19. Toxicology Using Yeast

Driving Questions

Toxicology is the science of assessing chemical hazards.

- How can yeasts be used in toxicity studies?
- Which is more toxic to yeast cells—household bleach or household vinegar?

Background

Toxicologists must concern themselves with the degree of toxicity—that is, the capacity to cause harm or death to living organisms—that exists for chemicals in the environment. Their studies must consider the dose (the amount of the substance that organisms are likely to be exposed to). They must also consider the genetic makeup of a species that might cause it to be sensitive to damage by a given chemical.

Toxicologists must select organisms for toxicity testing that are genetically similar to the organisms they want to protect from toxic chemical effects. Because of ethical considerations, substitutes for the organism of concern are often needed. Organisms further down in phylogeny that, nevertheless, have critical genetic characteristics similar to those of humans, are preferred test subjects.

Yeasts are good candidates for toxicology screening tests because 1) they are easy to grow, 2) they are relatively simple single-celled organisms yet are eukaryotic, 3) they have many metabolic processes that also occur in humans and other organisms further up in phylogeny, and 4) they have a known genetic code.

Materials and Equipment

For each student or group:

- CO_2 sensor
- pH sensor
- EcoChamber
- Magnetic stir plate and stir bar
- Beaker, 100-mL (for vinegar)
- Beaker, glass, 2-L
- Graduated cylinder, 25-mL or 10-mL
- Water, 1 L
- Graduated cylinder, 1-L or 500-mL
- Erlenmeyer flask, 125-mL (for bleach)
- Rubber stopper for Erlenmeyer flask
- Stirring rod
- Rapid-rise activated baker's yeast, 7-g packet
- Sugar, 100 g
- White vinegar, 50 mL
- Household bleach, half-strength, 50 mL

Safety

Add these important safety precautions to your normal laboratory procedures:

- If the room is not well-ventilated, handle open containers of bleach and bleach solutions under a ventilated hood.
- Wear safety glasses and a lab coat.
- Have running water or an eyewash station in close proximity.

Procedure

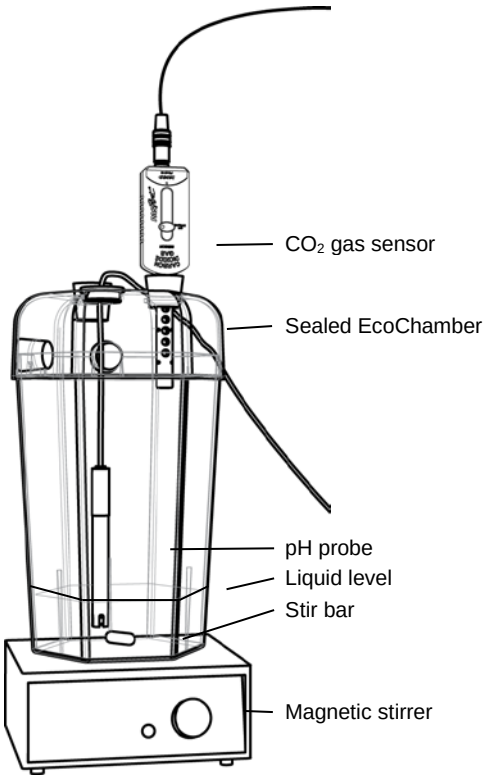
After you complete a step (or answer a question), place a check mark in the box (☐) next to that step.

Part 1 – Prepare the yeast culture

Set Up

1. ☐ Start a new experiment on the data collection system.
2. ☐ Connect the CO_2 sensor and the pH sensor to the data collection system to record pH and CO_2 gas simultaneously.
3. ☐ Display pH on the y-axis of a graph and CO_2 gas on the y-axis of another graph; show Time on the x-axes of these graphs.
4. ☐ Heat 1 L of water and 100 g of sugar in a 2-L beaker to about 40 °C on a hot plate.
5. ☐ Remove the beaker from the hot plate and turn off the heat.
6. ☐ Add a package of activated yeast, and stir thoroughly.
7. ☐ Allow the mixture to activate for 15 minutes, stirring occasionally.

