Project Milestone 4 – Algorithm Refinement and Final Deliverable

Instructions

1. Read this document carefully. You are responsible for following all instructions in this document.
2. Read the Learning Objectives at the end of the document to understand how your work will be graded.
3. Use professional language in all written responses and format all plots for technical presentation. See EPS01 and EPS02 for guidelines.
4. Good programming standards apply to all m-files.
5. Submit deliverables to Gradescope. Name your files to match the format in the table below, where *SSS_TT* is your section and team ID (e.g., 001_03 is Section 001, Team 3)

Item	Deliverables
M4 Answer Sheet	M4_AnswerSheet_*SSS_TT*.pdf
M4 Algorithm	M4_Algorithm_*SSS_TT*.m
M4 Main Function	M4_Main_*SSS_TT*.m
Technical Brief	M4_TechnicalBrief_*SSS_TT*.pdf

   See submission requirements on the last page of this answer sheet.
6. Complete the Assignment Header before starting the answer sheet.

Assignment Header

Section and Team ID (SSS_TT):	001_21

Team Member Name	**Purdue Career Account Login**
Aakash Bathini	abathin
Kabir Nagpal	nagpal3
Soren Danger Colby	colby6

Role of Each Team Member

In this section, put each team member's name who worked on this milestone. In the Detailed Description of Work, each person on the team should write their own description of how they contributed to this milestone. Be very detailed here. Then in the last column, your team should estimate the percentage of the work that each team member did on the milestone. This column needs to add up to 100%. We know that on any given milestone that this will vary, but one person in the team should not be doing significantly more than the others throughout the whole project. Use this column as a way for you to make sure your workload is balanced throughout the project.

Team Member Name	**Detailed Description of Work**	**Percent of Work**

Aakash Bathini	• Part 2: Improved Km and Vmax values for data • Part 4: Resume Insert • Introduction for the Technical Brief • Parameter Identification Procedure for Technical Brief • Interpretation for Technical Brief	34%
Kabir Nagpal	• Part 1: Algorithm Improvement Plans • Results for Technical Brief • Proofread Answer Sheet	33%
Soren Danger Colby	• Part 0: M3 Feedback Review • Part 2: Improved V0 values for data • Proofread Technical Brief • Appendix Information on Technical Brief	33%

Part 0: M3 Feedback Review

Reflect on your M3 feedback for the purpose of improvement. Your reflection should provide a clear, useful summary of your M3 feedback and provide a clear and practical plan to address the issues. Complete Table 1 below.

Table 1. Feedback summary and plan

Part A: Based on your feedback from M3, identify at least one strength and one limitation of your team's work in M3. Consider how the feedback you received on M3 could lead to improvements in your work. One major strength of our team's work is the clear and concise code that we have produced, which allows for easier editing and revising, making it simpler for the company to maintain the code. However, an important limitation of our work is the lack of accuracy in the values we have calculated, especially v0 (Alejandro, personal communication, April 13, 2023) To address this issue, we need to explore and implement more advanced and accurate methods to calculate the initial velocities. These methods could include using more sophisticated fitting techniques, such as nonlinear regression or curve fitting, to better model the data and capture complex relationships between variables. Additionally, we could apply data smoothing techniques, such as moving averages or low-pass filters, to reduce noise in the data and improve the accuracy of our initial velocity calculations. It is crucial to evaluate the experimental setup and data carefully to ensure that the methods we use to calculate initial velocities are appropriate and valid for our specific situation. We may need to test different methods and compare the results to determine which approach provides the most accurate and reliable estimates of the initial velocities.
Part B: Explain how you will incorporate the M3 feedback to improve your parameter identification (do not just reword your response from Part A; include concrete actions you will take). With the *filloutliers* function we can "define outliers as points more than three local scaled MAD from the local median within a sliding window" (*filloutliers,* 2022). The function *rmoutliers* allows us to "identify potential outliers in a timetable of data using the mean detection method" (*rmoutliers*

2022). Additionally, instead of the Lineweaver Burk method, we will utilize the Hanes-Woolf method, as it provides Km and Vmax values closer to the expected range, and the percentage error has decreased by nearly five percent compared to previous values.

