AC 2010-1373: FERMENTATION LABORATORY EXERCISE HELPS FIRST YEAR STUDENTS UNDERSTAND LOG-TRANSFORMED VARIABLES

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Fermentation Laboratory Exercise Helps First Year Students Understand Log-Transformed Variables in Linear Regression

Abstract

Lafayette College's Introduction to Engineering course offers students a chance to learn about five branches of engineering. The students spend approximately three weeks with faculty from each branch, and complete three laboratory exercises during that time. In the chemical engineering branch, the students (from all engineering disciplines) monitor and analyze a polymerization reaction, calculate friction losses in a piping system, and produce ethanol in a fermentation reaction. This article describes the fermentation experiment procedure and assesses the student learning that occurs as a result. The learning objectives of the laboratory experiment include:

- Gain an understanding of the fermentation process
- Learn to perform a regression of logarithmic data
- Calculate yield relationships and understand the implications

Introduction

For many years, the ChE department has surveyed students about their learning styles, using both the questionnaire by Felder and Rousseau[1], and the VARK questionnaire[2]. While engineering students cover the broad range of learning styles, a majority of engineering students prefer a kinesthetic or active learning style. Laboratory experiences are where much of the learning occurs for these students[3-5].

First and second year students often have trouble reading data from logarithmic plots (such as the friction factor chart) and many have not linearized nonlinear relationships (such as the Arrhenius equation). In the fermentation laboratory, the students are required to plot the cell number as a function of time, and obtain an appropriate equation. Since the fermentation experiment has been implemented, we have observed an improvement in the students understanding of logarithmic data in later courses

During the fermentation laboratory, the students grow yeast cells in an airlift reactor, measure glucose consumption and alcohol production. The laboratory equipment, which is elegant yet simple to operate, will be described in the paper, along with typical student results. Students must use their results to prepare a linear regression using natural logarithms, a task that many have never done before. Assessment data includes results on a multiple choice quiz, scores on a final quiz problem that is similar to the lab analysis, and information from sophomore students who took the course the year before.

Description of the Experiment

The fermentation experiment was a part of the Chemical and Biomolecular Engineering block of our Introduction to Engineering course. The experiment followed up on a lecture about fermentation in which students were introduced to the kinetics of fermentation as well as the various measurements of yield (*i.e.*, yield of cells on substrate and yield of product on substrate).

The students learned how to linearize the exponential growth equation $X = X_o e^{\mu t}$ by making plots of ln(X) *vs*. t. The plots were prepared using EXCEL®; the linear regression tool was used to determine the slope of the graph (equal to μ) and the regression coefficient was used as a tool to measure the quality of the regression. Prior to the laboratory exercise, the students completed a homework assignment in which they analyzed fermentation data. The homework assignment and its solution are given in the Appendix.

Details of the Experiment

Each student group of three was supplied with a 500 ml plastic bottle containing the following:

- D(+) Glucose (3.2 g)
- Magnesium Sulfate Heptahydrate (0.22 g)
- Sodium Phosphate Monobasic Anhydrous (2.7 g)
- Ammonium Sulfate (1.1 g)
- Baker's Yeast (*Saccharomyes cerevisiae)* (0.43 g)

Three hours prior to the start of the laboratory, the jar was filled with warm water and agitated. This three-hour time had been previously determined to be sufficient for the cells to adapt to their environment and begin multiplying.

At the start of the laboratory the students poured the solution into a Cytolift Kontes Bioreactor. Air was bubbled through the reactor at 1.25 SCFH and water at 40^oC was circulated through the jacket of the reactor.

The students used a syringe to remove approximately 2.5 ml of solution from the reactor. They measured the initial glucose concentration of the solution using a commercial glucometer (OneTouch Untra Mini®). Next, 2.0 ml of their initial solution was diluted with 8.0 ml of distilled water and placed in a cuvette. They measured the turbidity of this diluted solution with a turbidimeter manufactured by Wissenschaftlich-Technische, then used a calibration curve to calculate the concentration of cells from the turbidity measurement.

