

AC 2009-1273: UTILIZING DIVERSITY IN A BIOPROCESS ENGINEERING COURSE FOR A GROUP PROJECT TO DESIGN AND CHARACTERIZE A BIOREACTOR TO CONVERT CELLOBIOSE TO GLUCOSE

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**Utilizing Diversity in a Bioprocess Engineering Course for a
Group Project to Design and Characterize a Bioreactor
to Convert Cellobiose to Glucose**

Introduction

The field of bioprocess engineering includes the use of engineering principles to design, characterize, and optimize processes that use bioactive agents. This is a highly transdisciplinary field that involves principles in both engineering: chemical, mechanical, electrical, industrial, agricultural, and environmental, and biology: biochemistry and microbiology. At our university, we offer an introductory course in Bioprocess Engineering to seniors and entering graduate students for any of the disciplines listed above. This course is co-taught by faculty in both chemical engineering (CHE) and biosystems and agricultural engineering (BAE). This class can be a challenge to teach due to the diversity of the students at different levels and from different disciplines.

As part of their grade for the course, students participate in a “hands-on” class project designed to give the students experience in bioreactor design and characterization. The goal of the project is to design and characterize a batch enzyme reactor to convert cellobiose to glucose. The class project is divided into two parts: experimental and modeling. For the experimental part, students utilize a temperature-controlled bioreactor to measure the product formation from the enzymatic breakdown of cellobiose to glucose. For the modeling part, the students develop a mathematical model to predict the conversion of cellobiose to glucose in the bioreactor. They have one, three-hour laboratory period to collect data from the reactor. The students then compare their mathematical models to experimental data from the bioreactor and determine if the model is acceptable or not. Students report their findings in both a written report and an oral presentation given to the class by each group.

Students work in teams of three or four to complete the project. We capitalize on the diversity of the students within the class by ensuring that each team has a combination of undergraduate and graduate students from different majors. All team members actively participate in each part of the project and are encouraged to lead in areas of their strengths and to learn in areas of their weaknesses within the group.

Bioprocess Engineering Course

The Bioprocess Engineering course has been designed in such a way to provide students the ability to meet the following learning objectives. At the end of the semester, the students should be able to:

1. Understand the basic role of engineering in bio-processing applications.
2. Obtain a basic understanding of how cells work and become familiar with the environmental conditions (i.e. nutrients, pH, etc.) required for applications of biological components (cells or enzymes) to bio-processing systems.
3. Understand and model enzyme kinetics and apply the models for analysis of immobilized enzymatic bioreactors.
4. Utilize material balances to evaluate cell growth and substrate/product utilization in bioreactors.

5. Design bioreactors to achieve desired results (i.e. specified cell concentration, production rates, etc.)
6. Understand and apply scale-up methods for designing bioreactors.
7. Become familiar with principles of recovery and purification techniques of bioprocesses.
8. Enhance team skills, particularly working with inter-disciplinary majors and graduate students.

The required textbook for the course is Bioprocess Engineering by Michael L. Shuler and Fikret Kargi.¹ The instructors use this text for the majority of course material and supplement it with relevant current journal articles and their own research experiences. To provide students with experience in the course topics, the course includes homework assignments, exams, a team project, and the graduate students in the class each give a seminar over a topic relevant to the course, usually about their research project or a review of a journal article. Homework assignments include combinations of conceptual, direct problem solving, and open-ended design problems. Some homework problems and the project also require the use of computer tools. Students are given instruction to use Polymath software to solve nonlinear equations, nonlinear regression, and ordinary differential equations.

Project Description

After approximately five weeks into the semester, the students were divided into groups and given a memorandum describing the details of the group project, which is shown below. At this point in the semester, the instructors have covered course material that will be required for the successful completion of the project, including enzyme kinetics and batch bioreactor design. The students have also completed several homework assignments over these subjects.

