Aydin Seifi 2025 - 2026 CYSF Logbook

Project Topic: Analyzing the Effects of Temperature, pH and Inhibitors on Catalase Activity and Stability for Food Preservation and Industrial Applications

Discuss how pH might affect the enzyme's thermal stability. Does catalase denature *faster* at pH 4 when heated compared to pH 7?

Timeline

12/08/2025: Made a plan for the online section of the Project

l=long, Long= The longest Sections

Section	Depth	Work Dates
Hypothesis	**Long**	**Dec 8 – Dec 20**
Research (background + molecular + industrial)	**Long**	**Dec 21 – Jan 10**
Variables	short	**Jan 11 – Jan 13**
Procedure (precise steps + measurement reliability plan)	short	**Jan 14 – Jan 18**
Observations (experiment trials, photos, notes)	**long**	**Jan 19 – Jan 31**
Analysis (graphs, reaction rates, trends)	**Long**	**Feb 1– Feb 14**
Conclusion (normal start → deepen later)	**long → Long**	**Feb 14 – Feb 23**
Applications (expanded, not one paragraph)	**long**	**Feb 24 – Feb 27**
Sources of error + reliability improvements	short	**Feb 28**
Final polish, proofreading, submission formatting	**long**	**Mar 1 – Mar 2**

Section	Depth	Work Dates
SUBMISSION	—	**Mar 2**

12/21/2025: Finished hypothesis
12/23/2025: Started work on the research section of proteins
1/25/2026: Finished Research Section
1/27/2026: Changed manipulated variables, from 3 sources of inhibitors to only one, ethanol.
2/13/2026: Completed all trials in one day

- Changed a strategy in the experiment to save time while maintaining accuracy, and conducted two tests at once

2/14/2026: Begin to record observations in the platform
2/14/2026: Finished observations section
2/15/2026: Began work on the analysis
2/21/2026: Completed Analysis
2/22/2026: Began work on Conclusion.

Brief background Research (to adequately formulate my hypothesis)

- Proteins are essential macromolecules in the human body that are composed of amino acids, of which there are 20
- They perform a wide variety of functions, and our body relies on them for specific tasks.
- Enzymes are a specific type of protein that are catalysts, as in speeding up the reaction rate of essential life reactions, which would otherwise occur too slowly.
- They work by building specific substrates (reactant molecules) at an active site, and the active site works to position the substrate to weaker bonds, allowing the reaction to happen.
- Using this knowledge, we infer that the enzyme must be specific to the reactant molecule, which is indeed the case.
- They only bind to specific substrates in certain reactions
- Enzymes are not used up during a reaction; furthermore, they are unchanged and remain reusable after a reaction has completely taken place
- Catalase is a specific protein which is found in almost all living things
- It is crucial in the protection of a cell, because it specializes in breaking down harmful hydrogen peroxide (H_2O_2) into harmless hydrogen and oxygen ($H_2O + O_2$)
- This has a balanced reaction equation of $2H_2O_2 \Rightarrow 2H_2O + O_2$
- Hydrogen peroxide is a toxic by-product of normal cellular metabolism
- Catalase is very effective, with one of the highest turnover rates of any enzyme
- Natural enzyme inhibitors are compounds which block enzyme activity to a certain extent
- They work by blocking the substrate-binding process.
- In general, an increase in temperature of an enzyme speeds up the reaction because it increases the molecular kinetic energy.

- Denaturation is when proteins lose their 3-dimensional structure and essentially fall apart.
- This is what could happen if the temperature exceeds the optimum
- The effects of temperature on catalase, precisely on catalase, remain to be tested
- Each enzyme has its own optimal pH, while some carry out their function while at a neutral pH of 7, others are adapted to more acidic or alkaline environments
- Otherwise, substrate binding can be drastically reduced, and denaturation may occur.
- Catalase is mainly found in red blood cells and the liver
- Natural enzyme Inhibitors are substances that bind to an enzyme and block its activity
- It does this by binding to the active site of the enzyme, hence not allowing the substrate to bind
- Another way that the enzyme inhibitor hinders this process is when the inhibitor binds to another site so that the enzyme's catalysis is blocked.
- They can bind reversibly or irreversibly.

