2.1) ASSESSING POLLUTION AND REMEDIATION WITH YEAST

Objective: In this experiment, you apply a dose-response of a harmful substance to measure effects ranging from minor biological impairment to complete mortality. Potency of other pollutants and remediation methods are also evaluated. **Video instructions**: https://vimeo.com/70199307

Introduction: The most comprehensive measure of toxicity for a given substance is the dose-response. This data is usually summarized in terms of LD-50 or ED-50. LD-50 refers "Lethal Dose 50%." This is the dose that results in approximately 50% mortality. ED-50 refers to "Effective Dose 50%. This is when 50% of the organism is affected by the pollutant. The term ED-50 is used when a response other than mortality is being evaluated.

Dose-response experiments can be carried out *in vivo* or *in vitro*. *In vivo* refers to testing that is carried out on whole living organisms. *In vitro* literally means "in glass" and involves applying the treatment to cells or tissues that are kept alive in test tubes or incubation wells. Usually *in vitro* is more convenient, but testing on cells or tissues sometimes fails to tell us how the substance behaves in a whole organism.

Part 1: Crude Dose-Response for Yeast

1) Put on your goggles. *WARNING: Sodium hypochlorite is a strong oxidizer. Flush with running water if it comes in contact with the skin. Do not wear open-toed shoes. Be forewarned that this is also a powerful bleaching agent, so do not let it come in contact with your clothes.*

2) Prepare a 2% sodium hypochlorite solution. You do this by adding 38 mL of 5.25% solution to a graduate cylinder, diluting it to 100 mL with dechlorinated tap water, then pouring it into a labeled beaker. Rinse the graduate cylinder thoroughly.

3) Starting with this 2% solution you made, prepare a serial dilution. You do this by adding 10 mL of your 2% solution to your graduate cylinder then diluting it to 100 mL to obtain a concentration of 0.2%. Pour this new dilution into a labeled beaker and rinse the graduate cylinder thoroughly. Repeat this process with your 0.2% solution to obtain a concentration of 0.02%. This will give you three concentrations of sodium hypochlorite; 2%, 0.2%, and 0.02%. The addition of yeast suspension will cut dilutions in half.

4) Pour some dechlorinated tap water into a labeled beaker to serve as the control.

Adding the Yeast Suspension:

Fig. 1

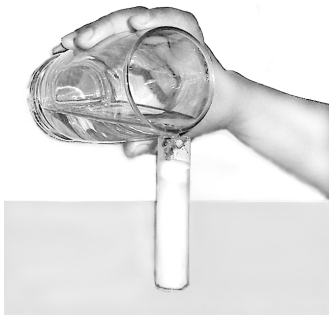

Fig. 2

5) Fill the incubation vial halfway with the yeast/sugar suspension (Fig. 1).

6) Fill the remaining space in the vial with your treatment (Fig. 2).

Mixing the Suspension with the Treatment Dose:

Fig. 3

Fig. 4

7) Pour your suspension and treatment mixture into a 50-mL beaker (Fig. 3) and mix thoroughly.

8) After mixing, pour back into the vial (Fig. 4).

Assembling the Apparatus:

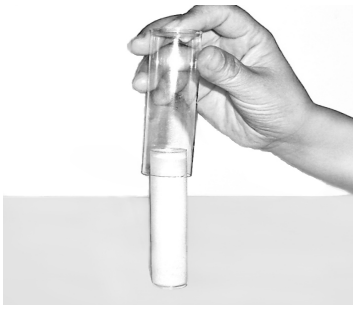

Fig. 5

Fig. 6

9) Add the other half of the incubation apparatus (Fig. 5).

10) Carefully pick up the incubation apparatus as indicated (Fig. 6).

Carbon Dioxide Measurement:

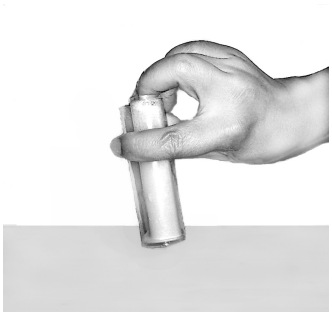

Fig. 7

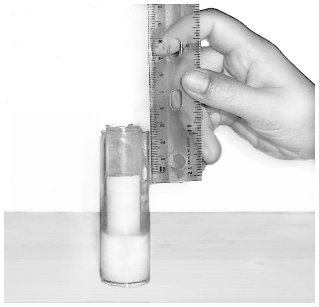

Fig. 8

11) Then turn it over, without spilling the contents, into the larger vial (Fig. 7). Set it on the table for 1-3 hours.

12) After the specified length of time, measure the length of the space created by carbon dioxide as indicated (Fig. 8).

Alternate Methods for Measuring Carbon Dioxide:

Fig. 9

Fig. 10

Option 1: You can measure the level of liquid that spills over to the larger vial if the gas space has too much foam (Fig. 9).

