min(V_{\max},\, v_{6,\max}), \tag{54}$$

provided $D \leq U$.

Saturation function

From the provided equation for v_6 we define the saturation function:

$$\phi(x; K_m) := \frac{x}{K_m + x}, \qquad x \geq 0,\ K_m > 0. \tag{55}$$