Project Milestone 4 – Technical Brief

To: President Avery D. Lion of NaturalCatalysts Inc.

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RE: Enzyme Performance Assessment

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Introduction

NaturalCatalysts has asked our team to assess the performance of their kinetic enzymes by creating an algorithm that determines the Vmax, Km, SSE, and v0 for each enzyme. The criteria for success were for us to recommend the enzyme with the fastest reaction time, while requiring the lowest concentration value. The constraints we were presented with was that we must use MATLAB while creating our algorithm, were directed to use linear regression, a variation of linearization, and the Michaelis Menten equation in order to determine our results.

Our Algorithm organizes the test data provided to us and uses it to preform parameter identification. This is achieved through multiple instances of Linear Regression, the utilization of data smoothing techniques, Lineweaver-Burk Linearization, and the Michaelis Menten Equation.

The first decision was to use only 35 points in our v0 calculations instead of 50. The second decision was to use the movmean() function to smooth the data. These steps achieved their goal of producing more accurate initial velocities and eliminating noise, respectively. These decisions also impacted the Vmax and Km, which were calculated from v0s. The third decision was to use the MM model instead of the previous method. This was done to more accurately reflect the project's requirements.

Parameter Identification Procedure

The first step of our algorithm was to format our data for use in our UDFs. This allows the algorithm to repeat a series of steps for each Enzyme Set. These Steps are:

1. Smooth each Test's Data - Our algorithm achieves this by preforming the 'Moving Average' method (Investopedia, June 2021). This means we iterate through every data point, and average the product concentration before, at, and after to achieve a new concentration, then replace the point with this new concentration.
2. Finds the v0s – for each concentration in an enzyme set, our algorithm uses the first 35 points and a built –in linear regression function to calculate the slope. This gives the initial velocity, which is then averaged with the result from the duplicate test for each enzyme. This step is repeated for each enzyme, giving 10 v0 values for each enzyme.
3. Find the Lineweaver Burk Model(LWB) – LWB is a Linearization method that allows our algorithm to easily find the Km and Vmax of a given enzyme. First, the inverse of each v0 (y-axis) and enzyme concentration (x-axis) must be found. This linearizes the data. From here, the algorithm uses linear regression to determine the slope and intercept of a model for the linearized data.

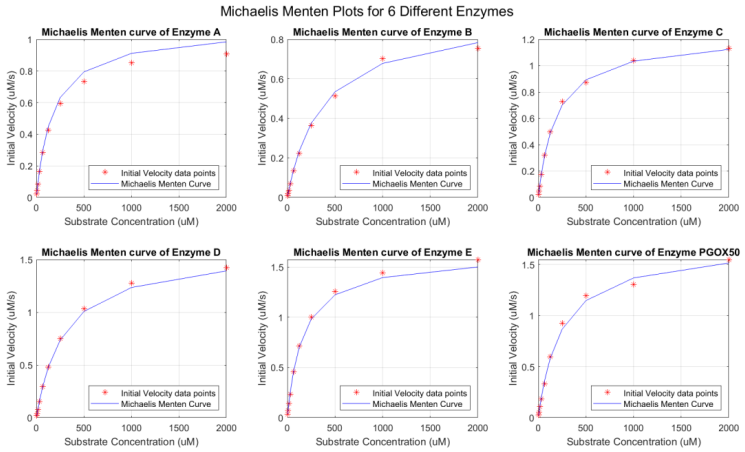
4. Use the LWB to find Vmax and Km – Using the previously calculated Slope and Intercept, Vmax and Km are calculated by the algorithm.

$$Vmax = \frac{1}{y\ intercept} \qquad Km = slope * Vmax$$

5. Move to the next Enzyme Set and Repeat.

Results

Figure 1. Michaelis Menten Plots



Interpretation

The error characterized in this process is caused by the heavy amount of test data given to us by NaturalCatalysts, in which they ran a total of 100 test. In our parameter identification algorithm, we first decided to smooth the data using the 'moving average' method mentioned above, then after finding the parameters this allowed us to use the Lineweaver Burk model to linearize our data without much error. We then used the Michaelis Menten model and calculated the SSE using all the parameters found. This in-depth process allowed us to have an accurate an quality experiment.

NaturalCatalysts can honestly claim that the manufacturing of their products is efficient and consistent. Along with this, the tested SSE value is similar to the reference values, as well as to 0. This means that from the experiments ran, we can conclude that the percent of error is extremely low, and the results we received very close to identical to the regression line (Stanford). This supports our claim that the manufacturing of these enzymes has stayed consistent and are reliable for this company to use for their clients.

