Enzyme Action (Optical Dissolved Oxygen Sensor)

How does the catalyzed decomposition rate of hydrogen peroxide compare with the uncatalyzed spontaneous decomposition rate?

# Objectives

* Compare the spontaneous hydrogen peroxide decomposition rate with the catalyzed rate.
* Relate the role of enzymes to the maintenance of homeostasis in living things.

# Materials and Equipment

* Computer or mobile device
* Wireless Optical Dissolved Oxygen sensor
* Photosynthesis Chamber with bar magnet
* Graduated cylinder, 100-mL
* 0.03% Hydrogen peroxide (H2O2), 120 mL
* Catalase suspension, 5-10 mL
* Disposable pipet
* Rinse bottle with distilled water

# Safety

Follow these important safety precautions in addition to your regular classroom procedures:

* + Wear safety goggles at all times.

# Procedure

*Part 1 - Setup*

1. Slide the transparent tank Ⓐ in the base Ⓑ as shown in Figure 1. If the tank does not fit, rotate it and try again.
2. Set the system upon the magnetic stirrer as shown. Add the magnetic stir bar to the inner chamber Ⓒ.
3. Squeeze both sides of the lid and align the lid tab Ⓓ with the base notch Ⓔ to close the chamber.

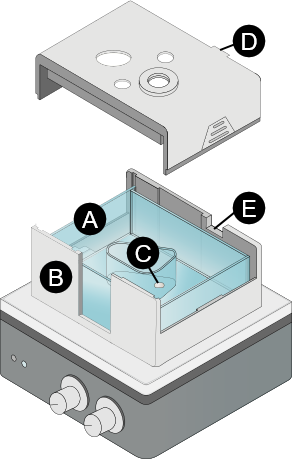


Figure 1. Photosynthesis chamber and lid

1. Remove the rubber boot from the end of the probe by turning it clockwise while looking at the probe. Do not let anything contact the end of the probe except the water samples in Part 2.
2. Use the O-ring Ⓕ as shown in Figure 2 to set the probe about 1" deep into the inner chamber. Observe the probe through the side opening Ⓖ to check for the correct depth. There should be enough space for the magnet to avoid striking the probe, and the probe should not be touching the tank floor.

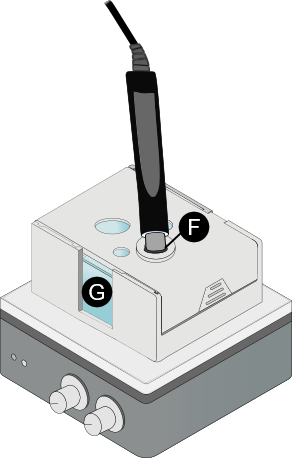


Figure 2. Use O-ring to set probe at 1" depth

1. Remove the probe. Hold the base steady, then gently squeeze the sides of the lid to remove it. Practice opening and closing the chamber lid until you are comfortable working with it.

*Part 2 - H2O2 Decomposition Rate With and Without an Enzyme*

1. Select **Sensor Data** in SPARKvue.
2. Connect the optical dissolved oxygen (ODO) sensor to your computer or mobile device. Make sure only the DO2 Concentration (mg/L) measurement is selected  and select the Graph template .
3. Set up the Photosynthesis Chamber as described in Part 1. Open the lid.
4. Add 60.0 mL of 0.03% hydrogen peroxide (H2O2) solution to the inner chamber.
5. Align the system so the bar magnet is centered on the magnetic stirrer. Turn on the stirrer to a medium speed.
6. Close the chamber and insert the Optical Dissolved Oxygen (ODO) probe in the lid. Check to make sure the probe is submerged about 1" deep into the inner chamber.
7. Allow the system to rest for 1 minute, then start collecting data . Stop collecting data after 3 minutes .
8. Use the Coordinates Tool  to determine the initial and final dissolved oxygen concentration and time elapsed. Enter the results in Table 1.
9. Turn off the stirrer. Remove the ODO probe from the chamber and rinse it thoroughly with distilled water.
10. Empty the contents of the chamber into the waste beaker. Retrieve the bar magnet. Rinse the chamber and magnet with distilled water.
11. Repeat steps 3 through 7; during the (step 7) 1-minute rest time, stir the catalase suspension and collect about 1 mL of it in a disposable pipet.
12. Start collecting data, then immediately add 2 drops of catalase suspension to the inner chamber through a lid hole.
13. Stop data collection after 3 minutes. Repeat steps 8-10.