Note: The formation of bubbles indicates the yeast is activating.



Part 2 –Test the toxicity of vinegar

Set Up

8. ☐ Set up the EcoChamber to measure pH and CO_2 gas simultaneously, as follows:
   a. Place a stir bar in the chamber and set it on a magnetic stirrer.
   b. Make sure the end of the pH probe is about 1 cm above the bottom of the chamber.
   c. Arrange the CO_2 gas sensor so the end is completely inside the container, but will not get wet.
   d. Using the graduated cylinder, measure and pour 500 mL of the yeast cell culture solution into the chamber and seal it airtight.
   e. Turn on the magnetic stirrer.

Collect Data

Important! Record data continuously until you have added all 50 mL of vinegar.

9. ☐ Start data recording. Adjust the scale of the graph so the data fills the screen.

10. ☐ Measure 10 mL of vinegar using the graduated cylinder while data is recording.

11. ☐ After recording data for 3 minutes, remove the stopper from a port at the top of the chamber and add the 10 mL of vinegar. Replace the stopper.

    Note: Do not stop recording data!

12. ☐ Record data for 3 additional minutes and again add 10 mL of vinegar.

    Note: Do not stop recording data!

13. ☐ Repeat this procedure until a total of 50 mL of vinegar has been added.

14. ☐ Record data for 3 additional minutes.

15. ☐ Stop recording data.

16. ☐ Name the data run appropriately.

17. ☐ Discard the yeast solution as instructed by your teacher, and rinse the chamber and pH sensor.

18. ☐ Predict which will be more toxic to the yeast cells—half-strength bleach or full-strength vinegar? Explain your reasoning.

19. ☐ Why are you measuring the CO_2 gas concentration?

20. ☐ Why are you measuring the pH of the solution?

Part 3 – Test the toxicity of half-strength bleach

Set Up

21. ☐ Set up the EcoChamber to measure pH and CO_2 gas simultaneously, as follows:
    a. Place a stir bar in the chamber and set it on a magnetic stirrer.
    b. Make sure the end of the pH probe is about 1 cm above the bottom of the chamber.
    c. Arrange the CO_2 gas sensor so the end is completely inside the container, but will not get wet.
    d. Using the graduated cylinder, measure and pour 500 mL of the yeast cell culture solution into the chamber and seal it airtight.
    e. Turn on the magnetic stirrer.

Collect Data

Important! You should record data continuously until you have added all 50 mL of half-strength bleach.

22. ☐ Start data recording. Adjust the scale of the graph so the data fills the screen.

23. ☐ Measure 10 mL of half-strength bleach using the graduated cylinder while data is recording.

24. ☐ After recording data for 3 minutes, remove the stopper from a port at the top of the chamber and add the 10 mL of vinegar. Replace the stopper.

    Note: Do not stop recording data!

25. ☐ Record data for 3 additional minutes and again add 10 mL of half-strength bleach.

    Note: Do not stop recording data!

26. ☐ Repeat this procedure until a total of 50 mL of half-strength bleach has been added.

27. ☐ Record data for 3 additional minutes.

28. ☐ Stop recording data.

29. ☐ Name the data run appropriately.

30. ☐ Discard the yeast solution as instructed by your teacher, and rinse the chamber and pH sensor.