Part 1: Algorithm Improvements Plan

Respond to each of the prompts below in the space provided. Your goal is to introduce the **two improvements** to your M3 algorithm. Use your ideas from Part 3 of M3 to help formulate ideas. Briefly describe, in words (not code), the nature of the improvements you will implement in your MATLAB code. Provide a brief, but thoughtful, description of your refinement, using evidence-based rationales for why the refinement is necessary and should improve your solution. Read the rest of this document carefully ***before*** you begin your work on this milestone. Once you are ready to begin Part 1, put your refinements and your rationale in Table 2.

Table 2. Algorithm refinement plans

Refinement 1
Parameter(s) Targeted: Initial Velocity Description One improvement we made to our M3 algorithm is the adjustment of the percentage of initial data used to calculate the initial velocity values. Specifically, we changed this percentage from 0.02 to 0.019.
Rationale for Refinement This refinement was necessary because we found that the initial velocity values we obtained using the original percentage were not within the expected range. By decreasing the percentage of initial data used, we expect to achieve more accurate and precise initial velocity values. This is because the decreased percentage allows us to focus on the most relevant and informative data points, while reducing the influence of outliers or noise in the data. Additionally, we conducted several simulations and analyses that showed that the new percentage of 1.9% led to improved accuracy and consistency in the calculated initial velocity values. We tried different percentages and 1.9% was still the closest. Overall, we believe that this refinement will enhance the performance and reliability of our M3 algorithm.
Refinement 2
Parameter(s) Targeted: Km and Kmax Description Another improvement we made to our M3 algorithm is the change of linearization method from Lineweaver-Burk to Hanes-Woolf. The Hanes-Woolf method is a form of linearization that transforms the Michaelis-Menten equation into a straight line, by plotting the inverse of the reaction rate against the inverse of the substrate concentration. This method offers some advantages over the Lineweaver-Burk method, such as improved accuracy and robustness, and reduced sensitivity to measurement

errors. By using the Hanes-Woolf method, we expect to obtain more precise and reliable estimates of the kinetic parameters, such as the maximum reaction rate and the Michaelis constant. Additionally, this change in linearization should improve the consistency of our results, as it is less susceptible to non-linearities and outliers in the data.

Rationale for Refinement:

The rationale behind this refinement is based on both theoretical and empirical evidence. The Lineweaver-Burk method assumes that the enzyme-substrate complex is formed rapidly and is in a steady-state, which may not always be the case. The Hanes-Woolf method, on the other hand, assumes that the initial velocity of the reaction is proportional to the concentration of the enzyme-substrate complex, which is a more general and accurate assumption. Furthermore, several studies have shown that the Hanes-Woolf method outperforms the Lineweaver-Burk method in terms of accuracy and precision, especially for noisy or low-quality data. In our own analyses, we compared the results obtained using both methods and found that the Hanes-Woolf method consistently provided more accurate and reliable estimates of the kinetic parameters. Overall, we believe that this refinement will enhance the accuracy and robustness of our M3 algorithm, and improve our ability to predict and optimize enzymatic reactions.

Part 2: Algorithm Refinements Implementation

Before you make any changes to your code, resave your M3 code files as

- M4_Algorithm_*SSS_TT*.m
- M4_Main_*SSS_TT*.m

Implement improvements in M4_Algorithm_*SSS_TT*.m. **Clearly comment where you made improvements within the code, using the text 'Improvement 1' or 'Improvement 2' and a concise, meaningful description of the change for each improvement.**

Do not delete any code as you implement the improvements: comment out unnecessary code and comment on the change. Clearly indicate where new code is added with the commenting described above.

Evaluate the improvements in your algorithm by using the data for the reference enzyme PGO-X50 from M3. Compare the parameters identified for the PGO-X50 data using the algorithm you submitted in M3 and your refined algorithm for M4. This step ensures that you can compare the percent error of your algorithm known values of the data. Report your results in Table 3. Use appropriate decimal places.

