Overview

Biophysical chemists insert the DNA that encodes a protein of interest into *E. coli* bacterial cells. These cells can be grown to high density and induced to produce the protein of interest. Once this protein has been produced, it is harvested and purified from the bacteria in order to be used for biochemical and biophysical experiments. This process of growing and harvesting bacteria for protein production is a bread-and-butter procedure for biochemists and is performed on a regular basis. Every step of this process requires monitoring by the scientist, often including on-the-spot calculation of dilutions. This lesson guides students to mimic these on-the-spot calculations and highlights the interconnected nature of the sciences and mathematics.

Common Core Standards

**Understand ratio concepts and use ratio reasoning to solve problems.**

CCSS.Math.Content.6.RP.A.1
Understand the concept of a ratio and use ratio language to describe a ratio relationship between two quantities. *For example, "The ratio of wings to beaks in the bird house at the zoo was 2:1, because for every 2 wings there was 1 beak." "For every vote candidate A received, candidate C received nearly three votes."*

CCSS.Math.Content.6.RP.A.2
Understand the concept of a unit rate a/b associated with a ratio a:b with b \(\neq 0\), and use rate language in the context of a ratio relationship. *For example, "This recipe has a ratio of 3 cups of flour to 4 cups of sugar, so there is 3/4 cup of flour for each cup of sugar." "We paid $75 for 15 hamburgers, which is a rate of $5 per hamburger."*

CCSS.Math.Content.6.RP.A.3
Use ratio and rate reasoning to solve real-world and mathematical problems, e.g., by reasoning about tables of equivalent ratios, tape diagrams, double number line diagrams, or equations.

CCSS.Math.Content.6.RP.A.3.a
Make tables of equivalent ratios relating quantities with whole-number measurements, find missing values in the tables, and plot the pairs of values on the coordinate plane. Use tables to
compare ratios.

CCSS.Math.Content.6.RP.A.3.b
Solve unit rate problems including those involving unit pricing and constant speed. For example, if it took 7 hours to mow 4 lawns, then at that rate, how many lawns could be mowed in 35 hours? At what rate were lawns being mowed?

CCSS.Math.Content.6.RP.A.3.c
Find a percent of a quantity as a rate per 100 (e.g., 30% of a quantity means 30/100 times the quantity); solve problems involving finding the whole, given a part and the percent.

CCSS.Math.Content.6.RP.A.3.d
Use ratio reasoning to convert measurement units; manipulate and transform units appropriately when multiplying or dividing quantities.

1 Expectations for unit rates in this grade are limited to non-complex fractions.

Focus Question

How can one calculate the proper dilutions necessary to determine the concentrations of biological samples to monitor progress of bacterial growth?

Objectives

Students will learn how to determine the appropriate use of dilutions in finding concentrations. Students will perform calculations using mixed fractions and decimals and discover the important connections between mathematics and science.

Background

From previous activities or lessons, students may have seen bacterial colonies growing on agar plates. In order to grow enough bacteria to purify a protein expressed by them, it is necessary to transfer a colony from a culture plate into liquid media. The liquid media (bacterial food) is initially transparent. This flask of liquid media is then warmed and shaken to introduce oxygen. As the bacteria grow in the liquid media, it becomes more and more cloudy as more bacteria are present.

Bacteria grow exponentially, doubling in quantity in a given amount of time. There are many factors that go into the amount of time that it will take for the bacteria to double. Some of these factors include the type of bacteria, the amount of media (food) that they are given, the temperature they are grown at, the amount of oxygen that they receive, etc. This is why one must monitor the bacterial growth rather than simply assuming a calculated rate of growth.

The amount of bacteria that are present in the liquid media can be monitored by spectroscopy. Since the more bacteria that are present in the liquid, the cloudier it is, the amount of light that passes through a sample of the bacterial media can be used to determine the density of the bacteria.

The detector of the spectrometer has a limited range of values that it can read. This working range is 0.01-1.0. Therefore, students must make dilution calculations using mixed fractions and decimals in order to prepare solutions that the instrument can read.

