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Simulating Bacterial Chemostat Growth as an Introduction to Rate Modeling with Calculus

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Abstract

The bacterial chemostat is a device which enables experimenter control over population growth rate by restricting access to nutrients. Replacement of a small volume of fully-grown, nutrient-starved culture with an equal volume of sterile nutrient medium constitutes a continuous dilution process enabling new cell production at the rate of culture replacement. Population growth rate is directly proportional to both culture replacement rate and cell concentration but is inversely proportional to culture volume. In order to illustrate the utility of rate modeling for biology, this work describes the theoretical basis of chemostat growth in terms of both algebra and calculus. We also describe a simple and non-biohazardous dye dilution laboratory activity that illustrates the modeling. The laboratory activity may be implemented in as little as one hour as a demonstration or student activity. This activity is appropriate for an undergraduate junior-level microbiology laboratory or as a first-semester calculus demonstration.

Keywords: mathematical modeling; chemostat; interdisciplinary connections

Link to Supplemental Materials: https://doi.org/10.37590/able.v44.sup9

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INTRODUCTION

High school and undergraduate science students are often taught biology without exposure to the mathematical concepts that underpin the life science disciplines. Many students who enter life science majors receive limited mathematical training and typically learn mathematical concepts through physics examples. Additionally, their life science courses do not integrate the concepts and procedural skills that were taught to these students within their mathematics courses (Usher, 2010). The 2012 report from the United States President's Council of Advisors on Science and Technology stated that integrating mathematics concepts into life science courses would be more meaningful for students. Eaton (2017) added that incorporating quantitative skills into biology courses is one legitimate way for a program to help its students develop their quantitative fluency. By making apparent the connections between calculus and biological applications, institutions may help students value calculus and to be less likely to question why it is part of their degree program (Aiken, 2021). We use the example of chemostat bacterial population growth to illustrate the application of algebra and calculus to biology. In addition, we describe an associated laboratory activity of adjustable length which can be implemented as either an instructor demonstration or student wet lab.

Student audience

We recommend this activity as appropriate for either junior-level undergraduate microbiology students or freshman-level, first-semester calculus students. A significant conceptual topic in microbiology is bacterial growth by binary fission, which can be described mathematically as an exponential process. Therefore, the concepts of exponents and logarithms should be familiar to these students. All should have completed algebra-based mathematics, and many biology students will have completed a first semester of calculus. Finally, students should have a functional knowledge of computer-assisted linear regression. Performance of the laboratory activity requires only proficiency with pipetting 1 mL volumes and reading the absorbance values in a spectrophotometer. These skills may be learned with five minutes of instruction and an additional five minutes of student practice.

Instructor preparation

Once the required equipment is procured and the (stable) methylene blue dye stock solution is prepared, setup time is less than thirty minutes. The lab activity may be designed to run for one to three hours according to instructor preference. It is recommended that the mathematical modeling theory be taught in a period prior to the lab activity; pipetting practice may also be performed beforehand. Students may further practice pipetting technique and be shown how to take spectrophotometer readings while the activity is in progress. Down time during activity performance may be used by the instructor to field student questions.

STUDENT OUTLINE

Objectives

Understand the difference between exponential and linear growth of bacterial populations
Mathematically model linear bacterial population growth rate in the chemostat
Perform liquid removal operations with a pipette and pipetting device
Perform absorbance readings with a spectrophotometer
Use Microsoft Excel® software to perform linear regression for chemostat flow rate calculation

Mathematical Descriptions of Bacterial Population Growth

Bacterial population growth by binary fission occurs as individual cells increase in size and then multiply into two smaller cells which in turn repeat the process. In the laboratory, binary fission is enabled by ideal conditions of optimum temperature, nutrient excess and oxygen excess. Cell concentration increases exponentially at its maximum rate under these conditions and can easily be measured by a spectrophotometer as optical density:

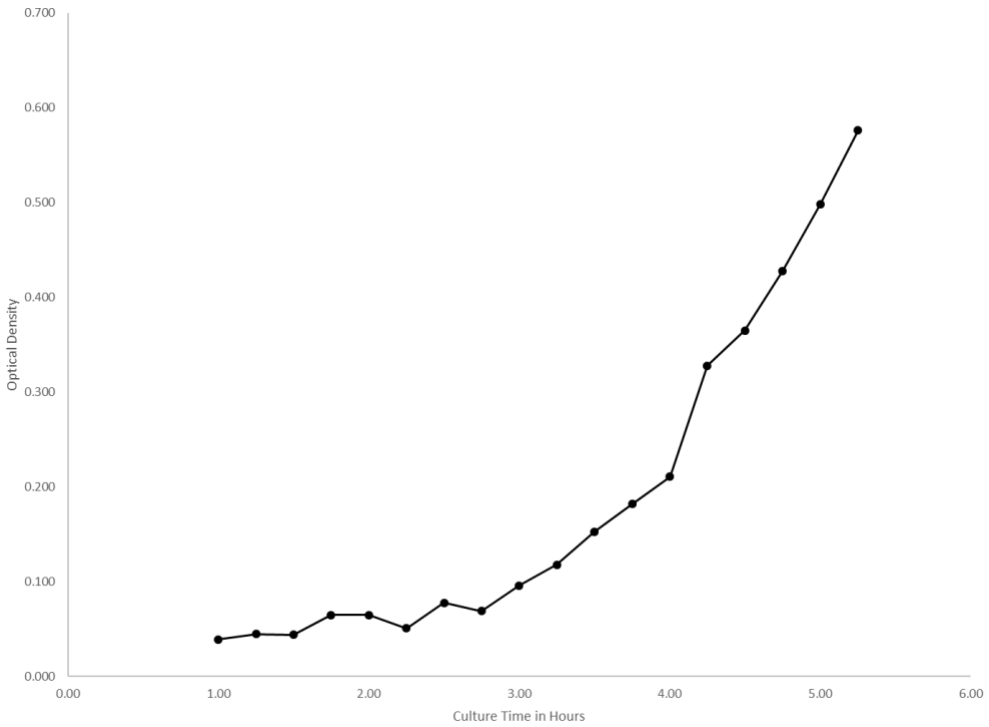

Figure 1. Exponential growth of a bacterial culture measured as optical density increase at 750 nm.

Logarithmic transformation of optical density data is often employed to provide linear rates of change which are easily compared across various growth conditions. Notice in the following graph that the same data set produces greater rates of change when reported as natural logarithms versus base ten logarithms.