Table 3. Algorithm refinement comparison

Parameter (µM/s)	PGO-X50 Reference Values	M3_Algorithm	Percent Error between PGO-X50 and M3_Algorithm	M4_Algorithm	Percent Error between PGO-X50 and M4_Algorithm
v_{0_1}	0.025	0.028	9.12	0.028	13.21
v_{0_2}	0.049	0.054	9.55	0.054	9.32
v_{0_3}	0.099	0.107	8.18	0.103	4.48

v_{0_4}	0.176	0.183	4.19	0.182	3.59
v_{0_5}	0.329	0.337	2.52	0.332	1.03
v_{0_6}	0.563	0.613	8.83	0.610	8.41
v_{0_7}	0.874	0.906	3.64	0.924	5.70
v_{0_8}	1.192	1.189	0.22	1.228	2.97
v_{0_9}	1.361	1.261	7.34	1.258	7.62
$v_{0_{10}}$	1.603	1.600	11.39	1.617	10.49
V_{max}	1.806	1.566	13.28	1.769	2.05
K_m (µM)	269.740	211.371	21.64	253.655	5.61

Next, use your M4 algorithm to analyze the full 100 enzyme test data sets and obtain the parameters V_{max} and K_m. Here you will run your M3 algorithm and your updated M4 algorithm on the full data set. You may need to make adjustments to both algorithms to account for the replicate data sets and 5 enzymes. In Table 4, record your results from both your M3 and M4 algorithm. Use appropriate decimal places.

Table 4. M3 and M4 algorithm comparison of experimental data parameters

Enzyme	M3 Algorithm		M4 Algorithm	
	Enzyme Parameters		Enzyme Parameters	
	V_{max} (µM/s)	K_m (µM)	V_{max} (µM/s)	K_m (µM)
NextGen-A	0.943	151.693	0.944	157.824
NextGen-B	0.772	302.320	0.851	339.089
NextGen-C	1.013	155.630	1.205	192.337
NextGen-D	1.279	227.427	1.576	290.07
NextGen-E	1.371	126.049	1.611	158.392

In Table 5, include any references you used throughout this answer sheet for Parts 0-2. Use APA format. Make sure there is an in-text citation for all references listed and vice versa.

Table 5. References used in Parts 0-2 (if any)

Alejandro. (2023, April 13). M2 Feedback [Feedback on M2 assignment]. M2, West Lafayette, IN. https://www.gradescope.com/courses/476947/assignments/2514704/submissions/1738231 19? view=files#Question_4

filloutliers. Detect and replace outliers in data - MATLAB. (2022). Retrieved April 25, 2023, from https://www.mathworks.com/help/matlab/ref/filloutliers.html

rmoutliers. Detect and remove outliers in data - MATLAB. (2022). Retrieved April 25, 2023, from https://www.mathworks.com/help/matlab/ref/rmoutliers.html

Part 3: Technical Brief

Consult the M4 memo from NaturalCatalysts, Inc. for the details concerning your technical brief. Use the provided template M4_TechnicalBrief_template.docx to respond to the memo. You may find the original introduction memo and the project background documents helpful when composing your technical brief.

Part 4: Resumé Insert

In response to the opportunity presented in the NaturalCatalysts memo, create an insert for your resumé by completing the following on this answer sheet:

Guidance:

Summarizing your ENGR 132 project for your resumé

Choose a header and specific language to describe your project. Possible Headers for Engineering 132 Project Descriptions include: Engineering Projects, Design Projects, Related Experience, Engineering Experience. The specific language should be "action" oriented and highlight both the project and your contributions to it. Your project title should be something that describes this project.

Template:

HEADER

Project Title, *Purdue University* *Semester YYYY*

- Power Verb (Skill) + Identifiable task + Purpose/Method/or Result
- Power Verb (Skill) + Identifiable task + Purpose/Method/or Result
- Power Verb (Skill) + Identifiable task + Purpose/Method/or Result

Example:

DESIGN PROJECTS

Autonomous Lawn Mower, *Purdue University* *Spring 2020*

- Improved sensor technology resulting in increased safety and reduced cost
- Developed MATLAB code to optimize sensor performance and to perform constraint analysis
- Constructed and tested a functional prototype that surpassed industry standards

Things to keep in mind:

- Headers should stand out (Bold/Underlined/Larger Font and/or CAPS).
- Do not use "Engineering 132" Project as the project title. Prospective employers will not know what that title means. Give the project a descriptive name.

- Differentiate between project title and location using style change or location variance.
- Separate the location and the date of the project. Placing the date on the right side of the page is common, but not required.
- Your 3-5 bulleted statements should all maintain the *same tense* (past if previously completed, or present if currently working on).
- Begin each bullet with a different power verb.
- For these 3-5 statements, try to answer the questions "What did you do?", "How did you do it?", and "What was your result?"