MEMORANDUM

TO: STUDENT GROUP
FROM: BIOREACTOR PROJECT MANAGER
SUBJECT: ENZYME BIOREACTOR
DATE: FEBRUARY 21, 2008

Our company needs to characterize the batch enzyme reactor used to convert cellobiose to glucose. Cellobiose is a disaccharide that is produced from the partial hydrolysis of cellulose, which is part of a process upstream to our bioreactor. We would like to use our bioreactor for

the complete conversion of cellobiose to the final product, glucose. To do this, we will utilize the enzyme, cellobiase in the reactor. However, since the enzyme is expensive, our use is limited to only 200 U per batch.

The rate of an enzyme reaction is strongly dependent on certain reaction parameters including, the concentration of various components (substrate, product, and enzyme), pH, and temperature. The optimal parameters are different for each enzyme and must be determined experimentally. Previous experimental data have suggested that the optimal reaction temperature and pH for cellobiase is 50 °C and 5, respectively. Develop a mathematical model to predict the conversion of cellobiose to glucose in the bioreactor located in the lab. Compare the mathematical model to experimental data from the bioreactor and determine if the model is acceptable or not. Data from previous experiments can be used to determine the kinetic parameters for the model. You will be given the following materials:

- Initial reaction data
- A temperature-controlled bioreactor with cellobiose and cellobiase
- Sodium acetate and acetic acid to prepare a sodium acetate buffer at a set pH
- Glucose and glucose assay reagent (G3293, Sigma-Aldrich)

For the enzymatic reaction in the bioreactor, assume that the enzyme forms a complex with its substrate, the “lock and key” theory, and that the rate equation can be derived by the Michaelis-Menten approach. Evaluate the Michaelis-Menten kinetic parameters by using the Langmuir plot, the Lineweaver-Burk plot, the Eadie-Hofstee plot, and nonlinear regression technique. In evaluating the kinetic parameters, do not include data points that deviate systematically from the Michaelis-Menten model and explain the reason for the deviation. Determine which technique results in the best prediction of kinetic parameters.

The instructors will serve as the technical advisors for this project and you should be working closely with them to define the scope of the project. You will need to start planning your project soon and submit a project preliminary planning report (see below) on March 4, 2008. You are on the agenda to present your work to the technical support group on April 22, 2008. The final report is also due on this date. A complete description of what is expected for the technical presentation and final report will be discussed later in class.

Preliminary Planning Reports

- Goals
- Experimental design (including background and theory)
- Safety
- Specific experiments to be conducted
- Methods of processing data

Project Groups

Project groups consisted of three or four students per group. Students were assigned to groups by the instructors, based on their major (CHE, BAE, or other) and graduate standing (undergraduate or graduate student). The undergraduate students consisted mostly of senior

level students; however, some junior level students were allowed to take the course after approval by the instructors. To increase group diversity, the instructors ensured a mix of students from different majors and graduate standing within each group. For example, each group consisted of one undergraduate student and one graduate student from BAE and one undergraduate student and one graduate student from CHE. Occasionally, there were students from “other” majors, which included chemistry, food science, industrial engineering, and mechanical engineering that would also be distributed throughout the groups to ensure group diversity. The major and graduate standing of the students were determined by the instructors from university records and by an informal questionnaire given to the students on the first day of class asking for such information.

The goal of creating diverse groups was to have students with different levels of experience and backgrounds to interact during a group project exercise. Each team member contributes their own expertise and prior experiences to the group so that each team member can learn from each other, as well as improving the overall project outcomes. Typically, graduate students have more experience than the undergraduates do, since they have already completed their undergraduate degree requirements and have started working on their thesis projects. The differences in course requirements for BAE and CHE that would lead to students with different backgrounds and expertise for the group project include the following:

For BAE students

- Biochemistry – General principles of chemistry in living organisms
- Microbiology – General principles of the biology of microorganisms
- Microbial Technologies in Biosystems Engineering – Introduction to engineering applications of industrial microbiology, including fermentation systems and enzyme kinetics

For CHE students

- Chemical Reaction Engineering – Principles of chemical kinetics rate concepts and data treatment, and elements of reactor design
- Rate Operations – Development and application of phenomenological and empirical models to the design and analysis of mass transfer and separations unit operations
- Chemical Engineering Laboratory – Application of CHE fundamentals and unit operation principles to the analysis of bench and pilot-scale equipment. Primary reaction and mass transfer processes. Design of experiments on non-ideal units to generate credible data useful for validation of principles and for engineering decisions. Interpretation of experimental data and presentation of results.

