# AP Biology Lab: Enzyme Reaction Rates Using Toothpickase

## Introduction:

Enzymes are proteins made by living cells. They act as catalysts and affect the rate of a chemical reaction. For example, the enzyme „amylase“ in your saliva speeds up the breakdown down of starch (the substrate) into simple sugars. The enzyme itself does not get used up during the chemical reaction.

This lab will serve as a **pre-lab activity** for **AP Biology Lab #2 on enzyme catalysis**. In this activity you will become the enzyme called "toothpickase", which breaks toothpicks (the substrate) in timed intervals. Calculations will be completed to determine how many toothpicks are broken per second to determine the rate of reaction of the enzyme toothpickase.

## Materials:

- Flat Toothpicks (250)
- Twist-ties from garbage sacks

## Time Required:

- 30 minutes

## Procedure:

The scientific vocabulary of an enzyme reaction will be modeled with your hands and toothpicks. The **enzyme** will be your hands with a particular shape – it demonstrates the **quaternary structure** with the two subunits coming together. The **active site** is the portion of the thumb and index finger which form a space within which the toothpicks fits. The **substrate** is the toothpick. A toothpick can be broken into two **products**.

The rate of the reaction will be measured by counting the amount of product produced, or by counting the amount of substrate remaining. There are many factors that affect the rate of an enzyme reaction. Break a toothpick.

1. Can a toothpick be broken faster?_____

2. Can it be broken infinitely faster?_____

3. Given a pile of toothpicks (substrate) and ideal conditions, it still takes some time for the enzyme to break the toothpick...that is the enzyme's **V-max**. If the room were filled with toothpicks, the rate would not increase, at least initially.

4. Would it take longer to break the toothpick if it was across the room on the floor?_____. This would be **lower substrate concentration**.

5. Would it take longer to break the toothpick if it was surrounded by look-a-likes?_____. These would be **competitive inhibitors**.

6. What would happen to the amount of time to break toothpicks if two people were breaking at the same time? _____ This would be enzyme **concentration**.

**For the next section, work with your lab partner.**

1. Select a pile of approximately 250 toothpicks.
2. Divide them into six piles of 40 toothpicks each, with a few left over.
3. Obtain a few "competitive inhibitors" (twist ties or another similar object).
4. Blindfold the person who will be acting as the toothpickase enzyme.
5. Using a new pile of 40 toothpicks each time, break the toothpicks for six selected time intervals:
   **0 sec., 10 sec., 30 sec., 60 sec., 120 sec.,** and **180 sec.**

6. Record your results in the attached data table noting time and toothpicks metabolized. (Data Table #1) Also count how many toothpicks remained in your stack of 40-this is the substrate remaining.
7. In Section #2, graph the number of toothpicks metabolized (M) over time (T) in seconds to establish the different rates of reactions in the different time intervals.
   - Do the rates change?_____
   - How can you conclude this?_____

8. Calculate the average rate of reaction at each of the time intervals using the formula for a slope. ( See attached #3):

   $$M_2\text{-}M_1 / T_2\text{-}T_1$$

9. An average initial rate of reaction is 0.7 toothpicks / sec. If taken to 180 seconds, the rate of reaction for the interval between 120 and 180 seconds is almost always 0 toothpicks / sec. When you are breaking toothpicks for 60 seconds and you run out of toothpicks, you have reached the point that your rate will decrease because it becomes harder to find toothpicks to break. This is one of the factors that affects en enzyme.

   Explain this concept using the example of the reaction rate of **salivary amylase enzyme** in your mouth when you eat a piece of bread.
   _____
   _____
   _____
   _____

10. Repeat the above experiment using the 30 or 60 second time intervals only, but **alter** the conditions this time. Choose from the list below: You may try as many as time allows. Compare these conditions to your first original 60 sec. reading.

    Begin with 40 toothpicks each time:

    **A. Put your hands in ice water for a minute before trying to break toothpicks.**
    What happened to your time vs. amount of substrate broken down?
    _____
    What did you simulate?_____

    **B. Break into groups and all should break the 40 toothpicks at once.**
    What happened to your time vs. amount of substrate broken down?
    _____
    What did you simulate?_____

**C. Add many competitive inhibitors to your stack.**
What happens to your time vs. amount of substrate broken down?

_____

What did you simulate?_____

**D. Denature your enzyme by crossing your fingers while you break.**
What happens to your time vs. amount of substrate broken down?

_____

What did you simulate?_____

**E. Start off with fewer toothpicks.**
What happen to your time vs. amount of substrate broken down?

_____

What did you simulate?_____

**F. Search for scattered toothpicks around the room.**

**G. Your ideas?**

_____

_____

_____

**Summary:**
In a paragraph below, summarize in detail, what you learned about enzymes, using scientific concepts and terms.

**Data Table #1:**

Record the data from you experiment with toothpickase in the following table:

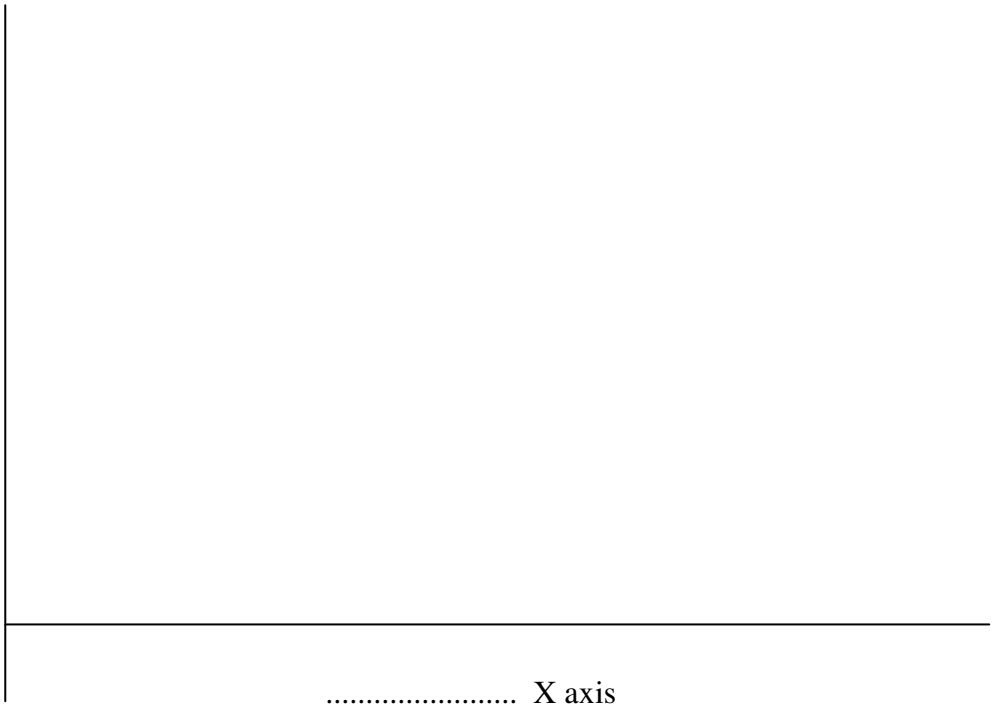
| Time (in sec.) | Toothpicks metabolized |
|---|---|
| 0 | |
| 10 | |
| 30 | |
| 60 | |
| 120 | |
| 180 | |

**Section #2: Graph**

Title _____

Y axis

……



..... X axis

Calculations #3

Calculate the initial and final rates of the reaction using the following formula: $M_2$-$M_1$/ $T_2$-$T_1$
Be sure to show your formula's setup to receive credit.