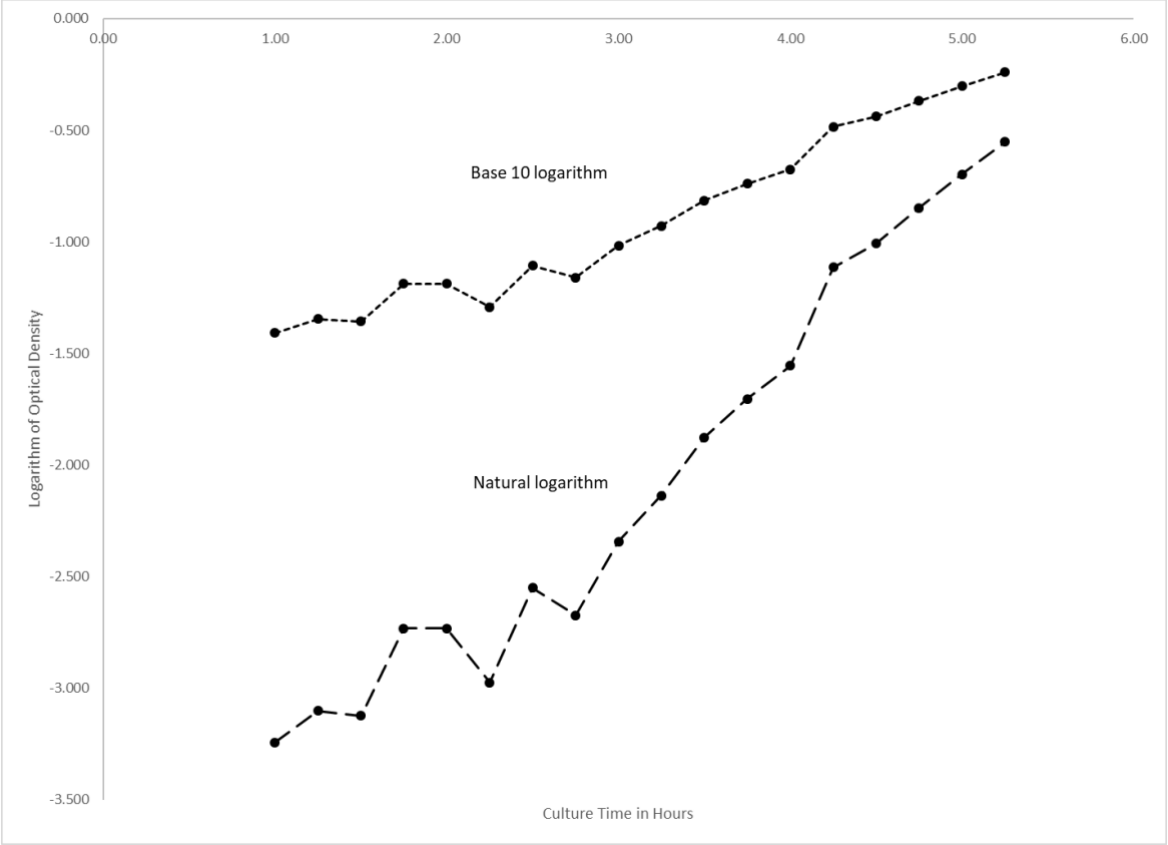

Figure 2. Logarithmic transformations of the Figure 1 data.

Where possible, natural logarithms rather than base ten logarithms should be used to model microbiological process. The former are more useful in calculus applications, and the latter tend to obscure variation.

Microbes may also be cultivated under continuous, growth rate-controlled conditions by restricting population access to liquid nutrients. The bacterial chemostat was invented for this purpose by Novick and Szilard in 1950, and one such device illustrating the growth of red-pigmented bacteria is pictured in Figure 3.

Figure 3. Chemostat growth of *Serratia marcescens* bacteria.

Beginning with a fully-grown bacterial culture whose growth rate is zero, growth rate can be modulated between zero and its maximum under the ideal conditions described above. Growth rate control is accomplished by replacing a volume of grown culture (red frothing liquid) with an equal volume of growth medium (yellow-brown liquid) at an experimenter-defined rate. Population growth, defined here as the production of new cells, then becomes a linear process that is directly proportional to the rate of culture replacement while the total cell concentration in the culture remains roughly constant. It can be assumed that cell density is approximately constant provided that culture replacement volume is a small fraction of the total culture volume.

The rate of bacterial growth in the chemostat may be most simply modeled algebraically using common laboratory units. To begin, we define chemostat growth rate as new cell concentration increase over time. In units:

$$\text{rate} = (\text{cells/mL/hr}) \qquad (1)$$

Note that this quantity is algebraically positive due to continuous cell multiplication. As indicated earlier, the chemostat population growth rate is limited by culture replacement with sterile medium and is directly proportional to it. In units:

$$\text{rate } \alpha \text{ (mL/hr)} \qquad (2)$$

Furthermore, bacterial cells multiply exponentially; this means that the chemostat growth rate is also directly proportional to the cell concentration itself. In units:

$$\text{rate } \alpha \text{ (cells/mL)} \qquad (3)$$

Combining expressions 1 through 3 produces the following upon unit cancellation:

$$\text{rate} = (\text{cells/mL/hr}) \neq (\cancel{\text{mL}}/\text{hr}) \cdot (\text{cells}/\cancel{\text{mL}})$$

This inequality suggests that one final parameter involving volume must be considered to fully model chemostat growth rate. Although chemostat volume is held constant in practice, population growth rate would certainly change with volume. That is, a volume increase would decrease growth rate by lowering cell concentration and vice versa. Therefore, chemostat growth rate is inversely proportional to culture volume. In units:

$$\text{rate } \alpha \text{ (1/mL)} \qquad (4)$$

Now, combining expressions 1 through 4 produces a unit-balanced equation and indicates that all factors which influence chemostat growth rate under given conditions of temperature, growth medium and oxygen have been incorporated. In units:

$$\text{rate} = (\text{cells/mL/hr}) = [(\cancel{\text{mL}}/\text{hr}) \cdot (\text{cells}/\cancel{\text{mL}})] \,/\, \text{mL} \qquad (5)$$