References

Devcic, J. (2021, June 15). *Weighted Moving Averages: The Basics*. Investopedia. https://www.investopedia.com/articles/technical/060401.asp

Stanford. (n.d.). *Error Sum of Squares (SSE)*. Error sum of squares. https://hlab.stanford.edu/brian/error_sum_of_squares.html.

Appendix: Figures and Tables

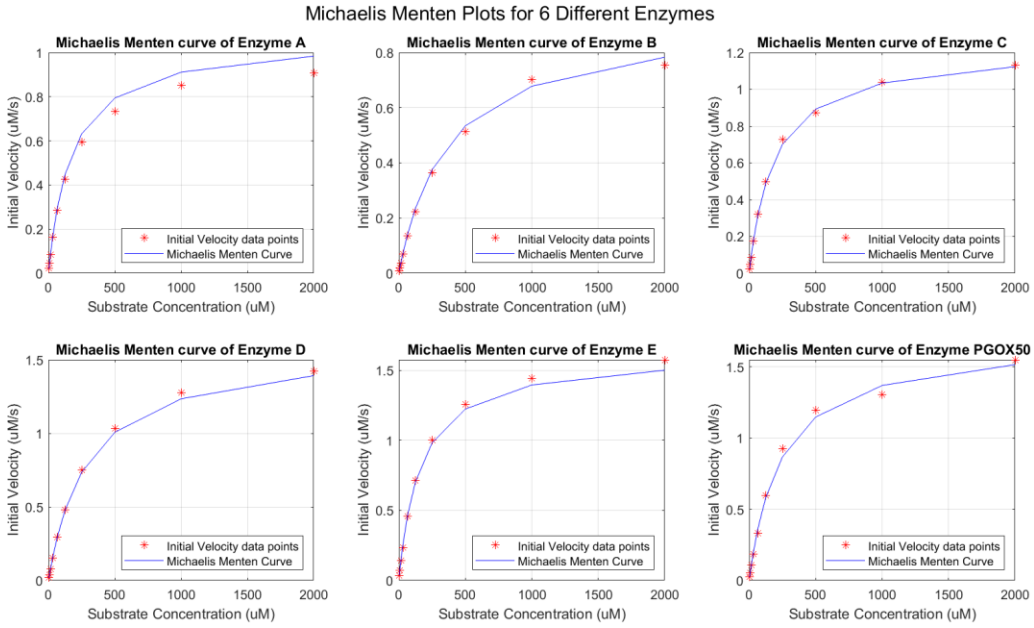


Figure 1. Michaelis Menten Curves

Figure 1 displays the 6 Michaelis Menten graphs, one for each of the five enzymes that are being tested as well as one for the PGOX50 data that was provided to us. From Visual analysis you can see that there is not a very large difference between all of the different enzymes or even the PGOX50 data. This fact makes it more important for our analysis of the numerical data, however there are some slight changes that are visible such as enzyme D and E reaching a higher maximum value or enzyme E having the greatest initial slope. From this information it is possible just from observing the figures that enzyme E has the highest increase in initial velocity across the concentrations as well as the final highest initial velocity as the only one to reach ~1.5 uM/s.

Figure 2. Command Window Output

Data on KE Enzyme A: Vmax: 1.07 | Km: 173.12 | SSE: 0.0156

v0 values: 0.023 0.046 0.085 0.165 0.286 0.426 0.593 0.733 0.850 0.906

Data on KE Enzyme B: Vmax: 0.93 | Km: 367.20 | SSE: 0.0022

v0 values: 0.009 0.019 0.037 0.069 0.135 0.223 0.365 0.513 0.702 0.753

Data on KE Enzyme C: Vmax: 1.23 | Km: 188.48 | SSE: 0.0014

v0 values: 0.024 0.047 0.088 0.174 0.320 0.498 0.727 0.871 1.039 1.130

Data on KE Enzyme D: Vmax: 1.60 | Km: 292.18 | SSE: 0.0035

v0 values: 0.020 0.039 0.076 0.154 0.296 0.481 0.752 1.031 1.276 1.421

Data on KE Enzyme E: Vmax: 1.62 | Km: 163.41 | SSE: 0.0099

v0 values: 0.036 0.070 0.144 0.233 0.457 0.715 1.002 1.258 1.445 1.576

Data on PGOX50 Enzyme: Vmax: 1.70 | Km: 239.26 | SSE: 0.0121

v0 values: 0.026 0.050 0.107 0.185 0.334 0.597 0.927 1.196 1.306 1.550s