Title: Enzyme—Catalase Activity

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
- to determine the effect of pH 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 mediated by catalase, an enzyme present in most cells. The balanced equation for the reaction is $2H_2O_2$ -----> $2H_2O + O_2$. 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, but you could also use catalase from potatoes, carrots, plant leaves, chicken liver, or steak….to name just a few of the many places where you can find catalase. Use your textbook as a source of additional information about the function and sources of catalase as well as information about the effects of H_2O_2 on cells.

Materials:

hydrogen peroxide	water bath
yeast catalase	stop watch or timer
graduated cylinder	water
small beaker	HCl
test tubes	NaOH
filter paper discs	pH paper
forceps	wax marking pencil

Procedure:

Observation of Catalase Activity:

1. Pour about 10 mL H_2O_2 into small beaker
2. Soak 1 filter paper disc in catalase solution for 5 seconds and then air dry
3. Drop the catalase-soaked disk in the beaker of H_2O_2
4. Record what happens
5. Repeat if necessary

Basic Catalase Assay Procedure

1. Pour 20 mL H_2O_2 into a 25 mL graduated cylinder
2. Pick up 1 filter paper disc with forceps
3. Soak the filter paper disc in catalase solution for 5 seconds and then air dry
4. Drop disc into graduated cylinder and start stopwatch as soon as the disc hits the surface of the H_2O_2
5. Stop the stop watch when the disc reaches the top of the liquid in the graduated cylinder
6. Measure the total distance in mm the disc travels
   Note: use the mL markings as if they were mm
   Note: for some of the trials, the disc may not reach the bottom of the cylinder!
   Note: total distance is distance the disc travels down and back up!
7. Record the time in seconds
8. Calculate rate as *mm/s*
   (Note: the disc traveled 20 mL down and then 20 mL back up to the surface!)
9. Repeat 3—5 times and calculate average rate and range

Effect of Concentration

1. Obtain 100% catalase solution
2. Make a series of catalase dilutions as follows:

Final Quantity	Concentration	mL Catalase	mL Water
10 ml	100%	10	0
10 ml	80%	8	2
10 ml	60%	6	4
10 ml	40%	4	6
10 ml	20%	2	8
10 ml	0%	0	10

3. Perform basic catalase assay using 100%, 80%, 60%, 40%, 20%, 10% and 0% catalase solutions
   Note: 100% solution is defined as 100 catalase units/mL
4. Construct a graph of rate vs concentration

Effect of Temperature

1. Perform basic catalase assay using catalase solutions at 0 ^{o}C (ice water bath), 25 ^{o}C (room temperature bath), 50 ^{o}C (warm water bath), 75 ^{o}C (warm water bath), and 100 ^{o}C (boiling water bath).
2. Record temperature of water bath just BEFORE performing the assay
3. Construct a graph of rate vs temperature

Effect of pH

1. Perform basic catalase assay using pH-adjusted catalase solution
2. Record pH of catalase solutions BEFORE performing the assay
   (Use pH paper)
3. Construct a graph of rate vs pH

Results: *4 data charts and 3 graphs:*

Observation of Catalase Activity
Effect of Concentration on Catalase Activity
Effect of Temperature on Catalase Activity
Effect of pH on Catalase Activity

Discussion: *Follow lab grading guidelines*

Remember to support your statements with specific results.
Explain your observations about concentration, temperature, and pH with reference to the protein nature of enzymes.
Remember to discuss sources of error and include suggestions for improvement.
Would catalase function in the mammalian stomach? In the mammalian intestine? Explain.
Ectothermic organisms have body temperatures that vary with the temperature of their surroundings. How might this affect the function of catalase in these organisms? Suggest some ways ectothermic organisms might cope with this problem.

Conclusion:

3 testable statements about effects of concentration, temperature, and pH
You may also want to state the optimum temperature and pH for yeast catalase activity

Reflection: *Personal statement about what you learned from the activity*