Mathematical Model

To meet the objectives of this project, students needed to develop a mathematical model for the process. The first step was to use given data from previous experiments (shown in the Appendix) to determine the enzyme kinetic rate constants for a given set of experimental conditions (temperature, pH, etc.). The next step was to use these rate constants within the design equation for a batch bioreactor to determine the change of substrate and product concentration over time. In addition to these main objectives, students also performed the

following data analyses: 1) an error analysis to determine the error involved in the preparation of the initial solutions (sodium acetate buffer, cellobiose, and cellobiase) and in the glucose assay, and 2) a statistical analysis to determine which technique results in the best prediction of kinetic parameters and how well the mathematical model fits the experimental data.

Derivation of the Rate Equation:

The mechanism of a substrate-enzyme reaction can be expressed as



S = Substrate

E = Enzyme

ES = Enzyme-Substrate Complex

P = Product

The Michaelis-Menten approach was used to derive the rate equation. For this approach, it is assumed that the product-releasing step is much slower than the reversible reaction. The reversible reaction involves the formation of an enzyme-substrate complex, which is based on a very weak interaction. It is reasonable to assume that the enzyme-substrate complex formation step is much faster than the product-releasing step, which involves a chemical change. The slow reaction step is then used to determine the rate, while the other is at equilibrium. This approach leads to the final, simplified rate equation, known as the Michaelis-Menten equation, shown below.

$$r = \frac{dC_P}{dt} = -\frac{dC_S}{dt} = \frac{r_{\max} C_S}{K_M + C_S} \quad (3)$$

r_{\max} = Maximum Reaction Rate

K_M = Michaelis Constant

Evaluation of Kinetic Parameters:

The relationship between the initial reaction rate and the initial substrate concentration can be used to estimate the kinetic parameters. This is done by performing a series of batch runs with different levels of initial substrate concentration with a constant initial enzyme concentration and determining the initial reaction rate for each. The results can be plotted graphically so that the values of the kinetic parameters can be determined. The Michaelis-Menten equation is linearized in order to develop expressions for the kinetic parameters that are related to the slope and intercept of the line. Three methods can be used to linearize the Michaelis-Menten equation that result in the following linear equations:

- Langmuir Equation (Hanes-Woolf):

$$\frac{C_S}{r} = \frac{K_M}{r_{\max}} + \frac{C_S}{r_{\max}} \quad (4)$$

- Lineweaver-Burk Equation:

$$\frac{1}{r} = \frac{1}{r_{\max}} + \frac{k_M}{r_{\max}} \frac{1}{C_S} \quad (5)$$

- Eadie-Hofstee Equation:

$$r = r_{\max} - K_M \frac{r}{C_S} \quad (6)$$

Another approach for the determination of the kinetic parameters is to use nonlinear regression, which produces a weighted least-squares estimate of the parameters. The advantages of this technique are:

- it does not require linearization of the rate equation,
- it can be used for complicated, multiparameter models,
- and the estimated parameters are reliable since this method produces weighted least-squares estimates.

Previous experimental data given to the students (shown in the Appendix) are used with the methods described above to determine the rate constants, r_{\max} and K_m . As described in the project memo, in evaluating the kinetic parameters, students are not to include data points that deviate systematically from the Michaelis-Menten model and must explain the reason for the deviation. Students must also determine which technique results in the best prediction of kinetic parameters by performing a statistical analysis (based on correlation coefficient).

