Enzyme Activity Lab

Part 1: Guided Inquiry

**Background:**

Enzymes are very important tools in biotechnical engineering. They can be used to build DNA (DNA Polymerase), cut DNA in specific places (restriction enzymes) or perform any number of essential chemical reactions in biologically compatible conditions. It is therefore essential that any biotechnical engineer know how to optimize enzyme-catalyzed reactions or troubleshoot if they are not performed.

Catalase is an enzyme present in most cells and found in high concentration in liver and blood cells. Hydrogen peroxide on its own is incredibly toxic to your body, and can cause severe damage tissues if not disposed of. It is formed as a by-product of chemical reactions in cells. The breaking apart of hydrogen peroxide into water and oxygen gas (two non-harmful substances) is the job of catalase. Catalase promotes the decomposition of hydrogen peroxide (H2O2) in the following reaction:

**2 H2O2 🡪 2 H2O + O2**

We will measure enzyme activity by measuring the generation of oxygen gas – a product in the reaction. Our source of catalase will come from yeast cells.

**Question:**

How can we measure the rate of the reaction between the enzyme catalase and the substrate hydrogen peroxide?

**Materials** (per team of 4):

50 mL beaker

10 mL graduated cylinder (2)

100 mL graduated cylinder

1 mL plastic pipets (2)

3% hydrogen peroxide (H2O2)

water pan

reaction chamber

ring stand and clamp

catalase solution

forceps

filter paper

In all experiments, make sure that your reaction chamber is **very clean**. Catalase could adhere to the sides of the chamber if not washed thoroughly making measurements inaccurate. Measure all substances carefully. Before you do the experiment, **read the directions completely.** Make sure that you have all the required materials on hand, that you understand the sequence of steps, and that each member of your team knows his or her role.

**Part A: General Procedure and Set Up**

1. Fill a pan with room temperature water.
2. Submerge the 100 mL graduated cylinder and fill it with water. Turn the graduated cylinder upside down keeping the open end under water at all times. The opening of the cylinder should be about 3 cm from the bottom of the pan. One member of the group can take responsibility for holding it in place.
3. Place a thermometer in the pan and record the temperature of the water. \_\_\_\_\_\_\_\_\_ degrees C.

There are two possible procedures. You can choose the one that you believe yields the most accurate results.

Procedure 1:

1. Obtain a small amount of stock catalase (yeast) solution in a 50 mL beaker. You will need 1 mL of this solution for your first trial. When you are ready, you will add it to the reaction chamber with the plastic vial.
2. Pour 10 mL of hydrogen peroxide into the reaction chamber. Pipette in 1 mL of stock catalase (yeast) solution and **IMMEDIATELY** stopper the reaction chamber tightly, submerge it in the water bath, and place the plastic tip of the reaction chamber directly underneath the opening of the graduated cylinder so the bubbles are feeding up towards the cylinder.
3. Measure the volume of gas being produced in 30 second intervals for 5 minutes. Record the levels in the provided data tables. You may perform a second trial if you choose.

Procedure 2:

1. Obtain a small amount of stock catalase (yeast) solution in a 50 mL beaker.
2. With a hole punch, punch 8 discs from filter paper. Using forceps, dip each disc in your catalase solution. Blot off any excess chunks on a paper towel and use the forceps to stick the discs to one side of the reaction chamber.
3. Rotate the reaction chamber so the discs are on the top and pour 10ml of hydrogen peroxide solution into the reaction chamber. Make sure to avoid contact with the discs! Carefully stopper the reaction chamber and submerge it in the water pan.
4. Position the 100mL graduated cylinder so that it is above the tube from the reaction chamber.
5. Rotate the reaction chamber so the discs make contact with the hydrogen peroxide and the reaction begins.
6. Measure the volume of gas being produced in 30 second intervals for 5 minutes. Record the levels in the provided data tables. You may perform a second trial if you choose.

**Data Table for Oxygen Gas Production by the Enzyme Catalase:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Trial One** | | **Control Trial** | |
| **Time (Min)** | **O2 produced (mL)** | **Time (Min)** | **O2 produced (mL)** |
| 0:30 |  | 0:30 |  |
| 1:00 |  | 1:00 |  |
| 1:30 |  | 1:30 |  |
| 2:00 |  | 2:00 |  |
| 2:30 |  | 2:30 |  |
| 3:00 |  | 3:00 |  |
| 3:30 |  | 3:30 |  |
| 4:00 |  | 4:00 |  |
| 4:30 |  | 4:30 |  |
| 5:00 |  | 5:00 |  |

**Questions:** *Answer as a group to guide your thinking for Part 2: Open Inquiry*

* What other factors may affect the rate of enzyme activity?
* How might we slow or stop enzyme activity?
* How might we speed enzyme activity?

Part 2: Open Inquiry

With your lab group, design an experiment to help you test the properties of catalase. You will present your procedure and findings to the class, so try to design and conduct your experiment with accuracy in mind.

Identify the following parts of your experiment in the space below:

1. Testable question:
2. Hypothesis:
3. Independent Variable:
4. Dependent Variable:
5. Control:
6. Constants: