Project Milestone 4 – Technical Brief Draft

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RE: Technical Brief of Final Results

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Introduction

For our assignment, NaturalCatalysts Inc. provided us with 5 enzymes to analyze and rank. They required us to develop an algorithm to analyze 100 total tests of the 5 enzymes and write a recommendation to determine which is the best.

Our algorithm consists of two sub-functions, one to calculate the initial velocity of the enzyme tests from the given data we were provided, and another function that calculates the maximum velocity and Michaelis constant of each enzyme. We also have a main function that organizes the data and calls the sub-functions before displaying the desired outcome.

Our team made multiple critical decisions in our parameter identification. Three of our critical decisions are described below.

1. We decided to calculate initial velocity (v0) using a slope equation and loop through values to find which x value would have the best r^2 value to use for our slope formula. The looping through of values to find the best r^2 will help minimize the effect of noise for our calculations.
2. We decided to use the Eadie-Hofstee equation to calculate the Michaelis constant (Km) and the max initial velocity (Vmax) from our v0 calculation. We made this critical decision because when we weighed out the pros and cons of all of the 3 equations to use to calculate the parameters and decided that this would be the most effective way to do it with the least amount of error.
3. The third critical decision we made was to set our y-intercept for our v0 calculations at 0. This is because our graphs start at (0,0) and it is important to calculate the slope from that starting point and have no y-intercepts that would impact our calculations.

Parameter Identification Procedure

To find the best estimate of the v0 of the substrate data, we found the slope of part of the function over a certain domain and found the r^2 value as a measure of accuracy of that slope. We then increased the domain by 1 data point each time (using a while loop) and stored the slope with the maximum calculated r^2 value into a variable. We then did the same process for the replicate data and calculated our v0 by taking the average of the two slopes.

After finding the v0s for 10 different substrate levels per enzyme, we used the Eadie-Hofstee linearization, which involves taking the reciprocal of the Michaelis-Menten equation, and then multiplying both sides by Vmax. Vmax was then the y-intercept and the Km is the slope times –1. We then used these new parameters in the Michaelis-Menten plot.

Results

In Figure 1, which is the Michaelis-Menten plot for enzyme 1, the algorithm fits the data almost perfectly, as the model hits every v0 that is part of the data. The same goes for Figures 3, 4, and 5. In Figure 2, there is a slight offset in the last 2 data points, but the difference is not extreme. All in all, the model calculated from the algorithm fit the data pretty well. Table 1 shows the calculated Michaelis constants and Vmax values calculated from our model. As seen in table 1, enzyme 2 has the highest Michaelis constant with a value of 353.15 µM while also having the lowest Vmax at 0.8635 µM /s. Enzyme 5 has the highest Vmax at 1.5933 µM /s. All the data fit in the expected range given by the client.

Interpretation

While there was much noise in the data, our error was relatively small when calculating our Michaelis constants for the data. Based on this error, NaturalCatalysts can say that their products have a Km consistent within 10.86% error (the maximum percent error for our analysis). Based on the analysis, if our error analysis were to fall within the acceptable range of values, then there would be no error in the analysis, since all values calculated were acceptable. For our parameter identification algorithm, we reported extremely accurate values, yielding errors within 6% and as low as 0.20%. This validifies the accuracy of our program and explains the slightly higher Km error.

If we were to give a safe estimation of the maximum error given from our algorithm to the exact value of the Michaelis constant, we would say that the Km lies within 15% error of the midpoint of the data, or if averaged, has an error of about 7.87% if averaged. Based on our results, if NaturalCatalysts selects the best enzyme to begin production and sale, they can claim that their products have a Km value of 353.15 µM with a possible 6.32% error. This is seen as a fairly consistent value since enzymes can be known to have higher error rates, putting them on par with the competition, with other enzymes having around 10% (Salameh & Wiegel, 2010).

References

Lion, Avery D. *Enzyme Analysis.* Personal Communication. 2022, March, 21

Lion, Avery D. *Enzyme Analysis.* Personal Communication. 2022, April, 18

Salameh, M. A., & Wiegel, J. (2010, March 5). *Effects of detergents on activity, thermostability and aggregation of two alkalithermophilic lipases from Thermosyntropha lipolytica*. The open biochemistry journal. Retrieved April 26, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2847205/

Appendix: Figures and Tables

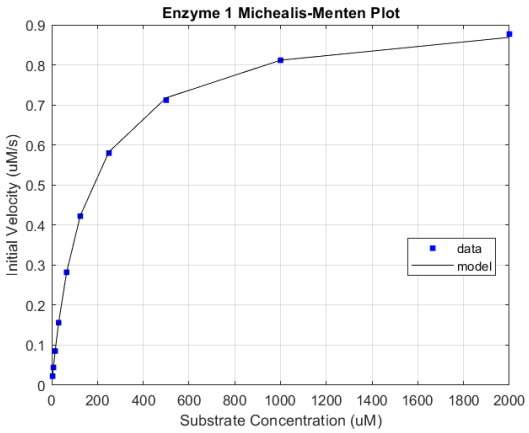

Figure 1 (Enzyme 1 Michaelis-Menten)

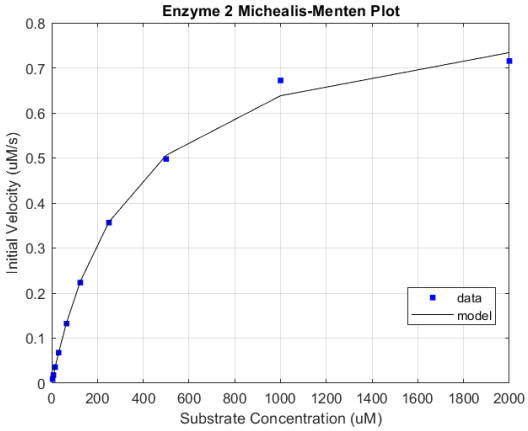

Figure 2 (Enzyme 2 Michaelis-Menten)

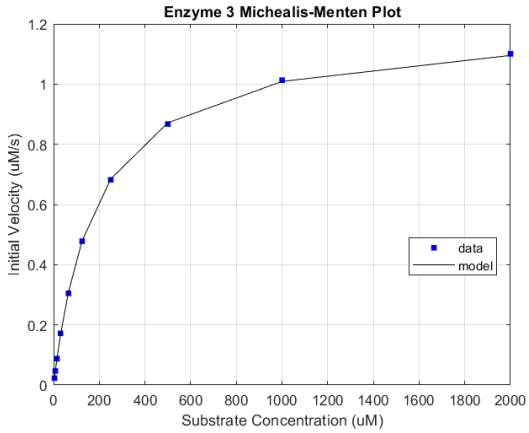

Figure 3 (Enzyme 3 Michaelis-Menten)

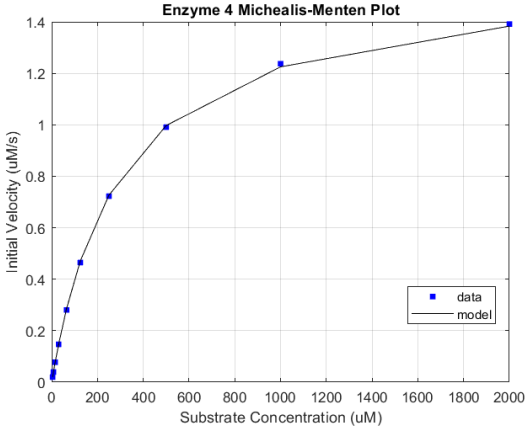

Figure 4 (Enzyme 4 Michaelis-Menten)

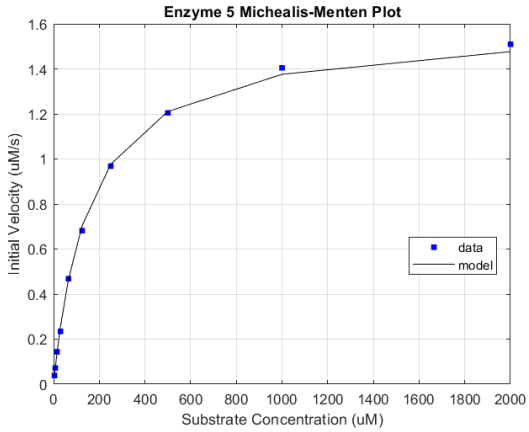

Figure 5 (Enzyme 5 Michaelis-Menten)

Table 1 (Table of enzyme Vmax and Km)

Enzyme Statistics					
	Enzyme 1	Enzyme 2	Enzyme 3	Enzyme 4	Enzyme 5
Vmax (uM/s)	0.9342	0.8635	1.198	1.5886	1.5933
Km (uM)	150.82	353.15	187.44	296.64	157.94