Determining the Change of Substrate and Product Concentration with Time:

Once the kinetic parameters for the rate equation are determined, the students use these along with the design equation for a constant volume enzyme batch bioreactor to determine the change of both substrate and product concentration with time. The design equation for an enzyme batch bioreactor is shown below.

$$-\frac{dC_S}{dt} = r = \frac{r_{\max} C_S}{K_M + C_S} \quad (7)$$

The equation can be solved to determine the change of the substrate concentration with respect to time by using the following boundary conditions:

$$t = 0 \quad C_S = C_{S_o} \quad (8)$$

$$K_M \ln \frac{C_{S_o}}{C_S} + (C_{S_o} - C_S) = r_{\max} t \quad (9)$$

Once the substrate concentration is determined for each time point, the product concentration can be determined by the following expression, taking into account that two moles of glucose are produced for every mole of cellobiose.

$$C_P = 2(C_{S_o} - C_S) \quad (10)$$

Experimental Protocol

As part of their preplan requirement, the students had to develop their own experimental protocol for the project. The instructors then reviewed the protocols and gave feedback prior to the laboratory session. The overall project experimental objectives are shown below. The experimental data would be used to compare to a mathematical model for the bioreactor in order to determine if the model is acceptable or not. The students were given one, three-hour laboratory period to collect their data.

The project experimental objectives included:

- To prepare a buffer solution at a set pH for the enzyme reaction within the batch bioreactor. A sodium acetate buffer at pH 5 was prepared and used for all experiments.
- To develop a quantitative method that can be used to determine either the substrate or product concentration in samples taken from the bioreactor. A glucose (hexokinase) assay was used to determine the glucose concentration in samples taken from the bioreactor. A glucose standard curve was prepared and used to determine the concentration of the unknown samples.
- To operate an enzyme bioreactor and measure the change in substrate and product concentration with time. A 1 L batch bioreactor with a water jacket to maintain a constant reaction temperature at 50 °C was used to convert cellobiose to glucose by using the enzyme cellobiase. The glucose concentration within the reactor was measured for set time points by using the glucose (hexokinase) assay and the corresponding cellobiose concentration calculated.

Mathematical Modeling Results

Students used experimental data that was given to them to calculate the kinetic parameters in the Michaelis-Menten rate expression. The data shows the change of substrate concentration with time for the following initial substrate concentration in the bioreactor: 2, 4, 6, 8, and 10 mM. Students were to use this data to determine the initial reaction rates corresponding to the initial concentrations. The reaction rate data as a function of substrate concentration was used with the

four methods described previously (Langmuir, Lineweaver-Burk, and Eadie-Hofstee plots and nonlinear regression) to determine the kinetic parameters K_m and r_{max} of the Michaelis-Menten rate expression. Table 1 shows the calculated values for K_m and r_{max} , along with the square correlation coefficient as a measure of how well the data fit the model used for each method. Figures 1 – 3 show each plot and the equation used to determine K_m and r_{max} .

Table 1. Kinetic Parameters for the Michaelis-Menten Rate Expression Determined by Various Methods

Method	K_m	r_{max}	R^2
Langmuir	9.97	1.73	0.80
Lineweaver-Burk	12.65	2.03	0.97
Eadie-Hofstee	6.67	1.37	0.52
Nonlinear Regression	8.72	1.61	0.93