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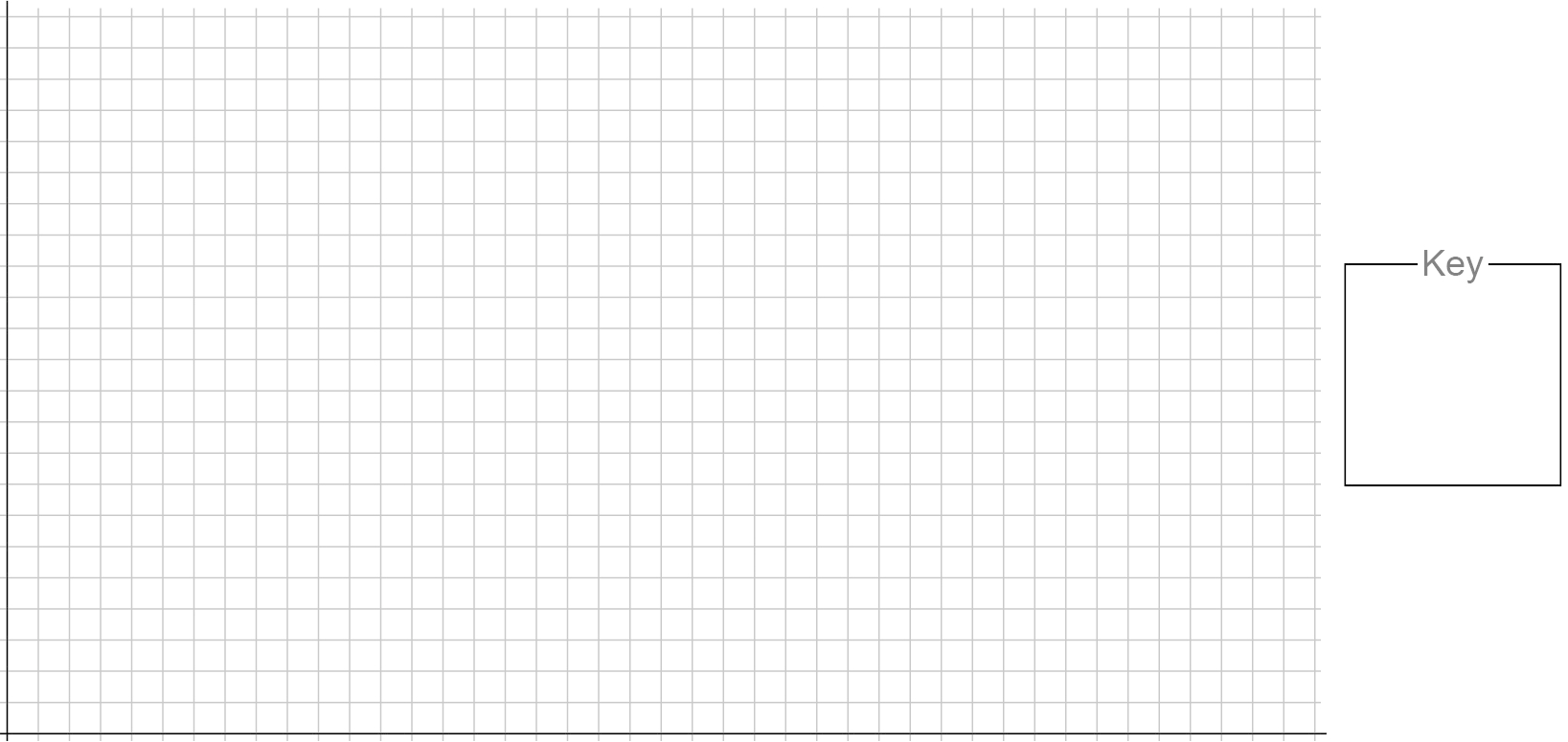
1. Select  both runs in the legend to show both runs on the same graph. Scale the graph; sketch it in Graph 1. Add a title and label the x- and y-axes. Use appropriate values and units. Fill out the legend.
2. Calculate the hydrogen peroxide decomposition rate (in mg/L∙s) for each run by dividing the change in DO2 concentration by the time elapsed. Enter the results in Table 1.

# Data Collection

*Table 1. H2O2 decomposition without and with enzyme*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Condition** | **Initial DO2 concentration (mg/L)** | **Final DO2 concentration (mg/L)** | **Time elapsed (s)** | **Change in DO2 concentration (mg/L)** | **Decomposition rate (mg/L∙s)** |
| Background Rate/ Control |  |  |  |  |  |
| Enzyme Added |  |  |  |  |  |

Graph 1:



# Questions and Analysis

1. Why does the DO2 concentration in the hydrogen peroxide solution change when the yeast suspension is added?
2. How much faster is the catalyzed reaction you observed compared to the spontaneous decomposition rate?
3. Explain why the reaction is so much faster when an enzyme is present.
4. Is the reaction rate constant for the entire time data is recorded? Support your answer with data.
5. If the reaction continued to run, do you predict the reaction rate to be constant? Explain your thinking.
6. What kinds of conditions could you test in your lab classroom that might affect the rate at which catalase breaks down hydrogen peroxide?