Procedure Draft

1) Gather needed materials to experiment:
    - Sweet Potatos - 5 - 650g-750g
    - Blender
    - 3% hydrogen peroxide - 400ml
    - Distilled water - 1.5 litres
    - Scale
    - Filter Paper
    - Liquid Soap (Surfactant) - One small bottle - 3 drops per test tube
    - Ethanol 100ml
    - Buffer solutions of varying pH - 120 ml of each
    - Identical Reaction Containers
    - Thermometer
    - Water baths of varying temperatures to heat the hydrogen peroxide
    - Stopwatch
    - Ruler
    - Camera
    - Safety Goggles and Gloves
2) Prepare Catalase Extract
    - Peel and cut the potato into 400 g.
    - Measure constant mass
    - Blend with measured volumes of distilled water (400ml) for exactly 30 seconds.
    - Filter mixture
    - Keep this master stock in a chilled water bath at about 5 degrees Celsius for the entire duration of trials.
3) Set up the reaction containers.

    - Label identical containers according to temperature, pH level, or ethanol treatment.
    - Ensure all containers are the same size and shape.
4) AddEqual amounts of Potato extract to each
    - 10 ml of master stock into the cylinder
    - 3 drops of soap
    - Add 10 ml variable if necessary
    - Measure the initial mass
5) Start the reaction by adding hydrogen peroxide at a consistent rate
6) Measure Catlase activity using foam, at 10, 30, and 60-second intervals
7) Repeat two more times with identical steps and Record

Rate of Activity = (Volume after reaction)- (Initial Volume)/60 seconds

=> to get an index of the activity

Materials needed:

Catalase Experiment Shopping List

- **Sweet Potatoes:** 5–6 medium potatoes (approx. 750g total)
- **Distilled Water:** 1.5 Litres
- **3% Hydrogen Peroxide:** 400 mL
- **pH Buffers:** 120 mL each of pH 4, pH 7, and pH 10
- **Ethanol (70%):** 100 mL
- **Liquid Dish Soap:** 1 small bottle
- **Graduated Cylinders:** 3 (100 mL capacity)
- **Filter Paper:** 1 pack of coffee filters or lab-grade filter paper
- **Digital Scale:** Must measure in grams (0.1g precision preferred)
- **Thermometer:** Digital or alcohol thermometer
- **Stopwatch:** Handheld or digital
- **Safety Gear:** 1 pair of safety goggles and 1 box of disposable gloves
- **Blender:** For creating the homogenate
- **Ruler:** For measuring foam height if cylinders aren't graduated
- **Camera:** For documenting your trials and final board display