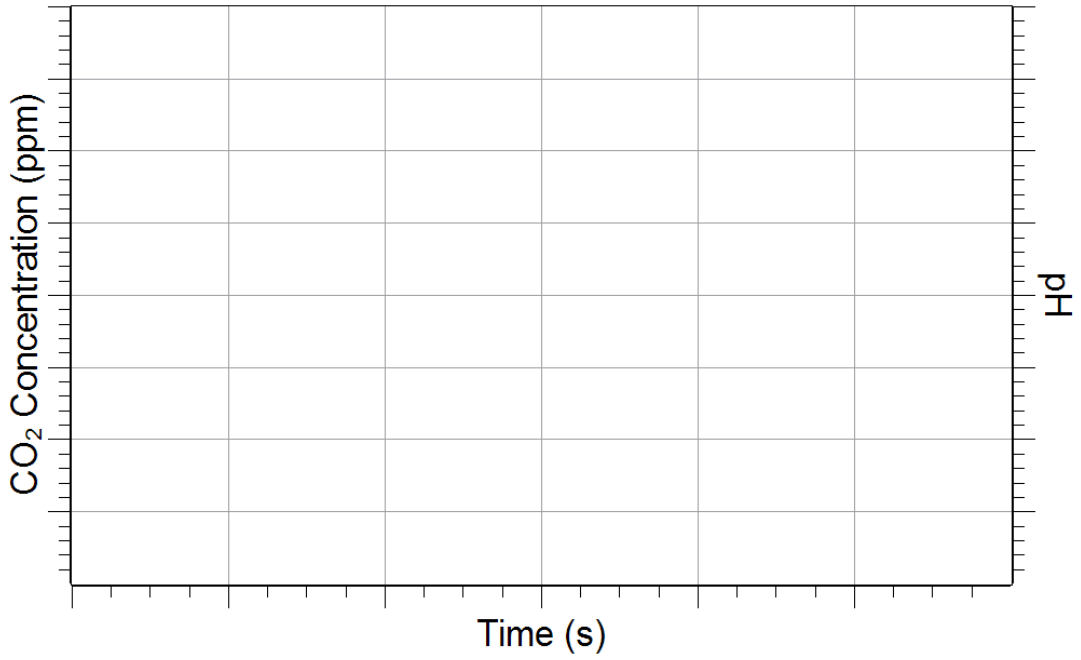
31. ☐ Save your experiment and clean up according to your teacher's instructions.

Data Analysis

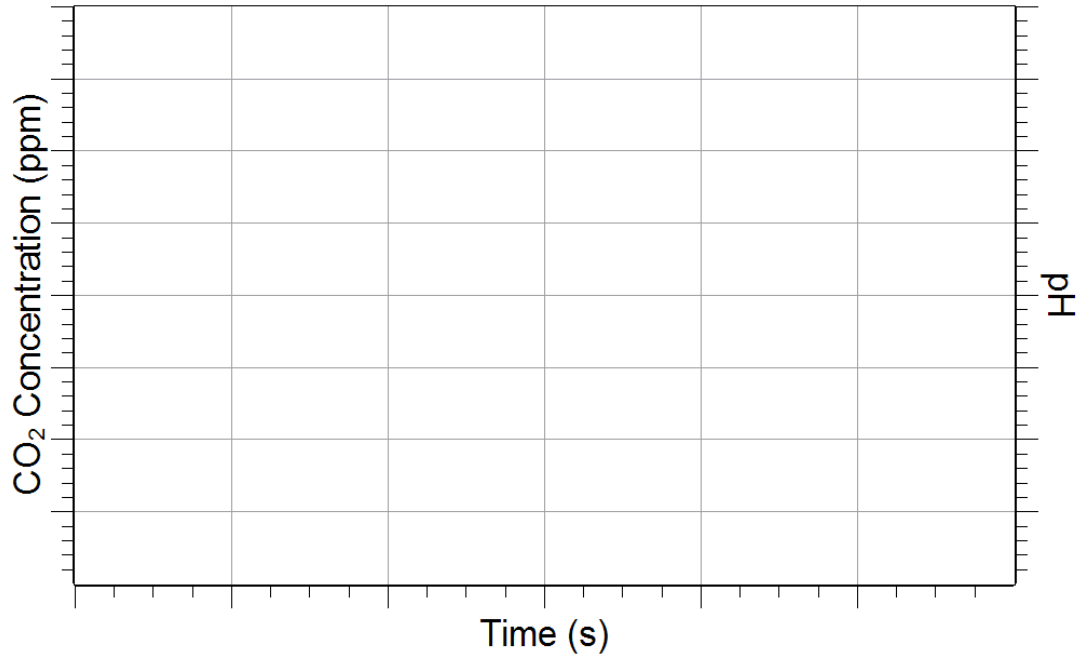
1. ☐ Display the dependent variable on the y-axis of a graph and time on the x-axis for both measurements (pH and CO_2 gas concentration) for each toxin.

