CB 5 Factors in Yeast Growth

How can we influence the growth of a yeast population? How can changes in the yeast's environment cause changes in their growth rate? When you buy yeast in a grocery store, you are buying living organisms that remain dormant as long as they are kept cool and dry. When water, food, and warmth are added, the yeast become active and begin to grow by budding. In this experiment you will grow different yeast cultures by feeding them food (corn syrup) and changing various factors which yeast need to grow.

When you look at the results of feeding the yeast with the corn syrup solution (a solution rich in sugars), you can form different hypotheses about the effects of the food on the yeast growth. In this inquiry, you will investigate different hypotheses about yeast growth. For example, one hypothesis you can test is: If the amount of sugar (food) is decreased, then the yeast will not grow as well. To test this hypothesis, you will set up several yeast cultures. All of our yeast cultures will be the same except for the amount of food (the independent variable) in each culture.

To determine the effects of this independent variable on the yeast cultures, you need to measure a dependent variable that will be affected by the amount of food. When organisms use food, they also produce waste products. In yeast, one waste product is carbon dioxide. You observed the production of carbon dioxide by yeast in the "Mystery Bags" Inquiry in CB 2. Review your Log records of comments about the "Mystery Bags." If you measure the amount of carbon dioxide produced by the yeast, you can get an indication of how active the yeast cultures are. The more carbon dioxide they produce, the more the yeast culture is exhibiting characteristics of growth.

Materials (for each group of 2-4 students)
- 5 large test tubes (such as 18x150 mm) and 5 small test tubes (such as 10x75 mm, without lips), so that the smaller tubes will easily fit inverted into the large test tubes
- 100 mL of 40% corn syrup solution (mix 40 mL of grocery syrup with 60 mL of water)
- 1 test tube rack
- 25 mL of yeast solution
- crayon/wax pencil (for marking on test tubes)
- 50 or 100 mL graduated cylinder
- 400 mL beaker
- beaker for discarded yeast solutions
- micropipette
- 5 pieces of foil
- metric ruler
- Casio fx2 Graphing Calculator: Input and display of data
- Casio Data Collector EA-100 and CO₂ probe: CO₂ concentration readings
- Casio QV2800 Digital Camera: Visual images of changes in fermentation tube
Procedure

Follow carefully and keep track of the procedures as you work. You will make a serial dilution where each test tube has progressively less syrup and more water.

Day 1: Make serial dilutions as follows.

1. Set up at all of the equipment and supplies you will need for your experiments at your laboratory station. Number the large test tubes from 1 to 5 (using the wax pencil).
2. Measure 15 mL of syrup solution with the graduated cylinder and pour into tube #1.
3. Pour 25 mL of syrup solution into the graduated cylinder. Add 25 mL of tap water. (There should now be 50 mL total) Mix by holding your hand over the mouth of the cylinder and shaking gently. Pour 15 mL of this solution into test tube #2. #2 now has half as much syrup as #1. Keep the remaining syrup solution.
4. Pour 10 mL of the syrup solution out of the graduated cylinder into the discard beaker. This will leave 25 mL of syrup solution in the cylinder. Add 25 mL of tap water and shake gently. Pour 15 mL of this solution into test tube #3. Tube #3 should now have half the syrup of #2. What percentage of Tube #1 would this be? Keep the remaining syrup solution.
5. Pour 10 mL of the syrup solution out of the graduated cylinder into the discard beaker (this will leave 25 mL of syrup solution in the cylinder). Add 25 mL of tap water and shake gently. Pour 15 mL of this solution into test tube #4. Keep the remaining syrup solution.
6. Pour 10 mL of the syrup solution out of the graduated cylinder into the discard beaker (this will leave 25 mL of syrup solution in the cylinder). Add 25 mL of tap water to the syrup solution in the graduated cylinder and shake gently. Pour 15 mL of this solution into test tube #5. Discard the remaining solution into the discard beaker. Steps 2-5 are illustrated in Figure 5.1.
7. Add the yeast to each solution as follows. Gently stir the yeast suspension with a micropipette before you add the yeast to each test tube. Using the micropipette, add 5 drops of yeast suspension into each of the five test tubes. Now, each tube has the same size population of yeast, but decreasing amounts of food. What do you predict will happen? Write your prediction in your Log.

8. Hold your thumb over the top of each test tube and gently shake each test tube to mix the yeast with the syrup solution. Steps 7 and 8 are illustrated in Figure 5.2, Diagrams B & C.
9. Place a small test tube upside down into each of the large test tubes numbered 1-5. Then, hold your thumb over the mouth of the large test tube. Turn the large test tube upside down so that the solution fills the small test tube completely (no air should be left in the small test tube).

10. Place each large test tube right side up into the test tube rack. Place a piece of foil over the top of each large test tube. Label the test tube rack with the names of all the lab partners. Place your rack into the designated place overnight.

Steps 9 and 10 are illustrated in Figure 5.3, Diagram D.

![Figure 5.3](image)

In your Log, write hypotheses for what you think will happen in each of the five tubes. Consider the independent and dependent variables in your experiment.

**Day 2:** Make observations and analyze data as follows.

1. Examine the small test tubes for any presence of gas.
2. Using your metric ruler, measure the length of any gas space in each of the small test tubes. Be sure to measure each tube the same way. Record your results for each test tube in your Log.
3. Construct a data table showing the results of your experiment.
4. The syrup solution you poured into test tube #1 was made by mixing 40 mL of syrup with 60 mL of water--this was a 40% solution of syrup. Calculate the % solutions for each of your test tubes below:

<table>
<thead>
<tr>
<th>Percent Syrup</th>
<th>mm of Carbon Dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube #1 40%</td>
<td></td>
</tr>
<tr>
<td>Tube #2</td>
<td></td>
</tr>
<tr>
<td>Tube #3</td>
<td></td>
</tr>
<tr>
<td>Tube #4</td>
<td></td>
</tr>
<tr>
<td>Tube #5</td>
<td></td>
</tr>
</tbody>
</table>

Clemson Biology Project
CB 5  Factors in Yeast Growth
Interpretations  Construct answers in your Log to the following questions.