Expressing chemostat growth rate in calculus terms is perhaps more useful because this approach uses differentials to describe growth rate at any point in the process rather than as an average rate calculated over an interval of time. We now define the following variables to facilitate this modeling: rate of culture replacement with medium *f*; cell concentration *c*, chemostat volume *v* and time *t*. Applying the logic of the algebraic rate model above produces the following equation for chemostat growth rate:

$$\text{rate} = dc/dt = fc/v \qquad (6)$$

Substitution of the appropriate units from Equation 5 for the variables in Equation 6 confirms that this starting equation is correct (not shown). In this model, the differential expression *dc/dt* must also be algebraically positive because the variables *c* and *t* are increasing during continuous culture. The quantities *f* and *v* are usually held constant and are positive as well. Algebraic rearrangement of (6) produces:

$$dc/c = (f/v) \cdot dt$$

Finally, integration of both sides yields an equation with both constants and measurable variables:

$$\int_{c1}^{c2} \frac{dc}{c} = (\frac{f}{v}) \int_{t1}^{t2} dt$$

And

$$\ln(c_2) - \ln(c_1) = (f/v) \cdot (t_2 - t_1) \qquad (7)$$

If the cells are present at an initial concentration c_1 at $t_1 = 0$, Equation 7 assumes the more familiar $y = mx + b$ linear form for any future point (t_2, c_2):

$$\ln(c_2) = (f/v) \cdot t_2 + \ln(c_1) \qquad (8)$$

In words, Equation 8 states that new cell concentration during continuous chemostat culture may be calculated in terms of the easily-measured values on its right side.

Chemostat Population Growth Simulation by Continuous Dye Dilution

Our mathematical model of chemostat growth incorporates the variables of culture replacement and new cell growth at constant culture volume and essentially constant cell density. As a partial conceptual corollary to the chemostat, consider an overflowing bucket of soapy water that is continually being diluted with water from a garden hose (Simon, 1986). How would the soap concentration change over time? This example reproduces the chemostat conditions of equal volume replacement at a user-defined rate and the maintenance of a constant volume over time. We describe here a similar dilution scheme to model chemostat function using simple laboratory materials.

This activity will simulate chemostat operation using the continuous process of non-toxic dye dilution with water. The dye methylene blue is used; its concentration in water solution can be measured indirectly as the absorbance at 664 nanometers (A_{664}) as recorded by a spectrophotometer. The apparatus for this activity is pictured in Figure 4.

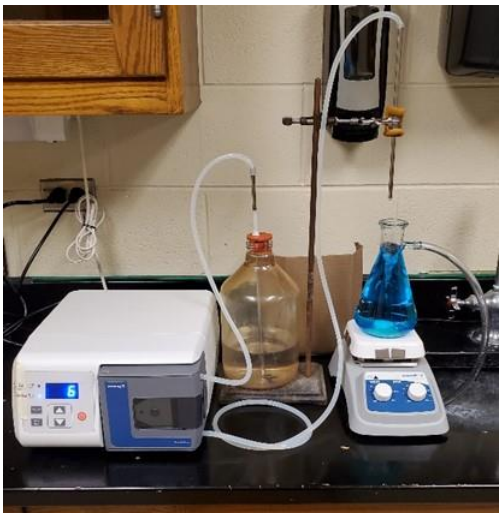

Figure 4. Methylene blue dye solution continuous dilution apparatus.