2. ☐ Adjust the scale of the graph so the data fills the screen.

3. ☐ Sketch the graphs on the blank graphs. Sketch one graph for the vinegar trial and one graph for the half-strength bleach trial. Indicate the appropriate scale on the axes. Indicate the points at which the toxins were added to the system.



Effect on CO_2 concentration and pH of adding aliquots of full-strength vinegar



Effect on CO_2 concentration and pH of adding aliquots of half-strength bleach

4. ☐ Use graph tools to find the value of data points to complete Table 1.

Hint: To find the rate of CO_2 production (parts per million/second) for selected regions of the CO_2 data plot, use the linear fit tool to determine the slope of a best-fit line. To find the pH value for selected regions of the pH plot, use the statistics tool to find the mean value.

Table 1: Enter the rate of production of CO_2 gas and the pH change for each of the toxins

	Rate of production of CO_2 gas (ppm/s)						**pH**					
Toxin (total volume added)	**0 mL**	**10 mL**	**20 mL**	**30 mL**	**40 mL**	**50 mL**	**0 mL**	**10 mL**	**20 mL**	**30 mL**	**40 mL**	**50 mL**
White Vinegar												
Bleach: water 1:1												

5. ☐ Toxicologists often report the strength of a toxin in terms of LD_{50} or ED_{50}. Which of these terms is most appropriate for the results of this experiment? Explain.

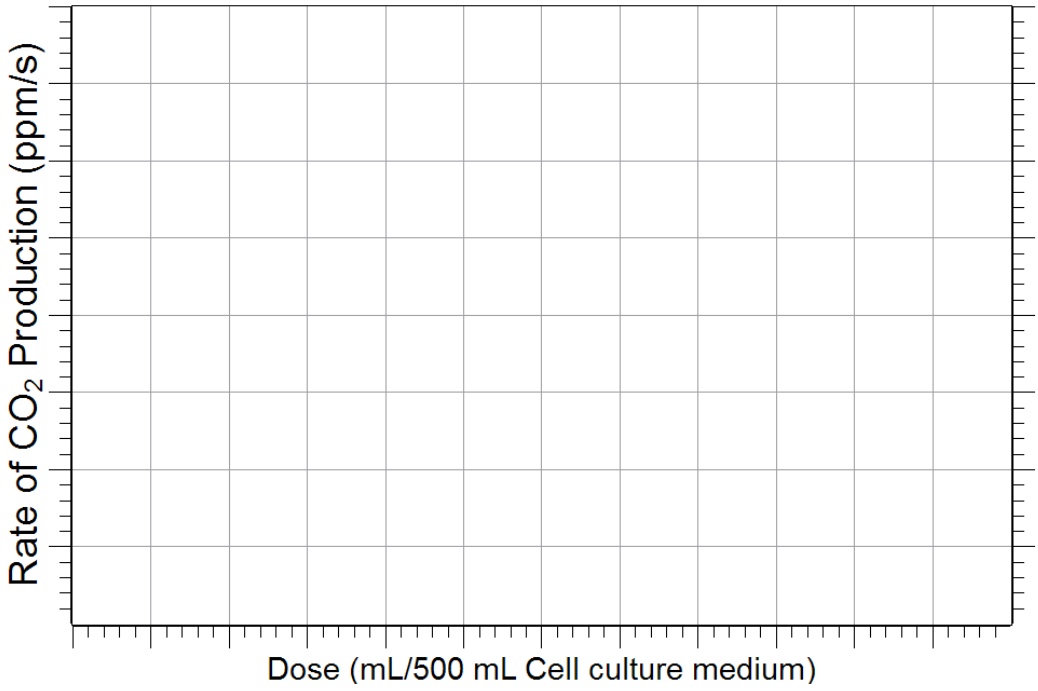
Note: The LD_{50} is the dose of a substance at which 50% of the organisms are killed—that is, the lethal dose for 50% of exposed organisms. In many cases, the end point of interest is a sublethal effect, such as affecting the ability to reproduce or inhibiting metabolism. In these cases, the strength of a toxin is reported in terms of ED_{50}—that is, the effective dose causing 50% inhibition.