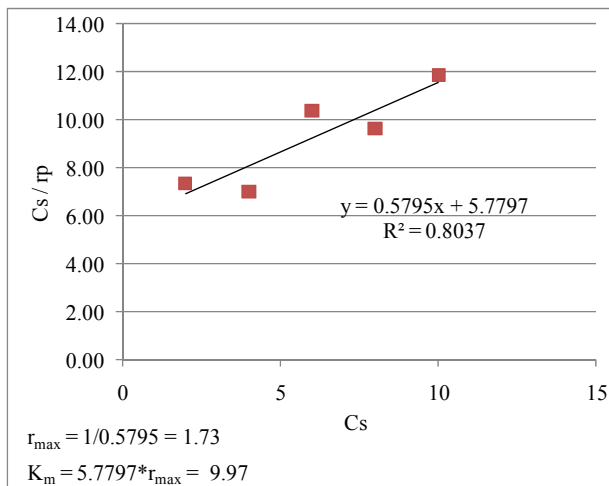


Figure 1. The Langmuir Plot

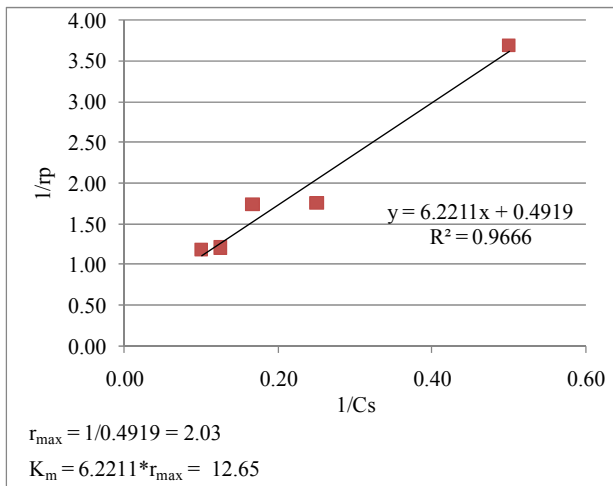


Figure 2. The Lineweaver-Burk Plot

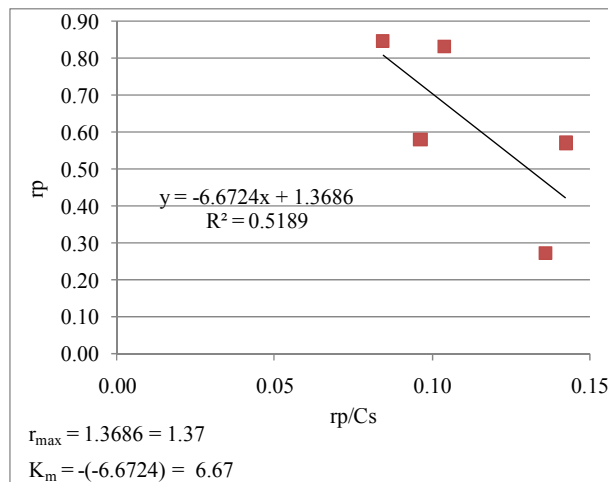


Figure 3. The Eadie-Hofstee Plot

The Lineweaver-Burk plot showed the best fit of the experimental data with a $R^2 = 0.97$. This plot is often used to determine the Michaelis-Menten rate constants because it shows a

relationship between the independent variable C_s and the dependent variable r_p . However, this plot also gives undue weight to measurements made at low substrate concentrations, which may not be as accurate as those made at high substrate concentrations. The Langmuir plot and the nonlinear regression technique give a more accurate weighing to measurements at all the substrate concentrations. These two methods also resulted in similar rate constant values. The Eadie-Hofstee plot resulted in the worst fit of the experimental data with a $R^2 = 0.52$. A disadvantage of this plot is that the rate of reaction, r_p , appears in both coordinates, while it is usually considered as a dependent variable. Any error in the r_p measurement will be weighed more in this plot than the other methods.

Experimental Results

Figure 4 shows the calibration curve for the glucose assay. A straight line was fitted to the data points that resulted in a square correlation coefficient of 0.9794. The equation of the straight line was used to calculate the glucose concentration of unknown samples by using the corresponding measured absorbance for the sample. The calibration curve is an important part of the experimental results due to all other measurements are based on this measurement. Since the glucose assay is essentially another enzymatic reaction, students were cautioned to follow the glucose assay procedures carefully, including accurate reaction temperature and time. Students were also to note and discuss steps where error could be introduced, such as pipetting very small volumes and thoroughly mixing samples. For many of the students, this was a totally new experience to work with new equipment, such as micropipettors, to measure small volumes accurately, and using such sensitive analysis techniques.

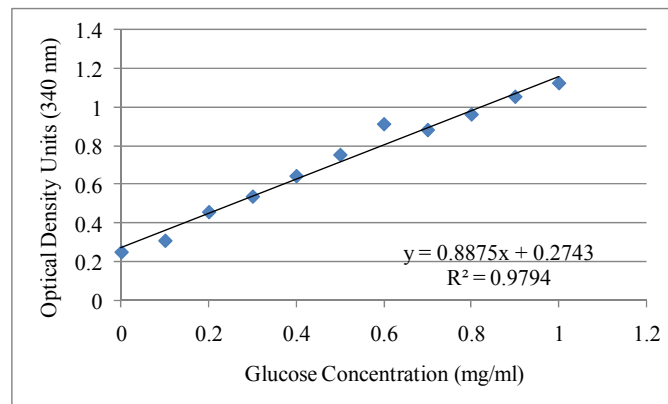


Figure 4. Glucose calibration curve used to determine the unknown glucose concentration in samples from the enzyme bioreactor.