Materials
Preparation

Prepare enough copies of the worksheet for each student to have their own to work on. Familiarize oneself with the background material.

Procedure

1.) Background: Approximately 10 minute power point presentation attached to guide the lesson introduction. Biochemists use bacteria to produce the proteins that they wish to study. In order to do so, they must grow large quantities of the bacteria while monitoring this growth to ensure optimal protein production. Give a brief explanation of spectroscopy and how it is used to monitor the growth of bacteria due to the relationship between bacterial density and darkness of solution.

2.) Students will be using the assumed OD600 of each reading to calculate what dilution factor would be necessary to use for each reading, and what the value obtained would be.

<table>
<thead>
<tr>
<th>Reading Time Increment</th>
<th>Starting OD600 Value</th>
<th>Desired OD600 Value to Reach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every 30 Minutes</td>
<td>0.1</td>
<td>2.5-3.5</td>
</tr>
</tbody>
</table>

3.) Guide students through the first few rows of the data sheet.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Post Induction (kick) Time Point</th>
<th>Expected OD600</th>
<th>Dilution Factor</th>
<th>Scan Reading Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0.0 hours</td>
<td>1/2 1/5 1/10 1/100</td>
<td>0.05 0.02 0.01 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 0.5 hours</td>
<td>1/2 1/5 1/10</td>
<td>0.2 0.1 0.04 0.02</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

To walk the students through the column of the worksheet:

Scan Number: Which reading of the scan they are examining.

Time Point: How much time has passed since the experiment began. Scan number 1 is the initial reading, therefore is Time point 0.0 hours. Readings must be taken every 30 minutes, therefore scan number 2 is taken at 0.5 hours.

Post Induction (kick) Time Point: At this point in the exercise, tell students to put a line through this column, we will come to this after we reach a value in the desired range.

Expected OD600: This is value of the OD600 if the density of the bacteria doubles every 30 minutes. It might be useful to instruct the students to write the desired range at the top of this column (2.5-3.5).

Dilution Factor: By what factor must the students dilute the Expected OD600 value in order to obtain a reading within the limits?

Scan Reading Value: If the students use the dilution factor in the corresponding column, what is the number that they would read upon placing the sample in the spectrometer? For example, if the expected OD600 is 0.1, a Dilution Factor of 1 would yield a Scan Reading Value of 0.1, a Dilution Factor of 1/2 would yield a Scan Reading Value of 0.05, a Dilution Factor of 1/5 would yield a Scan Reading Value of 0.02, a Dilution Factor of 1/10 would yield a Scan Reading Value of 0.01, and a Dilution Factor of 1/100 would yield a Scan...
Reading Value of 0.001. It may be helpful for students to write the scan limits of the spectrometer at the top of this column (0.001-1). The Dilution Factor of 1 is circled in green because it will give a Scan Reading Value of 0.1, which is within this desired range.

Once you have worked through a few rows with the students, allow them to work individually or in small groups until they reach the desired OD600 value (2.5-3.5).

This is the point at which the Post Induction (kick) Time Point column must be explained. Now that the bacteria have grown to a sufficient density, it is time to induce them to produce the protein of interest. In order to produce optimal amounts of protein, the bacteria should be allowed to grow for an additional 3 hours. From this point on, both the total growth time and the post-induction growth time should be recorded on the data sheet. Allow the students to continue working until they have reached this additional 3 hour time point.

Oh no! The Scan Reading Value for a Dilution Factor of 1/100 is still too large for the spectrometer to read! The students will have to determine what further dilutions to make in order to be able to confirm the OD600 at this point.

Assessment

Successful completion of the worksheet with all boxes filled should demonstrate competence in mixed fraction and decimal calculations.

Extensions

1. (Grades 6-12) Though for the initial exercise the students will assume that the bacteria will continue to double every 30 minutes after induction, the reality is that once the bacteria are producing the protein of interest, their growth will slow dramatically.


3. The worksheets can be tailored to different academic levels, appropriate dilutions can be provided or students must calculate themselves.