Option 2: If you lack incubation vials, you can incubate yeast in a test tube and collect gas through a hose to an inverted test tube filled with water (Fig. 10).

13) After 30-60 minutes, estimate the relative carbon dioxide produced from each vial by using a ruler to measure the length of the carbon dioxide bubble in mm. After incubation, enter your measurements into a table (Table 1):

Table 1: Tenfold Dilution of Pollutant

% NaClO solution	CO_2 generation (mm)	CO_2 generation as % of control
Control		
0.01%		
0.1%		
1%		

Part 2: Estimating LD-50
Prepare a new set dilutions based on the lowest dose that completely eliminated gas production and divide this dose each time by a factor of 2 (*not* 10). Consequently, your third test tube will be 1/4 strength. After incubation, enter your measurements into a table (Table 2):

Table 2: LD-50 Estimate

% NaClO solution	CO_2 generation (mm)	CO_2 generation as % of control
Control		
¼ dose		
½ dose		
Full dose		

Part 3:

Option A: Crude Dose-Response to Various Pollutants
Use the procedure used in Part 1 to evaluate two other pollutants provided by your instructor. Suggested pollutants include antifreeze and cleaning agents (like laundry detergent and ammonia). Enter results into separate tables and prepare one bar graph to compare differences between the three pollutants with the name of the pollutant on the x axis and the lethal dose on the y-axis. *WARNING: Follow all safety precautions on labels and avoid mixing ammonia with bleach.*

Option B: Hypochlorite Remediation
Follow the procedure below to evaluate the ideal dose of thiosulfate needed to neutralize the hypochlorite**.**

Introduction to Option B: Sodium hypochlorite is a powerful oxidizer. This is evident in the following half-reaction: $ClO^- + H_2O + 2(e\text{-}) \rightarrow Cl^- + 2OH^-$

The oxidizing power of the hypochlorite in can be neutralized with the thiosulfate ion:

$$ClO^- + 2S_2O_3^{2-} + H_2O \rightarrow S_4O_6^{2-} + Cl^- + 2OH^-$$

Based on this equation, at least 2 moles of thiosulfate are needed to neutralize 1 mole of hypochlorite. This works out to about 7 grams of $NaS_2O_3*5H_2O$ for each gram of NaClO.

Procedure for Option B:

1. Add 0.5 g sodium bicarbonate to 500 mL of distilled water and mix thoroughly. This is your "Solution A". The sodium bicarbonate is added to serve as a buffer.
2. Add 0.5 g sodium bicarbonate to 500 mL of 0.2 % sodium hypochlorite solution supplied by your instructor and mix thoroughly. This is your "Solution B."
3. Dissolve 10 g sodium thiosulfate in 100 mL of distilled water.
4. Prepare the following seven solutions based on the ratios indicated in Table B-1.

Table B-1: Thiosulfate/Hypochlorite Ratios

Ratio ($S_4O_6^{2-}$ / ClO^-)	A (mL)	B (mL)	$S_4O_6^{2-}$sol'n (mL)	Dist. Water (mL)
0/0 (neither additive)	100	0	0	16
8/0 ($S_4O_6^{2-}$ only)	100	0	16	0
0/1 (ClO^- only)	0	100	0	16
8/1	0	100	16	0
4/1	0	100	8	8
2/1	0	100	4	12
1/1	0	100	2	14

5. Prepare and incubate yeast suspension using the treatments listed in Table B-2 (50% yeast suspension, 50% treatment) and record data to the table.
6. Prepare a line graph with the ratios from 0:1 to 8:1 on the x-axis and CO2 generation as percent of 0/0 treatment on the y-axis.

Table B-2: Remediation Dose Response

Treatment	CO_2 generated (mm)	CO_2 as % of 0/0 treatment
0/0		
8/0		
0/1		
1/1		
2/1		
4/1		
8/1		

Questions:

1. Does 50% carbon dioxide production by yeast necessarily correspond to the LD-50?

2. How can you more accurately measure yeast survival?

3. Are yeasts a good model for examining the effect of hypochlorite on aquatic organisms? Why or why not?

4. Is this bioassay *in vivo* or *in vitro*? Explain:

Option A

5. Based on your graph, which pollutant is most harmful?

6. Based on your graph, which pollutant is least harmful?

Option B:

5. Why did you set up a test tube with thiosulfate alone?

6. Which test tube(s) served as your negative control? Explain:

7. Which test tube(s) served as your positive control? Explain:

8. If you are the owner of a factory that produces large amounts of hypochlorite waste, what ratio of thiosulfate would you use to minimize both your costs and your impact on the environment?

9. Why did you add 16 mL of water to the two controls?

Assignment Checklist:

1. Did you answer all the questions?
2. Did you graph the data (bar graph for option A, line graph for option B)?
3. Do all three graphs have titles, axis labels, and captions?
4. Are the graphs hand-drawn?