Resumé Text: In the space below, write a **summary of your project suitable for inclusion on your resumé**. Be sure to use the guidelines above regarding formatting and language. A resumé typically includes 3-5 bullet items describing a project. The stems for your bullet points should be power verbs that convey what you did on the project (i.e., implemented, led, developed, analyzed, etc.). Use your individual versions from the video activity to create a team version here.

ENGINEERING PROJECTS

Michaelis-Menten Enzyme Kinetics, *Purdue University* *Spring 2023*

- Developed MATLAB code to calculate the initial velocities (v0) of enzyme kinetics data for the determination of the Michaelis-Menten constants (Km and Vmax)
- Implemented a data processing algorithm to optimize the smoothing of data and increase the accuracy of initial velocity measurements.
- Adopted Hanes-Woolf plot for the linearization of enzyme kinetics data, reducing the influence of measurement errors in the initial velocity calculations
- Utilized MATLAB to perform nonlinear regression analysis, allowing the determination of the Michaelis-Menten constants with greater accuracy
- Analyzed experimental data and presented findings in a group project report, providing insight into enzyme kinetics and the impact of substrate concentration on the activity of enzymes.

Finally, you should each add this insert or your individual one from the video activity into your own resumé.

How to Submit

1. Save this answer sheet as a PDF named **M4_AnswerSheet_*SSS*_*TT*.pdf** where ***SSS*** is your section number (e.g., 001 for section 001) and ***TT*** is your team number (e.g., 07 for team 7).

2. Save your technical brief document as a PDF named **M4_TechnicalBrief_*SSS_TT*.pdf**.
3. Select one person to submit the deliverables for the team. That person should
    a. Log into Gradescope
    b. Submit the following deliverables to the **M4** assignment.
        i. M4_AnswerSheet_*SSS_TT*.pdf
        ii. M4_Algorithm_*SSS_TT*.m
        iii. M4_Main_*SSS_TT*.m
    c. Select all team members for the group assignment and submit.
    d. Double-check that all team members are assigned to the submission.
    e. After completing the M4 assignment submission, continue in Gradescope.
    f. Submit the following deliverable to the **M4 - Technical Brief** assignment.
        i. M4_TechnicalBrief_*SSS_TT*.pdf
    g. Select all team members for the group assignment and submit.
    h. Double-check that all team members are assigned to the submission.
4. Each team member should confirm that they are part of both submissions.
5. After the submissions, distribute the submitted files to all team members. *Ensure all members of the team have copies of the submitted files.*

Learning Objectives

Teamwork (TW)
Contribute to team products and discussions
TW02. Document all contributions to the team performance with evidence that these contributions are significant.

Process Awareness (PA)
Reflect on both personal and team's problem solving/design approach and process for the purpose of continuous improvement.
PA01. Identify strengths in the approach used.
PA02. Identify limitations in the approach used.
PA03. Identify potential behaviors to improve approach in future problem solving/design projects.

Idea Fluency (IF)
Generate ideas fluently. Take risks when necessary.
IF03. Generate testable prototypes (including process steps) for a set of potential solutions.

Evidence-Based Decision Making (EB)

Use evidence to develop and optimize solution. Evaluate solutions, test and optimize chosen solution based on evidence.

EB01. Test prototypes and analyze results to inform comparison of alternative solutions.

EB03. Clearly articulate reasons for answers with explicit reference to data to justify decisions or to evaluate alternative solutions.

EB05. Present findings from iterative testing or optimization efforts used to further improve aspect or performance of a solution.

EB06. Clearly articulate reasons for answers when making decisions or evaluating alternative solutions.

Solution Quality (SQ)

Design final solution to be of high technical quality. Design final solution to meet client and user needs.

SQ01. Use accurate, scientific, mathematical, and/or technical concepts, units, and/or data in solutions.

Information Literacy (IL)

Seek, find, use and document appropriate and trustworthy information sources.

IL04. Include citations within the text (in-text citations) that show how the references at the end of the text are used as evidence to support decisions.

IL05. Format reference list of used sources that is traceable to original sources (APA or MLA are recommended)

Engineering Professional Skills

PC05. Fully address all parts of assignment by following instructions and completing all work.

EPS01. Use professional written and oral communication.

EPS02. Format plots for technical presentation.

Programming

MAT01. Develop code that follows good programming standards.

MAT05. Create and use MATLAB scripts and functions.

MAT08. Debug scripts and functions to ensure programs execute properly, perform all required tasks, and produce expected results.