

Investigation: How Does Concentration Affect the Reaction Rates of Enzymes

Purpose:

- to observe the catalytic action of yeast catalase on hydrogen peroxide
- to determine the effect of concentration on yeast catalase activity
- to determine the effect of temperature on yeast catalase activity

Background information:

Hydrogen peroxide (H_2O_2) is a common but poisonous by-product of cellular metabolism, but H_2O_2 does not accumulate in cells because it is decomposed to water and oxygen gas. The decomposition of the hydrogen peroxide is facilitated by catalase, an enzyme present in most cells.



One molecule of catalase can catalyze the decomposition of approximately 4×10^7 molecules H_2O_2 per second!

In this lab activity, you will be using yeast catalase to observe how increasing and decreasing the concentration of the enzyme and substrate can affect the reaction rate.

Materials

Hydrogen Peroxide (3%) | Multiple beakers or cups (100-300 ml) or test tubes
Graduated cylinders or other measuring tools
Filter paper and Hole punch | Forceps | Water | Stopwatch or timer | Active dry yeast

Create your stock catalase solution

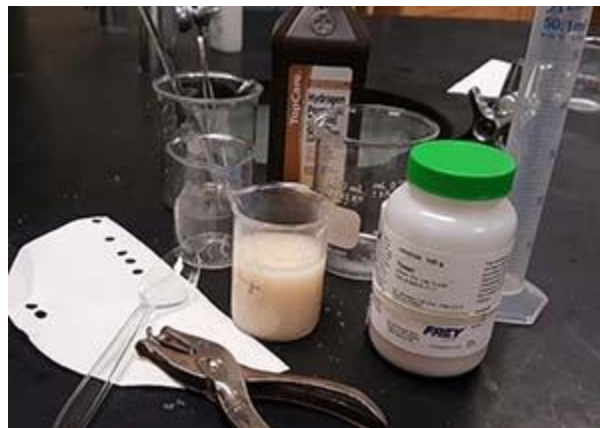
1. Dissolve 1 tsp (2-4 grams) of yeast in 200 ml of warm water.
2. Mix well and let sit for about 3 minutes
3. Test the activity of your yeast by adding a few drops to 5 ml of H_2O_2 - observe bubbles.

Observation of Catalase Activity

1. Pour 80 ml of H_2O_2 into a small beaker or test tube.
2. Cut a filter paper disk using a hole punch and soak this in your stock catalase, blot on a paper towel.
3. Drop the disk into the H_2O_2 .

Describe your observations:

If nothing happens (disk just floats) you may need to troubleshoot your experiment. Try stirring your catalase solution or dipping the disk in the H_2O_2 to break the surface tension. If you are still not seeing anything happen, consult your instructor.



4. Perform this procedure again and record the time it takes for the disk to drop and then raise to the surface. Perform multiple trials to perfect your technique. Convert all readings to seconds to take an average.

	Trial 1	Trial 2	Trial 3	Average
Time				

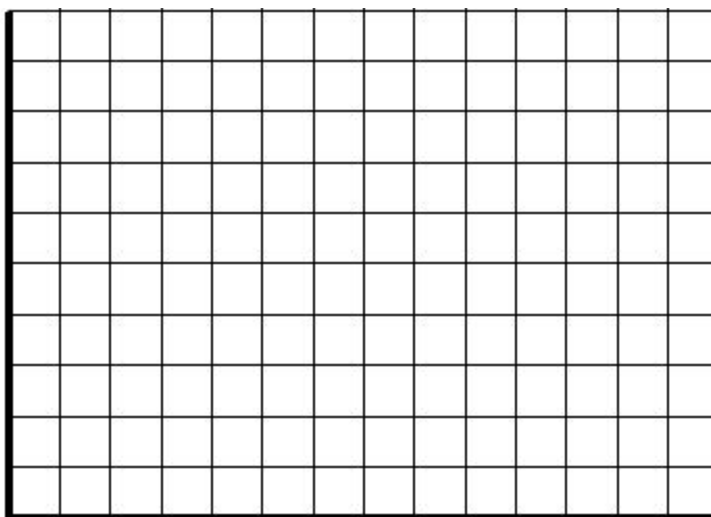
Effect of Substrate Concentration

Your H₂O₂ started at 3%. You will now dilute the peroxide in order to change its concentration.

1. Place 40 ml of H₂O₂ into a new beaker. Add 40 ml of water. H₂O₂ concentration = 1.5%
2. Place 20 ml of H₂O₂ into a new beaker. Add 60 ml of water. H₂O₂ concentration = .75%
3. Place 10 ml of H₂O₂ into a new beaker. Add 70 ml of water. H₂O₂ concentration = .375%
4. Perform the floating disk procedure for each concentration. You can copy your data for the 0.3% from the table above.

	0.3% H ₂ O ₂	1.5% H ₂ O ₂	0.75% H ₂ O ₂	0.375% H ₂ O ₂
Trial 1				
Trial 2				
Trial 3				
Average				

5. Create a graph that compares the averages for each concentration.



6. Use your graph and data to make a CLAIM that answers the question: How does decreasing the concentration of the substrate affect the enzyme reaction rate. This should be written in a complete sentence.

Effect of Enzyme Concentration

Your stock solution of catalase is your 100% solution. Create diluted solutions according to the ratios below and place each in small cups. These cups will be used to dip your filter paper disks.

1. 100% = 20 ml of catalase + 0 ml of water
2. 80 % = 16 ml of catalase + 4 ml of water
3. 60% = 12 ml of catalase + 8 ml of water
4. 40% = 8 ml of catalase + 12 ml of water
5. 20% = 4 ml of catalase + 16 ml of water.
6. Perform the floating disk procedure for each concentration. (We will not be taking averages this time.)

	100% catalase	80% catalase	60% catalase	40% catalase	20% catalase
Trial 1					

7. Use your graph and data to make a CLAIM that answers the question: How does decreasing the concentration of the enzyme affect the reaction rate.

8. Provide EVIDENCE for this claim by briefly summarizing your data or observations.

9. Consider how enzymes and substrates interact with each other. Suggest a REASON for your claim. This is where you consider what scientists understand about enzymes.

Synthesis

10. Which of the two variables (enzyme concentration or substrate concentration) seem to have the biggest affect on the reaction rate. How do you know?

Extension

Describe how you could use the same technique to test the effects of temperature on reaction rates.