6. ☐ Use your data to extrapolate the ED_{50} of the half-strength bleach solution and the full-strength vinegar solution. To do this:

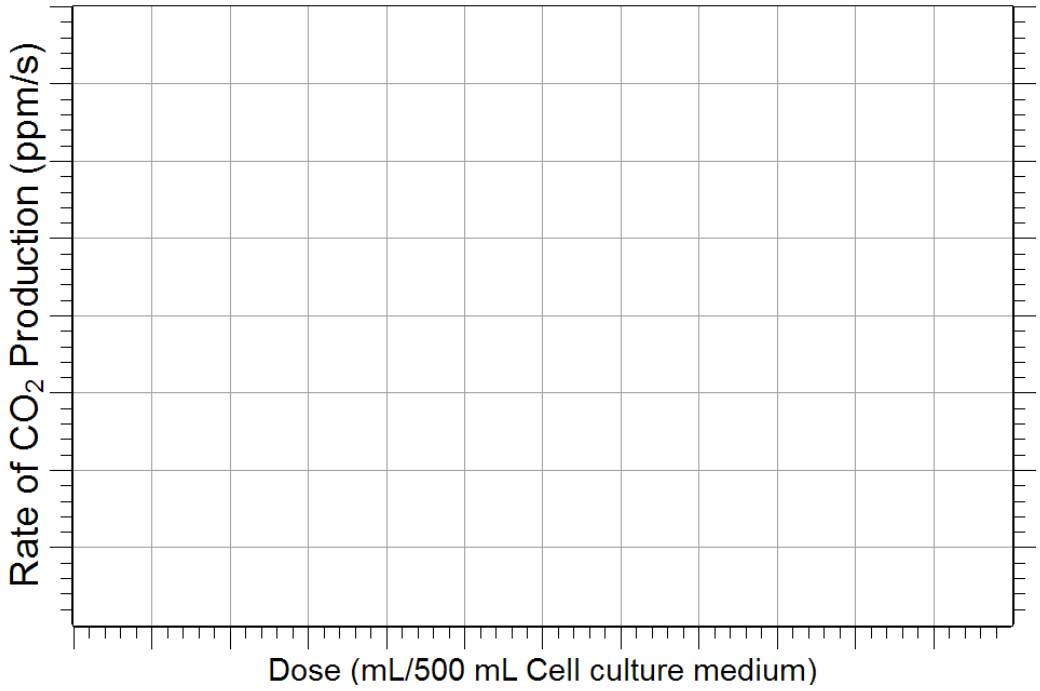
   a. Plot the rate of CO_2 gas production versus the dose administered (mL toxin solution/500 mL).

   b. Then connect the points and use this curve to extrapolate the volume of toxin (mL toxin/500 mL yeast culture) required to reduce the rate of CO_2 gas production in half (ED_{50}).

   c. Adjust the dose calculation to terms of mL toxin/L yeast culture.



Toxicity of full-strength vinegar



Toxicity of half-strength bleach

ED_{50} of full-strength vinegar _____

ED_{50} of half-strength bleach: _____

Analysis Questions

1. **What is the independent variable in this experiment?**

2. **What are the dependent variables?**

3. **What are the controlled variables in this experiment?**

4. **How do the data compare with your prediction?**

5. **What does the rate of CO_2 gas production indicate regarding the yeast culture? Explain why the rate of CO_2 gas production by a yeast culture can be used as an indicator of the toxicity of an added substance.**

6. **Why do you think pH was measured in this experiment? What was the relationship of pH to toxicity? Was a large change in pH necessary for a toxic effect to occur?**

7. **For the best interpretation of the data, what additional test should be run with this assessment? Explain.**

Synthesis Questions

Use available resources to help you answer the following questions.

1. **Determine the mean and standard deviation (SD) of the ED_{50} data obtained from the several trials conducted in your class. Compare the results of your trial to that of your classmates. Discuss possible reasons for the variations seen. Which value do you think is closest to the true value?**

2. **In what ways are yeast cells good subjects for toxicity studies?**

3. **What are some limitations of using yeast cells as subjects for toxicity studies?**