Turbidity measurements were taken every 15 minutes for the next 1.5 hours. Experience has shown that at this temperature the cells reached a stationary growth phase after about 1.5 hours. The students took an additional glucose measurement with the last sample. The final concentration of alcohol in the fermenting solution was measured with an ethanol sensor manufactured by Pasco Scientific. The sensor was calibrated so the students measured the concentration in g alcohol/liter.

Results from the experiment

Typical experimental results are shown in Table 1. The cell concentrations were calculated from the turbidity using the following previously developed equation:

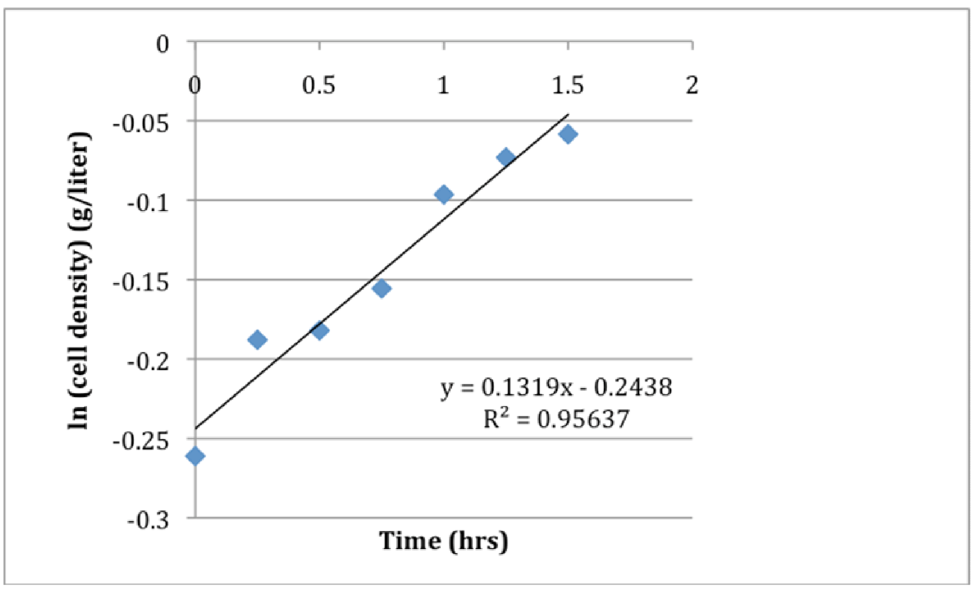
g cells/liter = 0.0204*NTU -0.035

A plot of ln(cell concentration) *vs.* time is shown in Figure 1 along with the results of a linear regression.

Table 1. Student results.

Time (hours)	Turbidity (NTU)	Cell Concentration (g/liter)	Glucose Concentration (g/liter)	Temperature (°C)	Alcohol Concentration (g/liter)
0.00	39.47	0.7702	4.91	32	0.00
0.25	42.34	0.8287		35	
0.50	42.58	0.8336		37	
0.75	43.67	0.8559		39	
1.00	46.23	0.9081		40	
1.25	47.28	0.9295		40	
1.50	47.95	0.9432	2.34	40	1.22

Figure 1. Plot of student data.



The slope of the line represents the specific growth constant for the process (0.132 hr^{-1}). This corresponds to a doubling time of 5.25 hrs. Calculations for the yields of the reaction are as follows:

Yield of cells on glucose = -Δ(conc. cells)/ Δ(conc. glucose) = 0.067 g cells/g glucose

Yield of ethanol on glucose = -Δ(conc. alcohol)/ Δ(conc. glucose) = 0.47 g alcohol/g glucose

Discussion of Results

Students wrote a group lab report for the experiment. Nearly all of the groups performed the regression correctly. The most common problem was scattered data, which resulted from poor dilution technique, or not taking care to use the same cuvette to measure the turbidity for each sample. Doubling times ranged from 2.7 ($r^2 = 0.8$) to 19 hours ($r^2 = 0.5$) – groups with better technique obtained results consistent with reported values (doubling time of 2.8 hours[6]). The students also measured the yield of cells on glucose and yield of ethanol on glucose. The yield of cells on glucose ranged from 0.04 to 0.18 g cells / g glucose. The results were lower than some reported data (0.5 g cells/g glucose [7]), but the growth conditions were not totally optimized. Ethanol yields ranged from 0.4 to 0.7 g ethanol/g glucose.

