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| ***Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ #\_\_\_\_\_ Block \_\_\_\_ Partners:***  **Enzyme Rate of Catalase** |  |

 ***Introduction:***

**Enzymes** are molecular substances found in cells.  Enzymes act as catalysts and most are **proteins**.  Enzymes bind temporarily to one or more of the reactants of the reaction they catalyze. In doing so, they lower the amount of **activation energy** needed and thus speed up the reaction.
 Not only do enzymes economize energy usage, but also provide a variety of other functions. Cells uses an enzyme (**catalase**) to rid itself of a poisonous substance (**hydrogen peroxide**). The rate at which this occurs depends on the amount of catalase that is available. In this lab we are going to measure the time it takes for a disc of filter paper, soaked with different concentrations of enzyme, to make its way to the top of a plastic vial filled with peroxide.  **Rate of enzyme activity = distance (depth of hydrogen peroxide in mm)/time (in sec).**

**Catalase** catalyzes the decomposition of hydrogen peroxide into water and oxygen.  One molecule of catalase can break 40 million molecules of hydrogen peroxide each second.

**catalase
2H2O2 -----> 2H2O + O2**

***Objectives:***

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| http://www.biologyjunction.com/images/BD14572_.GIF | Students will prepare various serial dilutions from a 100% enzyme solution. |
| http://www.biologyjunction.com/images/BD14572_.GIF | Students will determine how enzyme concentration affects reaction rate. |

***Materials****: (READ THOROUGHLY- may have to use modifications)*

-6 ‘medicine’ cups for catalase dilutions (label each concentration);

-Catalase stock solution

-clear plastic vial(s)- if only one vial is used, rinse with deH2O before starting a new concentration

-forceps

 -Filter paper & hole punch to make 18 disks (put in lid of a plastic Petri dish)

-1.5% H2O2 (\**if H2O2 is in the original container at 3%, use distilled water and dilute to 50%. Put a total volume of ~300 ml of diluted solution among each of 3 brown plastic bottles and label with labeling tape & sharpie*)

-paper towel(s)

-safety glasses

-stopwatch or watch with second hand (or smartphone)

-clear metric ruler

-calculator

\**micropipets and/or microtips may be needed (see below)*

**\*\***The enzyme has been prepared for you as follows: 100g of beef liver was mixed with 1000 ml cold distilled water and crushed ice and homogenized in a blender for 30 seconds. This extract was filtered through cheesecloth and enough cold distilled water was added to the filtrate to get a total volume of 1000 ml (volume needed for all classes). Extract stock concentration is arbitrarily set at 100%. **ENZYME SHOULD BE KEPT ON ICE OR IN FRIDGE AT ALL TIMES!**

***Procedure****:*

1. Using disposable pipets, make a series of dilutions of the enzyme catalase and put in plastic graduated vials (label the concentration of each with labeling tape & sharpie. See #2, below). It is recommended to only make one solution at a time and immediately run trial(s) before making the other solutions, as the enzyme should be kept on ice until use. Enter the quantities needed in the following table.

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| **Final Quantity Needed** | **Concentration of Final Solution** | **mL of Catalase** | **mL of Water** |
| **5 ml** | **100%** |  |  |
| **5 ml** | **80%** |  |  |
| **5 ml** | **60%** |  |  |
| **5 ml** | **40%** |  |  |
| **5 ml** | **20%** |  |  |
| **5 ml** | **0%** |  |  |

1. Use labeling tape & a marking pen, label the plastic vials as follows: 100%, 80%, 60%, 40%, 20%, and 0%. These will be used for catalase solutions. Again, only make one solution at a time, as the catalase MUST be cold.
2. Using 3% H2O2 (from original stock solution bottle), make 350 ml (total volume) of 1.5% H2O2 (use distilled H2O). After mixing, pour diluted solution among 3 brown plastic bottles and label each with labeling tape. Keep the bottles capped when not in use. Next, pour 20 mL of 1.5% hydrogen peroxide into a clear vial (*volume may be adjusted, depending on size of vial*). **\*\*The following steps will require teamwork and practice!**
3. Using your forceps, pick up one filter paper disk and submerge it in the 100% enzyme solution for 5 seconds. Continue to hold the disk with the forceps.
4. Remove the disk from the solution and (barely) blot it on a paper towel (just enough that it doesn’t ‘drip’).
5. *\*Alternate to steps #4 and 5: Using the forceps, pick up filter paper disk and hold it over a paper towel while you use a micropipet and add 10 µL of the catalase solution onto the disk.*
6. READ STEPS 7 & 8 carefully: Place the saturated disk on top (or just beneath) of the hydrogen peroxide surface and measure the time it takes for the disk to sink to its lowest point and rise back to surface.
7. Use the metric ruler to measure the distance the disk sinks into the hydrogen peroxide. *\*You should consider taping (or use a rubber band) to attach the clear metric ruler to the side of the vial to measure distance, as some discs will not go to the bottom before rising!* Multiply by two to determine the entire distance the disk traveled. Enter the time and distance the disk traveled in the column for Trial 1 in the data table below.

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| --- | --- | --- | --- |
| **% Catalase** | **Time in seconds** | **Distance in millimeters** | **Reaction Rate mm/s** |
|   | **Trial 1** | **Trial 2** | **Trial 3** | **Avg.** | **Trial 1** | **Trial 2** | **Trial 3** | **Avg.** |   |
| **100** |   |   |   |   |   |   |   |   |   |
| **80** |   |   |   |   |   |   |   |   |   |
| **60** |   |   |   |   |   |   |   |   |   |
| **40** |   |   |   |   |   |   |   |   |   |
| **20** |   |   |   |   |   |   |   |   |   |
| **0** |   |   |   |   |   |   |   |   |   |

1. Do 3 total trials & record results (\*use notebooks first!). Repeat the above steps for remaining solutions, using 20 ml **FRESH H2O2** for EACH TRIAL (discard used H2O2 in sink). Use a clean filter paper disk for each trial. RINSE PLASTIC VIALS THOROUGHLY when finished for the next class to use. Thoroughly clean all glassware. THROW USED DISKS IN TRASH!

***Analysis & Conclusions:*** *\*These may be answered on another page if space below is limited for your response.*

1. Which concentration of catalase had the fastest reaction time and which had the slowest reaction time? **Explain.**

 2. What is catalase & why is it important to cells in an organism?

 3. What 2 substances form when catalase breaks down hydrogen peroxide?

 4. Why was catalase kept on ice? **Explain.**

5. Based on what you have studied about enzymes, state 3 other factors/variables that could have affected the reaction rate.

6. Produce an appropriate graph of the above data to display the rates of the reactions. Determine the independent variable and the dependent variable and label each axis, including appropriate units. Include a Title and a Legend.

**Title**:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Legend:

7.  Based on the graph and overall slope of the line, analyze the results. What can you conclude about the effect of enzyme concentration on reaction rate? You should include any data and calculations to support and **justify** your response.

1. Each group will now design a controlled experiment, testing a variable of each group’s choosing, and observe the effect on catalase and the resulting reaction rates. Write down your ideas and your procedure in your lab notebook. I must approve the procedure prior to starting the lab.
2. A formal lab report will be required for this part only (group-designed experiment) by each person in the group and turned in with this lab sheet, along with the prelab. Order for turning in: 1) Prelab (on top), 2) this lab sheet, 3) formal lab report of group designed experiment. Typically, the lab will be due ~ 1 week after the last day in lab.