The peristaltic pump at left draws distilled water from the center bottle and adds it dropwise to a methylene blue solution in a sidearm flask. The sidearm is connected to tubing for overflow to waste as drops of water are added, while the solution itself is continually stirred on a magnetic plate to promote uniform mixing. Dye solution samples are withdrawn at periodic intervals for measurement of the methylene blue concentration as A_{664} in a spectrophotometer (not shown). Absorbance values are recorded in a spreadsheet, and the dye sample is returned to the stirring solution to avoid skewing the data by sample removal. The natural logarithm of A_{664} is then plotted versus time in minutes for slope calculation, and the final dye solution volume is measured with a one-liter graduated cylinder. The results are mathematically modeled below in terms of Equations 6 and 7 as adapted to the new experimental conditions.

Mathematical modeling of the dye dilution activity begins with a modified version of Equation 6. While the variable f in the chemostat context indicates culture replacement with growth medium, f here is equivalent to the dye dilution rate. Drops of water added to the dye solution continually dilute it while an identical volume overflows the flask to waste; this meets the chemostat condition of nearly exact volume replacement. Unlike bacterial growth in a chemostat, however, we note that the dye concentration is decreasing throughout the activity. This means that the differential expression must begin as algebraically negative:

$$-(dc/dt) = fc/v$$

Or

$$dc/dt = -(f/v) \cdot c$$

Following the same rearrangement and integration steps which produced Equation 7 creates a similar equation that has a negative constant term:

$$\ln(c_2) - \ln(c_1) = -(f/v) \cdot (t_2 - t_1) \quad (9)$$

Thus, a plot of the natural logarithm of methylene blue concentration (A_{664}; *y*) vs. time (minutes; *x*) will produce a straight line with a negative slope whose value is (*-f/v*). Line slope is then determined by linear regression using Microsoft Excel® spreadsheet software. The dye dilution rate *f* is easily calculated as the negative of the regression slope multiplied by the final dye solution volume, and *f* has the volume per time units used for data collection:

$$f = -(\text{slope}) \cdot (v) \quad (10)$$

As noted above, both the dye dilution rate *f* and the solution volume *v* are held constant throughout the activity.

Methods, Data Collection and Data Analysis

Methods Overview

A spectrophotometer is used for recording dye concentration measurements, and these are entered into a Microsoft Excel® spreadsheet template as they are generated during the activity. The spectrophotometer is a device which measures light absorbance at a specified wavelength by dissolved chemicals. Light absorbance is proportional to absorbing chemical concentration, and (if desired) it can be converted to a concentration by multiplying by a constant known as the molar extinction coefficient. This activity measures dye concentration at a visible light wavelength of 664 nanometers, which is the wavelength of maximum visible light absorbance by methylene blue. During growth of an actual bacterial culture, the spectrophotometer is used to measure cell concentration. Spectrophotometer measurements in that case indicate light scattering by a cloudy suspension of cells rather than true light absorbance. Cell culture readings would be recorded as optical density at a specified wavelength (Fig. 1).

Data Collection

All users should become proficient at transferring 1 mL samples of the methylene blue dye solution to a spectrophotometer sample container known as a cuvette. You will be allowed time to practice pipetting either in a pre-lab period or during the lab period itself. You will remove samples from the solution periodically as dye dilution proceeds, read the absorbance at 664 nanometers (A_{664}), record the values in the spreadsheet provided, and return the samples to the solution.

Data Analysis

Following data collection, your instructor will make the complete data spreadsheet electronically available to class members. Each student will then be responsible for performing linear regression to calculate the slope of the natural logarithm of absorbance at 664 nanometers vs. time in minutes plot. Use this value to calculate the flow rate *f* according to Equation 10. Example results and analysis from prior performance of this activity are presented in Figure 5. Equation 10 produced a dye dilution rate *f* of 23 mL/min.