Figure 5 shows the change in substrate concentration (cellobiose) over time measured experimentally and determined by using Eq. 9 with the values of K_m and r_{max} determined by the four methods. Figure 6 shows the change in product concentration (glucose) over time measured experimentally and determined by using the calculated values for the substrate concentration at each time point in Eq. 10. The model data using rate constants determined from the four methods resulted in very similar profiles. The Eadie-Hofstee plot showed the worst fit of the data in determining the Michaelis-Menten rate constants, but the use of these constants showed

the best fit of the model to the experimental time points (≤ 10 minutes). The model equation using rate constants determined by the nonlinear regression technique showed the next best fit to the experimental data for the early time points. None of the models fit the experimental data for the later time points (> 10 minutes). This could be a sign of either product or substrate inhibition occurring at the later time points, which the Michaelis-Menten model does not account for. The students were given instructional information about enzyme inhibition in class and were to use this knowledge to discuss other possible models to use to describe the experimental data.

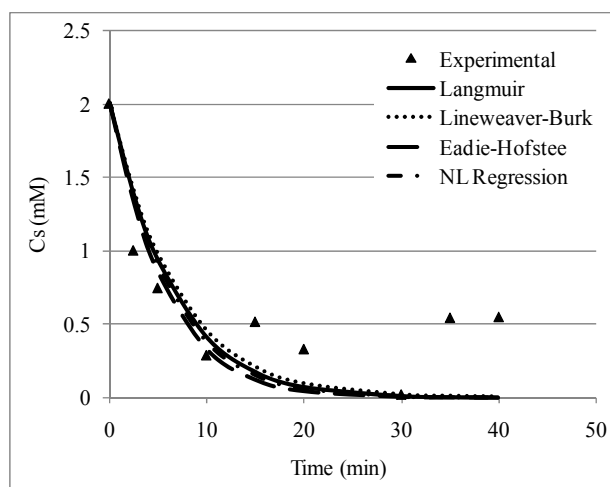


Figure 5. Change in substrate concentration with time measured experimentally and determined with the Michaelis-Menten rate expression with rate constants determined by four different methods.

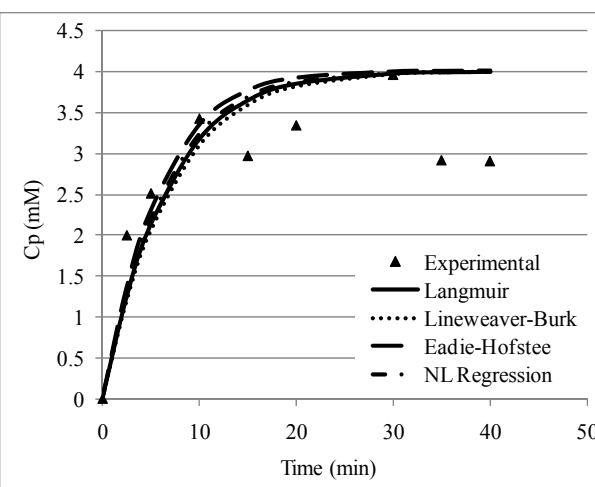


Figure 6. Change in product concentration with time measured experimentally and determined with the Michaelis-Menten rate expression with rate constants determined by four different methods.

Project Reporting

The first project deliverable from the students was a project preplan to be completed prior to beginning their experimental procedures in the laboratory. As described in the project memo, the preplan was to include the project goals, an experimental design, which includes specific experiments to be conducted and safety concerns, and methods for analyzing experimental data. The instructors reviewed the project preplans and gave the students feedback prior to the laboratory sessions to ensure the students would have a safe and productive laboratory experience. The project preplans were worth 25 of the 150 possible points for the project. After the laboratory sessions, students were given instruction on requirements for their project final report and oral presentations. Project final reports were graded on both technical content and communication, giving equal weight to each. The grading sheet used to grade the final reports is shown below. The oral presentations were graded based on the following components: focus, technical content, planning, speaker's manner, and visual aids, as shown in the grading sheet for the oral presentations, shown below. Both the BAE instructor and the CHE instructor graded each final report and oral presentation. The grades from each instructor were averaged to determine the final grade for each group. Students were given approximately four weeks to complete the final project deliverables, which were both due during the final week of the

semester. The final report was worth 75 points and the oral presentations were worth 50 points of the 150 possible points. Grades were given to project groups, so each project member received the same grade for the project. Students were not given the opportunity to formally evaluate each other's performance. Students were encouraged to discuss any group problems with the instructors. A formal group member performance evaluation will be added to the final project deliverables this semester.