One of the learning objectives of the experiment was to increase students understanding of logarithmic functions. Near the end of the semester, about forty students were asked four questions about logarithms and linearization both before and after performing the experiment. The multiple choice questions were:

1. Suppose you have plotted some data and fitted various equations to it. The regression coefficients for the equations are listed below. Which would you choose?
2. If you have the equation $y = a\,x^b$, which plot will give you a linear relationship?
3. If you have the equation $y = a\,e^x$, which plot will give you a linear relationship?
4. A fermentation experiment was done to determine the value of the growth constant. Is the regression line in the graph below a good choice for this data? [The regression line included the lag and death phases].

The results from these questions are summarized in Table 2. Unfortunately, this was done late in the semester, and only one set of data was obtained, and no statistical analysis could be performed. Most students already understood that regression coefficients should be close to 1.00. Fewer students knew how to linearize exponential equations, and there was some improvement after the experiment. Students understood that the death and lag phases should not be included when calculating the growth rate of the cells. From these results, we realized that while students understand the specific application of linearization, they may not understand the concept in general. This will be addressed in future offerings of the course.

Table 2. Results of multiple choice quiz.

	Percent answering correctly	
	Before experiment	**After experiment**
Regression coefficient should be close to 1.00	87%	91%
Ln (y) vs ln (x) will linearize $y = a\,x^b$	23%	29%
Ln (y) vs x will linearize $y = a\,e^x$	60%	65%
Calculate growth rate ignoring lag and death phases	97%	90%

The written laboratory reports showed us that students were able to correctly use Excel to obtain a specific growth rate. However, because the reports are written by groups of three students, it is possible that not all students understood the method. Individual students were tested for understanding on the final quiz (see Table 3). The students were given an Excel plot of ln (cell number) as a function of time with the regression equation for some fermentation data, and asked to identify the specific growth rate (the slope) and the doubling time. The students had seen this plot on the homework, and created one for the lab report. Over 60% of the students received a score of 8/10 or higher (losing points due to significant figures or units), showing they understood the concept. About 20% of the students did not answer the question correctly, not understanding or not remembering that the slope of the plot was the growth rate. The average exam score for these students was 81%, which indicates that these students understood most of the other chemical engineering concepts covered in the block.

Table 3. Student scores on final quiz.

Score	% of class	Average exam score
10/10	26.7	
9	23.0	
8	14.9	
7	13.0	
6	5.0	83.1
5	9.9	80.3
4	4.3	80.9
3	1.2	70.0
2	0.0	
1	0.0	
0	1.9	64.0

We have had first year students do this lab for the past seven years. We wondered if students remembered the technique later, so we asked some questions of sophomore chemical engineering students. During the semester, 38 students in the Material and Energy Balances class were given a homework problem from the textbook[8] (problem 4.13, 3rd edition). In this problem, the students must perform the same type of analysis as the fermentation lab, finding a regression equation for exponential data. The students were asked to use Excel to find the equation and also to plot the data on a piece of log-log paper. Surprisingly, most of the students had trouble creating the paper plot (but had no trouble with the Excel regression). Students who came to office hours and asked how to create the paper plot were shown, but we did not spend class time on it.

These students were given a simple anonymous quiz after their Material and Energy Balances final exam. The students were given a piece of 2 cycle log-log paper, and asked to mark four points on it [(0.25, 0.20); (0.82,12); (3.6, 400); (7.8, 640)]. Table 4 shows the results from this quiz. About a quarter of the students could plot the points, probably those who had asked during office hours. Apparently, many students do not know how to use log-log paper (although some may not have put full effort into the anonymous quiz immediately after a final exam). As they handed the quiz in, students made comments such as "This was harder than some of those questions" or "It's good that my name's not on this – it's embarrassing". Because students must read log and semilog graphs for engineering problems (such as friction factor plots), we will add this to the Introduction to Engineering course in the future.

Table 4. Sophomores using 2 cycle log-log paper

Sophomores marking log-log plot:		
	65.7%	Could not plot all four points correctly
	8.6%	Had more trouble plotting points < 1.0
	25.7%	Could plot all four points correctly

References

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3. A. W. Chickering, and Z. F. Gamson (1987). Seven Principles for Good Practice in Undergraduate Education." *AAHE Bulletin,* 39(7), 3–7.
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6. Shuler, M.L. and Kargi, F., Bioprocess Engineering, Prentice Hall, 2002.
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8. Felder, R.M. and Rousseau, R.W., Elementary Principles of Chemical Processes, Wiley, 2005.

A Lafayette student obtained the following data in the laboratory fermenting yeast to alcohol at 38°C:

Time (hr)	Cell concentration (g/liter)	Glucose Concentration (g/liter)	Alcohol Concentration (g/liter)
0	0.882	5.14	0.00
0.250	0.899		
0.500	0.883		
0.750	0.894		
1.00	0.879		
1.25	0.936		
1.50	0.980		
1.75	1.00		
2.00	1.04		
2.25	1.05		
2.50	1.09		
2.75	1.13		
3.00	1.12		
3.25	1.13		
3.50	1.14		
3.75	1.12	3.25	1.31

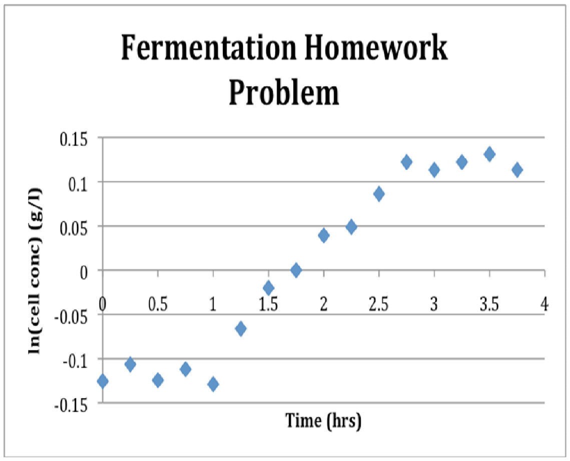
The Fermentation produced 1000 g of a solution containing 0.13% alcohol.

(a) Prepare a graph of ln(cell concentration) *vs*. time.
(b) What is the lag time for the fermentation?
(c) At what point in time does the stationary phase begins?
(d) **Use data from the exponential growth region** to determine the specific growth rate μ and the doubling time. Attach copies of any graphs and regressions that you use.
(e) Calculate the yield of cells on glucose.
(f) Calculate the yield of alcohol on glucose.

Figure A1. Homework problem given to the students after the lecture on fermentation.

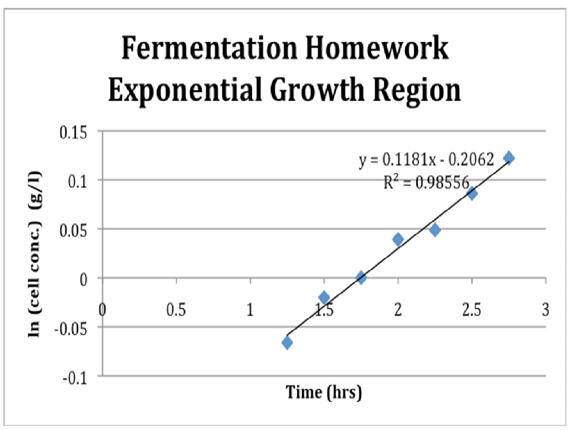
Solution

(a)



(b) From the graph it appears that the lag time is about 1 hour.

(c) From the graph it appears that the stationary phase starts after about 2.75 hrs.



(d) The regression results show that $\mu = 0.118\ hr^{-1}$. The doubling time, $t_d = \ln(2)/\mu = 5.9$ hrs.

(e) Yield of cells on glucose = -Δ(conc. cells)/ Δ(conc. glucose) = 0.126 g cells/g glucose

(f) Yield of alcohol on glucose = -Δ(conc. alcohol)/ Δ(conc. glucose) = 0.693 g alcohol/g glucose

Figure A2. Fermentation homework problem solution.