1. Graph your results in your Log. What does the graph suggest?
2. Place your group's results on the board/overhead so that a class average can be determined and graphed. Compare your graph with the graph of the class average.
3. Summarize what happened to the tubes.
4. How did your data support your hypothesis?
5. If your results did not support your hypothesis, what are some explanations that could account for your results?
6. What relationships can you see between the independent variable(s) and the dependent variable?

Applications

1. Design an additional experiment to test another limiting factor for the growth of yeast.
2. Describe a seasonal outbreak of plant or animal population growth in your community?
3. To what do you attribute the outbreak?
4. What eventually happens when populations run out of resources?
Casio FX2.0 Calculator Procedures for CB 5

Calculating the Syrup Percentages for Each Tube

To calculate the percent syrup in your solution of syrup and water used in the first tube, you need to divide the amount of syrup you used by the total amount of solution then multiply that result by 100. The total amount of the solution is the amount of syrup plus the amount of water, added together. See the formula below:

\[
\text{percent syrup} = \frac{\text{amount of syrup}}{\text{amount syrup} + \text{amount water}} \times 100.
\]

To enter this in the FX2.0 from the RUN-MAT menu, you will need to use two sets of parentheses. Enter the left \([ ( \) parenthesis, then the \(\text{amount syrup}\), then \([ ÷ ]\), followed by the left parentheses \([ ( \) again, the \(\text{amount syrup}\), the \([ + ]\), then the \(\text{amount water}\), then the right parentheses, \([ ) ]\) twice, then the \([x]\) times, then 100. Finally press \([\text{EXE}]\) for the answer.

For each of the successive tubes, the syrup solution is diluted by half, thus tube #2 will have half the percentage of syrup that tube #1 has. Tube #3 will have half the percentage that tube #2 has. Tube #4 will have half the percentage that tube #3 has. Tube #5 will have half the percentage that tube #4 has. So if tube #1 has a 40% solution, then tube #2 will have 20%, tube #3 will have 10%, tube #4 will have 5%, and tube #5 will have 2.5%.

Graphing the Results

First you need to determine which variable should be the independent and which should be the dependent variable. Since the percentage of syrup appears to affect the amount of carbon dioxide produced, then we can assume that the amount carbon dioxide produced is dependent upon the percentage of syrup. Recall that we enter the independent values in the first list and the dependent values in the second list. Although it does not really matter in which order you enter these, for the purpose of uniformity we do it this way. From the Main Menu choose STAT by pressing \([2]\). If you already have data stored in Lists 1 and 2, there are several different things you can do:

1) Delete the data from the lists.
   Move the cursor so that List 1 is highlighted. Press \([F4]\) for DEL-A (delete all). Then press \([\text{EXE}]\) for Yes. Do a similar procedure to delete data for other lists, highlighting the list name that you wish to delete entries from.

2) Use empty lists.
   Each File contains 20 lists, so you can cursor over until you find 2 empty lists side by side. Just be sure that you remember the name of the lists because when you set up the graph you will need to enter the correct list numbers into the XList and YList.

3) Change Data Files.
   You can change the "drawer" into which you are filing information. From the main stat screen, press \([\text{CTRL}]\) (the green button), followed by \([F3]\) for SET UP. Cursor down to the third line List File. Press \([F1]\) for FILE then enter a number 1 through 6, excluding from your choice the number of the current file listed on the screen. Then press \([\text{EXE}]\), then \([\text{ESC}]\). This will take you back to
the main stat screen. If there is new data listed, you can either delete it or follow the procedure again and change files.

For the sake of uniformity we will use List 1-2.

- Press [CTL] [F3] for SETUP. Be sure that Stat Wind is auto and Func Type is Y. If not, use the menu at the bottom of the screen and the function keys to adjust the line when it is highlighted.
- In List 1 enter the percent sugar amounts, remembering to press [EXE] after each entry.
- Move the cursor to List 2 and enter the amounts of CO₂ produced that correspond to the percentages in the first list. Press [EXE] after each entry.

Once you have entered your data, and you are ready to graph it, you will need to set up the graph information for the FX2.0.

- Press [F1] for GRPH, then [5] for Set. Choose which graph you want to use, 1, 2, or 3, by pressing [F1], [F2], or [F3] respectively, while the top line, StatGraph is highlighted. For this example we will choose [F1].
- Move the cursor down to Graph Type. You may want to graph either a scatter graph or a broken line graph, where the points are connected by lines. [F1] Scat will give you the scatter plot and [F2] XY will give you the broken xy-line graph.
- Move the cursor down to the XList. Press [F1] if you need to change the list number, then enter [1]. Press [EXE].
- Cursor down to YList and follow the same procedure, except enter [2] for the list number.
- Cursor down to Frequency, which should be 1, so if it is not, press [F1] to change it.
- Cursor down to Mark Type and choose the mark you prefer by pressing [F1], [F2], or [F3].
- Press [ESC] to return to the main stat screen.
- OPTION: You could choose [4] instead of [1], and press [F1] for On and [F2] for Off, turning on and off which ever graphs you choose for viewing. This feature allows you to view more than one graph at a time on the screen. Once you have turned on the graphs you want to view, press [F6] for DRAW.