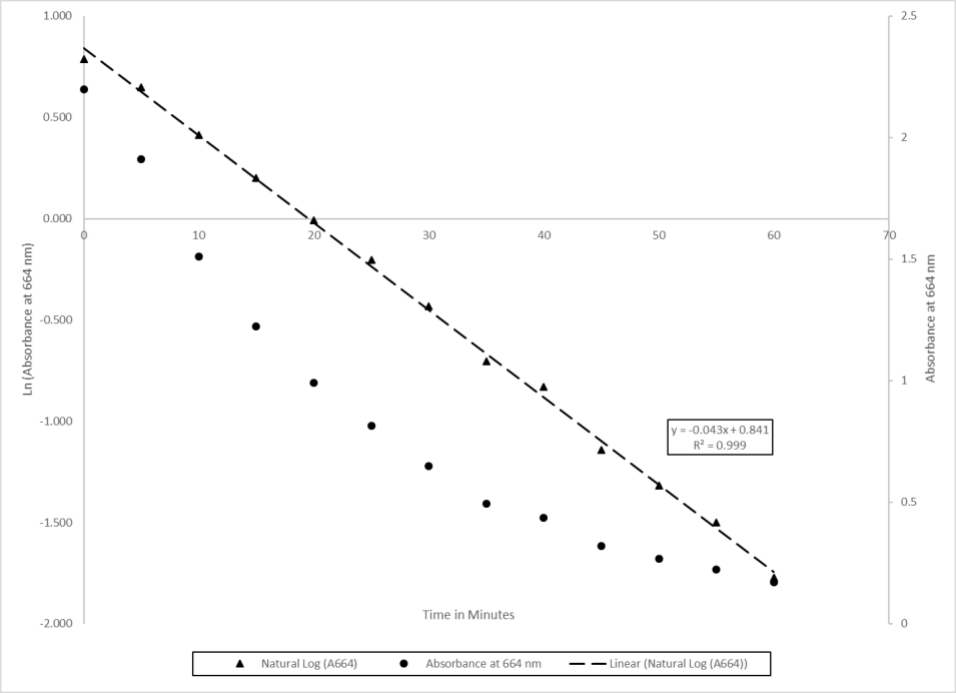

Figure 5. One-hour methylene blue dye dilution experiment.

Review and Discussion Questions

1. What is meant by a linear process? An exponential process? Which mathematical process describes bacterial population growth under conditions of nutrient excess, and which under nutrient limitation?
2. How does the bacterial chemostat operate to restrict the rate of population growth?
3. Beginning with an algebraic definition of bacterial chemostat population growth rate as cells/mL/hr, mathematically model growth in terms of units for cell concentration, culture replacement rate, and chemostat volume.
4. If you are familiar with calculus, mathematically model bacterial chemostat population growth as a differential rate equation using the variables cell concentration *c*, culture replacement rate *f*, and chemostat volume *v*. Solve the equation by integration to produce a plottable expression for calculation of *f*.
5. Advanced students may attempt the following more complex problem. Consider a modification to this activity that performs this dye experiment in reverse. A solution of methylene blue is added dropwise to distilled water in a sidearm flask, while the solution is drained to waste at the same rate of dye addition. How would this be mathematically modeled? *Hints: Consider the relationship between dye concentration and time and sketch a graph of this relationship to guide your modeling. Then, propose modifications to Equation 6 which could produce the predicted concentration increase. Keeping your model as simple as possible will avoid confusion and lessen opportunities for error.*

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MATERIALS

Tables 1 and 2 list consumable and non-consumable items for a class of 25 students.

Table 1. Non-Consumable Items.

Item	Quantity Needed (Class of 25 Students)
Peristaltic pump (VWR Scientific cat. # 76035-992 or equivalent)	1
Silicone tubing (VWR # 89068-436)	Ca. 3 m
Ringstand with test tube clamp	1
500 mL Erlenmeyer sidearm flask with drainage tube and 1inch stir bar	1
Magnetic stir plate	1
Laptop or desktop computer with Microsoft Excel® spreadsheet software; projection capability preferred	1
Visible wavelength spectrophotometer (absorbance range 0.000-2.200)	1
1 L graduated cylinder	1
2 to 3 L bottle for input distilled water	1
Lab bench with access to sink drain	1
Manual or electric pipetting device	1 per student
250 mL beakers	7 (shared in student groups for pipetting practice)

Table 2. Consumable Items.