Group Project Final Report Grade Sheet

Communication:

Content/Organization

- Summary of Project Goals and Objectives _____/10
- Introduction/Background _____/5
- Discussion of Model/Experimental Design _____/5
- Discussion of Safety and Environmental Issues _____/5
- Conclusions and Recommendations _____/5
- References, Figures, Tables, Attachments, etc. _____/10

Presentation

- Grammar and Spelling _____/5
- Clarity, Style, Neatness _____/5

Technical:

- Background/Theory _____/10
- Model/Experimental Design _____/10
- Data Analysis/Interpretation _____/10
- Statistical Analysis _____/5
- Sample Calculations _____/10
- Accuracy, General Impression, Response to Charge _____/5

Total: _____/100

Group Project Oral Presentation Grade Sheet

Presenters: _____

Presentation Title: _____

Qualitative Scale	Weak 1	2	3	4	Strong 5
Focus: Connection to project objectives Credibility					
Technical Content: Relevance Clarity Technical competence					
Planning: Organization Transitions Continuity					
Speaker's Manner: Voice Eye contact Gestures Confidence					
Visual Aids: Visibility Simplicity Appropriateness					
Total Score:	/ 25				
Final Grade (Total Score x4):	/ 100				

Comments:

Student Feedback and Assessment

Overall, students gave positive feedback about the project experience. They liked the hands-on experimental part of the project, giving them an opportunity to run an actual enzyme batch bioreactor and analyze data. However, some did not like that the project description was purposely open-ended and left to the students to work with the instructors to completely define the scope of the project. Too often, students get accustomed to having very explicit details given to them about procedures and deliverables. This project was designed to be more of a real world

situation where students must plan and work through problems as a team. After completion of the project, the students were asked, “What is your overall opinion about the class project? Would you recommend doing the project again, and if so, are there any changes you would recommend?” and some of the student comments were as follows:

- “It was a good project with hands on application. Yes, I would recommend doing the project again as it helped us apply a bit of what we learned in theory.”
- “The overall topic of the project was appropriate. However, the actual performance of the experiment was difficult because of lack of details in the project description.”
- “I enjoyed the project. It was very informative and nice to do a hands on learning experience. Yes and no changes.”
- “Clearer instruction of expectations”
- “I enjoyed working on the class project, although some parts of the project charge were difficult to follow.”
- “The project was helpful...and I would definitely recommend it. It would be a good idea to spend some more time in the lab.”
- “I recommend that it’s a very interesting, hands-on, and highly demanding project but it would become even more fun if the goals are explained to the students slightly more explicitly in the beginning of the semester. It was a little too overwhelming for students when everything was left onto them to interpret.”

Students also thought it was a worthwhile experience to compare actual data to a mathematical model and see how well (or not well) it fits the data. However, this was also the part of the project that most frustrated the students. Many did not like dealing with data that may have had high variability and the models would not fit with the data as expected by the students. This was a good learning experience for the students to understand how to deal with data that may have variability (i.e. dealing with outliers) and how to determine if a mathematical model appropriately describes the experimental phenomenon.

By personal communication with the students both during and after the group project experience, the following describes common themes about the students working in diverse groups. Students enjoyed the experience of working within diverse groups with those from different disciplines and graduate status. Students got the chance to learn more about other disciplines by finding out about the other’s experiences and understanding other’s strengths and weaknesses in working on various parts of the project. With the interaction between the undergraduate and graduate students, the leadership roles did not always default to the graduate students, but instead to those who had more experience in certain areas, which fell on the undergraduate students as well. It was also worthwhile for the undergraduate students to interact with the graduate students to learn more about graduate school as another option for after they complete their undergraduate degree.

Concluding Remarks

Having a course that involves a diverse set of students from different disciplines and graduate standing is a perfect opportunity to use a group project, as the one described in this paper, in order to maximize the exposure to diversity as a learning experience for the students. For many students, this has been their only opportunity to experience such a setting prior to entering their career fields, where they are often required to work in such diverse groups. The course project was also a valuable tool to identify the connection between experimentation in the laboratory and mathematical modeling from theory. Students learned that there can be difficulties within each and in the connection of the two, but through such experiences, they can become better at both.

References

1. Shuler, M. L. and Kargi, F., Bioprocess Engineering Basic Concepts, Upper Saddle River, Prentice Hall PTR, 2002: 553.

Appendix: Experimental Data to be Used to Calculate Michaelis-Menten Kinetic Parameters

Treatment 1: 2 mM Cellobiose Concentration

Cellobiose Concentration (g/L)= 0.5846

Time, min	Abs 1	Abs 2	Abs 3	Avg. Abs	C_p, gL^{-1}	C_s, gL^{-1}
0	0.125	0.128	0.121	0.125	0.086	0.599
2	0.490	0.480	0.478	0.483	0.329	0.356
4	0.605	0.626	0.645	0.625	0.425	0.259
6	0.716	0.674	0.743	0.711	0.483	0.201
8	0.828	0.773	0.837	0.813	0.552	0.132
10	0.859	0.803	0.878	0.847	0.575	0.109

Treatment 1: 4 mM Cellobiose Concentration

Cellobiose Concentration (g/L)= 1.369

Time, min	Abs 1	Abs 2	Abs 3	Avg. Abs	C_p, gL^{-1}	C_s, gL^{-1}
0	0.126	0.127	0.124	0.126	0.086	1.283
2	0.811	0.785	0.803	0.800	0.544	0.826
4	1.084	1.051	1.074	1.070	0.727	0.642
6	1.607	1.617	1.532	1.595	1.083	0.286
8	1.314	1.239	1.311	1.288	0.875	0.494
10	1.706	1.476	1.723	1.635	1.110	0.259

Treatment 1: 6 mM Cellobiose Concentration

Cellobiose Concentration (g/L)= 2.054

Time, min	Abs 1	Abs 2	Abs 3	Avg. Abs	C_p, gL^{-1}	C_s, gL^{-1}
0	0.127	0.125	0.131	0.128	0.088	1.966
2	2.304	2.268	2.363	2.312	1.569	0.484
4	1.948	1.950	2.045	1.981	1.345	0.709
6	2.073	2.120	2.007	2.067	1.403	0.651
8	1.946	1.823	1.878	1.882	1.278	0.776
10	1.788	1.456	1.736	1.660	1.127	0.927

Treatment 1: 8 mM Cellobiose Concentration

Cellobiose Concentration (g/L)= 2.738

Time, min	Abs 1	Abs 2	Abs 3	Avg. Abs	C_p, gL^{-1}	C_s, gL^{-1}
0	0.134	0.136	0.136	0.135	0.093	2.645
2	2.132	2.365	2.321	2.273	1.543	1.196
4	2.300	2.325	2.243	2.290	1.554	1.184
6	2.204	2.198	2.395	2.266	1.538	1.200
8	2.094	2.027	2.079	2.067	1.403	1.335
10	2.312	2.327	2.342	2.327	1.580	1.159

Treatment 1: 10 mM Cellobiose Concentration

Cellobiose Concentration (g/L)= 3.423

Time, min	Abs 1	Abs 2	Abs 3	Avg. Abs	C_p, gL^{-1}	C_s, gL^{-1}
0	0.372	0.355	0.340	0.356	0.243	3.180
2	1.091	1.073	1.123	1.097	0.745	2.678
4	1.407	1.381	1.133	1.307	0.888	2.535
6	2.228	2.173	2.063	2.155	1.463	1.960
8	2.292	2.282	2.369	2.314	1.571	1.852
10	2.473	2.295	2.331	2.366	1.606	1.817