CHEME 5440 - Final Exam
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Problem 4

PFK rate expression $\Rightarrow$ $\boxed{\hat{r}_1 = r_1 v(\ldots\ldots)}$,

where, $\boxed{r_1 = k_{cat} \cdot E_1 \cdot \left(\frac{FGP}{K_{F6P} + F6P}\right) \cdot \left(\frac{ATP}{K_{ATP} + ATP}\right)}$ and

$$\boxed{v(\ldots\ldots)_j = \frac{\sum_{i \in \{x\}} W_i f_i(\ldots\ldots)}{\sum_{j \in G_j} \omega_j f_j(\ldots\ldots)}} \; ; \; \boxed{f_i = \frac{(x/K_i)^n}{\left(1 + (x/K_i)^n\right)}}$$

Assumptions

→ conc of F6P = 0.1mM and is constant

→ Conc of ATP = 2.3mM and is constant

→ Conc of PFK (E_1) = 0.12μM and is constant

→ K_{F6P} = 0.11mM & K_{ATP} = 0.42mM

→ k_{cat} = $0.4\,s^{-1}$

a) Estimate W_1 and W_2 if 3-5-AMP is the activator.

→ Calculate r_1 (Kinetic limit).

⇒ $k_{cat} = 0.4\cancel{s^{-1}} \times 3600 \frac{hr^{-1}}{\cancel{s^{-1}}} = 1440\,hr^{-1}$

→ E_1 = 0.12μM

$\rightarrow \left(\frac{F6P}{K_{F6P} + F6P}\right) = \left(\frac{100}{110 + 100}\right) \frac{\cancel{\mu M}}{\cancel{\mu M}} = 0.476190476\,2\ldots$

$\Rightarrow \left(\frac{ATP}{K_{ATP} + ATP}\right) = \left(\frac{2300}{420 + 2300}\right) \frac{\cancel{\mu M}}{\cancel{\mu M}} = 0.8455882353\ldots$

$\therefore r_1 = 1440\,hr^{-1} \cdot 0.12\mu M \cdot \left(\frac{100}{110 + 100}\right) \cdot \left(\frac{2300}{420 + 2300}\right)$

$= 69.57983193\ldots$

$\therefore \boxed{r_1 \approx 69.5798\ \mu M/hr}$

[redacted]

→ From the data, we can use the rate when 3-5-AMP concentration is <u>zero</u> to estimate W_1. In this case, $\boxed{f_I \approx 0}$

⇒ When $[3\text{-}5\text{-AMP}] = 0$, $\hat{r} = \underline{\underline{3.003\,\mu M/hr}}$

$$\therefore \quad \hat{r} = r_1 \cdot v$$
$$= r_1 \cdot \left(\frac{W_1}{1+W_1}\right)$$
$$\hat{r} + \hat{r} W_1 = W_1 \cdot r_1$$
$$W_1 (r_1 - \hat{r}) = \hat{r}$$
$$\therefore W_1 = \frac{\hat{r}}{r_1 - \hat{r}} = \frac{3.003\,\mu M/hr}{(69.5798 - 3.003)\,\mu M/hr} = \underline{\underline{0.0451}}$$

→ Similarly, we can use the rate when 3-5-AMP concentration saturates to estimate W_2. In this case, $\boxed{f_I \approx 1}$.

→ When [3-5-AMP] saturates, $\hat{r} = \underline{\underline{68.653\,\mu M/hr}}$.

$$\therefore \quad \hat{r} = \left(\frac{W_1 + W_2}{1 + W_1 + W_2}\right) r_1$$
$$\hat{r}(1+W_1) + \hat{r} W_2 = (W_1 + W_2) r_1$$
$$W_2 (r_1 - \hat{r}) = \hat{r}(1+W_1) - W_1 r_1$$
$$\therefore W_2 = \frac{(\hat{r} + \hat{r} W_1 - W_1 r_1)}{(r_1 - \hat{r})} = \frac{\left(68.653 + (68.653)(0.0451) + (0.0451)(69.5798)\right)\frac{\mu M}{hr}}{(69.5798 - 68.653)\,\mu M/hr}$$

$$W_2 \approx \underline{\underline{74.030}}.$$

b) <u>Estimate binding constants (K) and order parameter (n).</u>

⇒ K and n were determined using Least squares error (attached in the Excel file). Initial estimates of [redacted] K=0.5 and n = <u>2</u> were taken, and solving for least squares yielded the following values:-

$$\boxed{K \approx 0.657}$$
$$\boxed{n = 2.49}$$

c) Plot of estimated & measured rate (with error bars) vs. 3-5-AMP concentration is shown in the following page.

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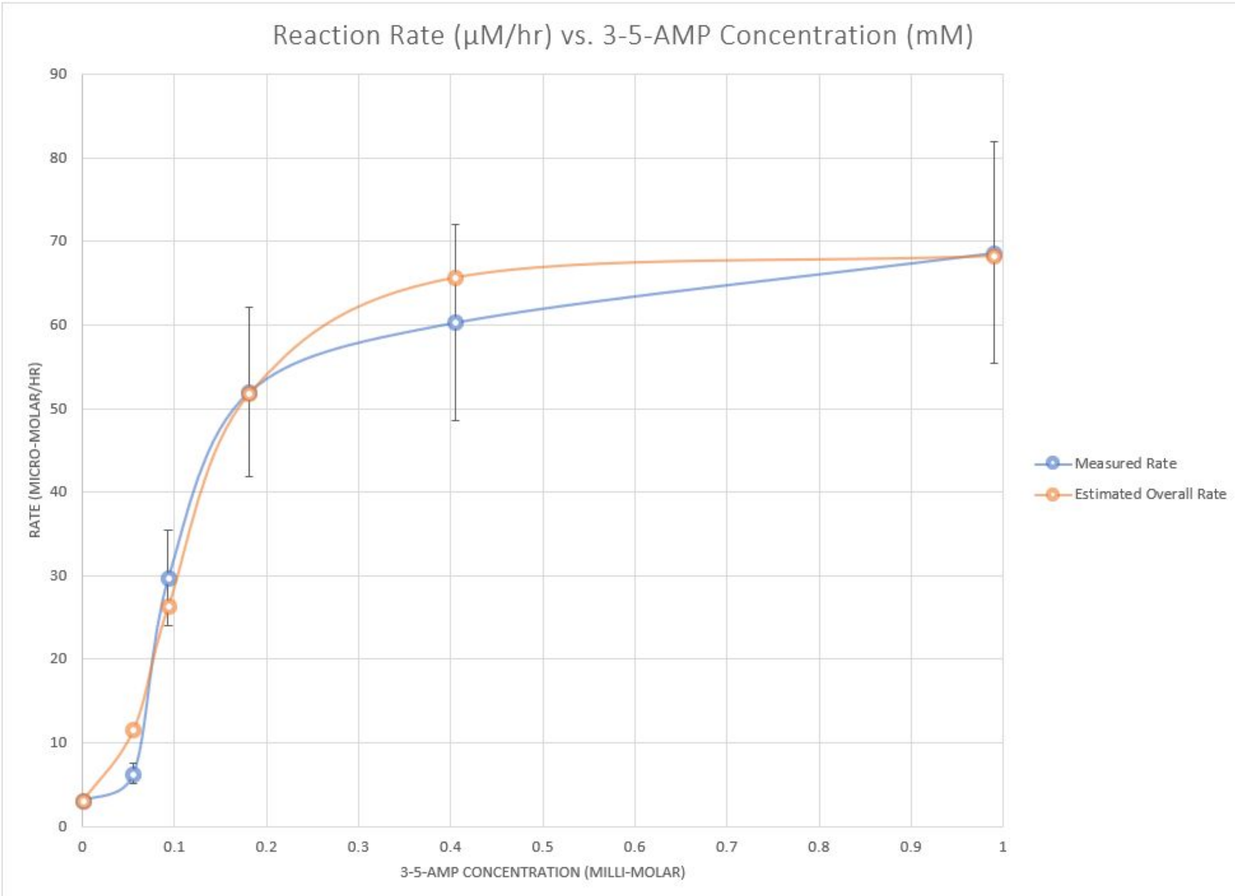
A plot of estimated and measured rate vs. activator concentration:



The graph shows that the proposed model seems to work quite well for the measured activator rates. The only place we see a large discrepancy would be at the lower concentrations of 3-5-AMP; namely when the concentration was 0.055 mM, which yielded a measured rate of 6.302 µM/hr, which was significantly lower than the 11.528 µM/hr that our model predicts. Apart from this point, which falls outside of the 95% confidence level, all other points seem to be in good agreement with our model, and so we can say that the measured data fits our model well.