Item	Quantity Needed (Class of 25 Students)
Methylene blue powder, 25 g. VWR # 34172-054	1
10X methylene blue dye stock solution (0.15 g/L; stable indefinitely when refrigerated or stored at room temperature)	1 L
1x methylene blue working solution (0.015 g/L; for use during the activity; stable indefinitely)	1 L
Disposable 1 mL semi-micro cuvettes	Ca. 50 (1 per student)
Cuvette holding trays	Ca. 25 (1 per student)
Disposable 5 mL pipettes	Ca. 50 (2 per student)

NOTES FOR THE INSTRUCTOR

This dye dilution activity may be presented either as a hands-on wet lab or as an instructor demonstration. Methylene blue dye solutions absorb visible light maximally at 664 nanometers, and many spectrophotometers have a linear absorbance range of zero to approximately 2.200. Therefore, following preparation of the dye working solution, a sample of it should be measured to ensure that the initial A_{664} lies near the maximum absorbance value. If the starting A_{664} exceeds the spectrophotometer's linear range, the first few points will be outliers with a combined slope of roughly zero. Similarly, dilution below the instrument's detection minimum of approximately 0.150 will produce outliers in the low dye concentration range. We have found it is most instructive to have a spreadsheet template open for direct entry of activity data as they are generated. The template embedded in the Appendix includes a graph whose points are plotted as the data are entered. Students may be instructed on pipetting technique and given time to practice while dye dilution is occurring. Alternatively, beakers of water may be made available with 5 mL pipettes and a pipetting device for practice prior to student sampling.

Table 3 summarizes the peristaltic pump flow rates *f* for performance of this activity over various lengths of time. Data for this table were determined for a VWR pump cat. # 76035-992 and a dye solution volume of 530 mL. If a different peristaltic pump and/or solution volume are used, similar data should be obtained beforehand for calibration of system function.

Table 3. Dye dilution activity operational parameters

Activity Duration, hr	VWR Pump Speed Setting	Slope from Plot of ln(A_{664}) vs. Time in Minutes (*n*, r^2)	Approximate Dilution Rate *f*, mL/min
1	18	-0.0431 (13, 0.999)	23
2	9	-0.0225 (13, 0.998)	12
3	6	-0.0140 (13, 0.993)	7.4

The limited data of Table 3 show that, as expected, activity duration is inversely proportional to pump speed. Pump speed may therefore be calculated for any desired activity duration according to the following equation:

Pump speed setting = (18.0) / (activity duration in hr)

Finally, a solution to Review and Discussion Question #5 is not obvious and requires considerable thought. Describing the system in terms of as many constants as possible will simplify subsequent integration steps. Again, the fundamental chemostat relationship (Eq. 6) is the best starting point:

$$\text{rate} = dc/dt = fc/v$$

A sketch of the actual dye concentration *c* vs. time *t* relationship suggests that *c* increases to a limit equal to the input dye concentration. The appearance of the curve is roughly logarithmic, but a logarithmic model fits actual data poorly (not shown). Another function to describe the increasing portion of the curve is a half-parabola which reaches the maximum dye concentration c_{max}. This constant value is measurable and is equivalent to the input dye concentration. The model must also include a c^2 term to fit a quadratic relationship. Realizing that the rate of concentration increase actually declines to zero over time suggests a *c* variable multiplier which eventually stabilizes to the constant c_{max}. A candidate multiplier is (c/c_{max}), which itself approaches one and drops out of the relationship as c_{max} is reached. Importantly, multiplying the "*c*" of Eq. 6 by this term produces a quadratic equation which increases at a progressively slower rate as c_{max} is approached:

$$dc/dt = f\,[(c/c_{max}) \cdot c] \div v$$

Or, with *f, v* and c_{max} as a collected constant, we have:

$$dc/dt = (f/[c_{max} \cdot v]) \cdot c^2$$

And the integrated form of this equation for all $c_1 \neq 0$ is:

$$1/c_1 - 1/c_2 = (f/(c_{max} \cdot v)) \cdot (t_2 - t_1)$$

Substitution of algebraic units into this final equation shows that the integrated relationship is correct. Furthermore, a plot of $(1/c)$ vs. time is predicted to form a straight line with a positive slope equal to the three collected constants. Since dye concentration increases over time to a constant positive value, alternative mathematical models which include a negative c^2 term, which might be expected for a downward-pointing parabola, are excluded.

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