

Biomanufacturing: An inquiry lesson in growing cells

Growing Living Cells lab

Problem: What factors influence the growth of cells in sterile media?

Background:

This experiment models how different variables can affect cell growth in a bioreactor. Media provides the nutrients that cells need to grow under optimum conditions. In this inquiry, you will pick one independent variable in the growth of your cells to influence. What you do with your cells will be entirely up to you and your research partner. Everyone will use the same types of containers for media growth and the same techniques.

Procedure:

The class will be growing yogurt bacteria in skim milk media. Yogurt bacteria have been cultured for centuries around the world and originated from wild bacteria. Yogurt bacteria produce lactic acid which denatures and precipitates milk proteins. Lactic acid will be the “product” we are trying to make in our biomanufacturing process.

You will be growing your culture in tubes. Every tube will receive 20 mL of sterile milk media and be inoculated with 2 mL of the “mother” culture (yogurt bacteria).

You will have one control group that you will do nothing to and you must have at least 3 test groups that you will use to test your independent variable in different amounts.

You will be evaluating the growth of your bacteria in three ways.

- 1) You will measure the pH before you inoculate, after you inoculate, after 24 hours and after 48 hours.
- 2) You will note changes in the culture using qualitative observations after 24 and 48 hours.
- 3) You will complete a serial dilution of one of your cultures and evaluate the growth on agar plates.

Hypothesis:

If I change _____,
then the bacteria will grow (faster/slower) _____,
because _____.

Independent Variable: _____

Test Groups: Control, 1. _____, 2. _____, 3. _____

Things to keep constant (things I won't change between the groups): _____

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Procedure:

Materials:

4 culture tubes "Mother" culture Skim Milk media _____
pH paper Sterile 20 mL and 2 mL measurement tools _____
Sterile swabs

1. Label 4 culture tubes with initials, class number, and date. Label 1. "Control", 2. "Group 1," 3. "Group 2," 4. "Group 3."
2. Add 20 mL of sterile milk media to each tube.
3. Add 2 mL of the mother culture to each tube.
4. To group 1, I will _____
5. To group 2, I will _____
6. To group 3, I will _____
7. .
8. .
9. Cap and mix well by inverting several times.
10. Using a different sterile swab for each tube, dip into the culture and place some of the culture solution onto pH paper and record the starting pH. Using your microscope, record any observations of the culture.
11. Loosen the caps to allow air flow and place your culture tubes in a cup in the incubator.
12. After 24 hours, repeat step 10 and 11.
13. After 48 hours, repeat step 10 and 11 and complete the "Serial Dilution" procedure.
14. Discard cultures as directed by your teacher.
15. Record bacterial counts from your serial dilution.

Data:

Quantitative Observations:

Group	Starting pH	24 hours pH	48 hours pH
Control			
1			
2			
3			

48 hour Bacterial Count for Group _____ = _____

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Qualitative Observations:

Group	Starting observations	24 hours obs.	48 hours obs.
Control			
1			
2			
3			

Line Graph:

pH vs. Time



Control
 Group 2

Group 1
 Group 3

Data Analysis:

What happened to the pH for each of the groups?

Was it the same for all of the groups? If not, how was it different?

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How were the observations for each of the groups different?

Was there bacterial growth in the tube you chose to sample? How many bacteria were present?

Conclusions:

Were there any differences between the control and the test groups?

Did your independent variable (what you tested) affect the dependent variable (cell growth)? How do you know?

Was your hypothesis supported by the data?

What mistakes did you make?

What other variables do you think could affect cell growth?

What would you change if you had a chance to do this experiment again? What do you think would help the cells grow or keep them from growing more than what you did?

What have you learned about the challenges of growing cells in a bioreactor to make a biological chemical?