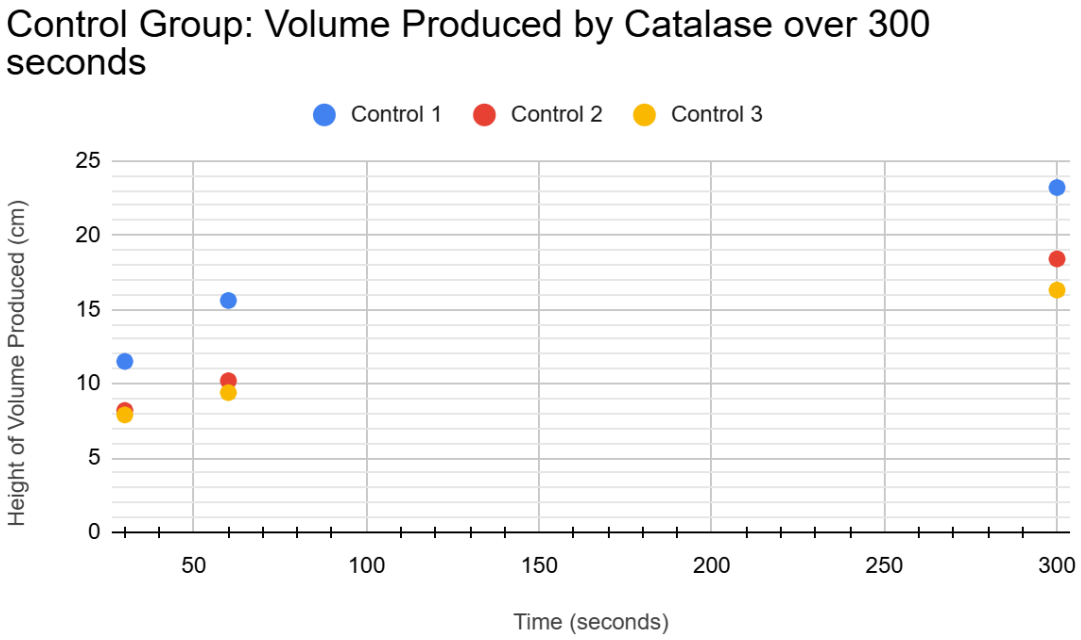
1. Gather required materials to experiment:
    - 5 sweet potatoes: 650g-750g
    - Blender
    - 3% hydrogen peroxide: 400.0 mL
    - Distilled water: 1.5 litres
    - Scale
    - Filter Paper
    - Liquid Soap (as Surfactant): 3 drops per test tube
    - Ethanol: 100.0 mL
    - pH buffers for pH values 4, 7, 10: 120.0 mL of each
    - Identical Reaction Containers (100.0 mL, graduated cylinder)
    - Thermometer
    - 4 Water baths of temperatures 5, 14, 37, and 60 degrees Celsius
    - Stopwatch
    - Ruler
    - Camera
    - Safety Goggles and Gloves
    - Pipettes
2. Peel and cut Sweet Potatoes to a mass of 400.0 grams.
3. Blend 400 grams of the mass achieved above with 400.0 mL of distilled water for exactly 30 seconds.
4. Filter this mixture through filter paper once.
5. Keep the outcome in the water bath at five degrees Celsius as the master enzyme stock. This is done for uniformity of the catalase source, ensuring accuracy.
6. Label reaction containers according to temperature, pH, ethanol treatment, or control (Ensure all containers are the same shape and size).
7. Place the calculated amounts of hydrogen peroxide for each temperature level into the corresponding water baths to heat or cool to the desired temperature.
8. Pipette 10.0 mL of the master stock, three drops of liquid soap, 10.0 mL of pH buffer or ethanol (if applicable) and 10.0 mL of distilled water into the reaction cylinder.
9. Measure exactly 10.0 mL of 3% hydrogen peroxide into a separate, clean test tube to keep the substrate isolated during the heating process (temperature as manipulated variable test).
10. Submerge both the reaction cylinder and the substrate test tube into the designated water bath for ten minutes to get to thermal equilibrium. (temperature as a manipulated variable test).
11. Pour the pre-heated hydrogen peroxide into the reaction cylinder to start the enzyme-substrate interaction at the target temperature.
12. Start the stopwatch immediately upon contact to record the foam volume at 10, 30, and 60-second intervals for precise data collection.
13. Repeat two more times to guarantee accuracy, yielding 36 tests in total. (3 control, 3 inhibitor, 3 pH, 3 temperature x3)

Temperature levels:

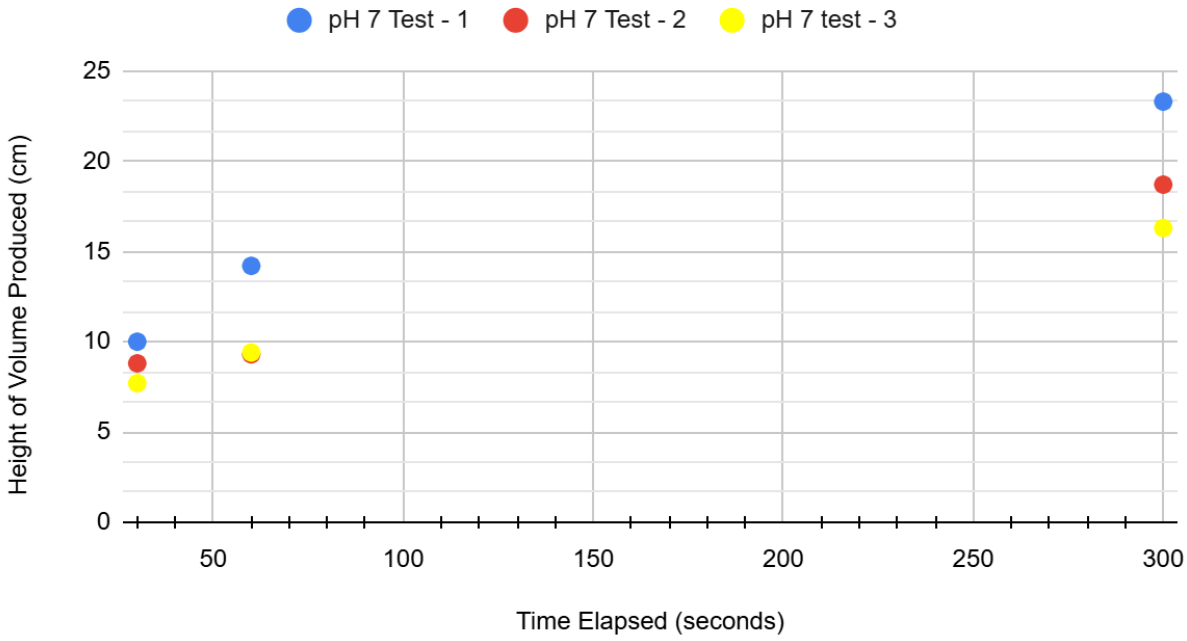
37, 60, 14

Analysis Graphs

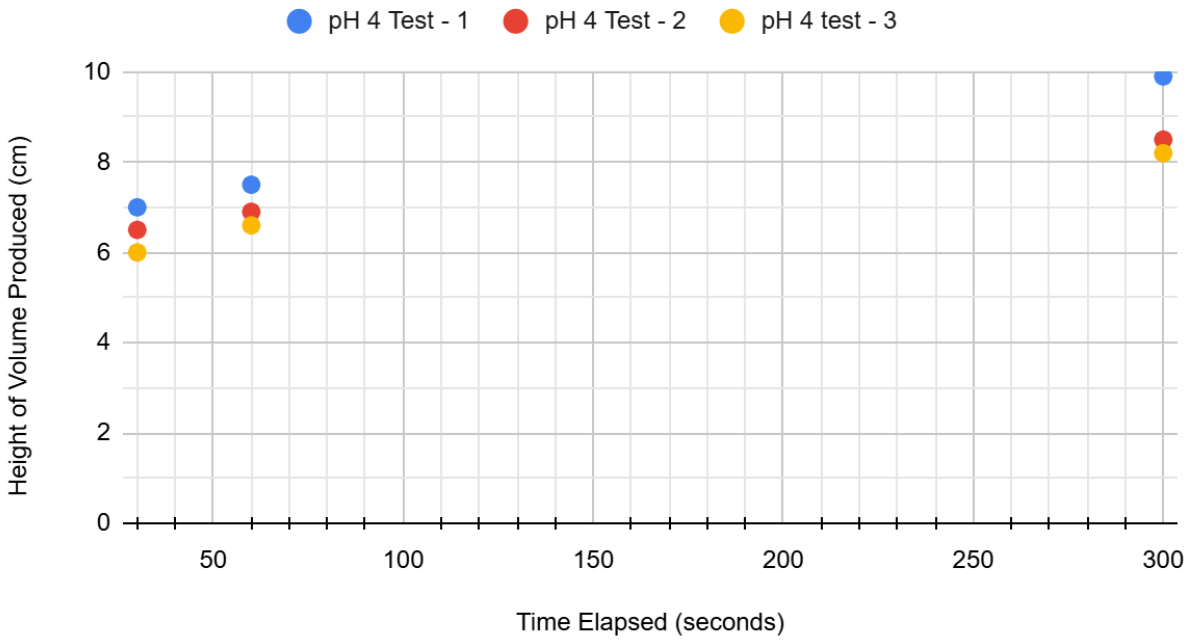
Controls



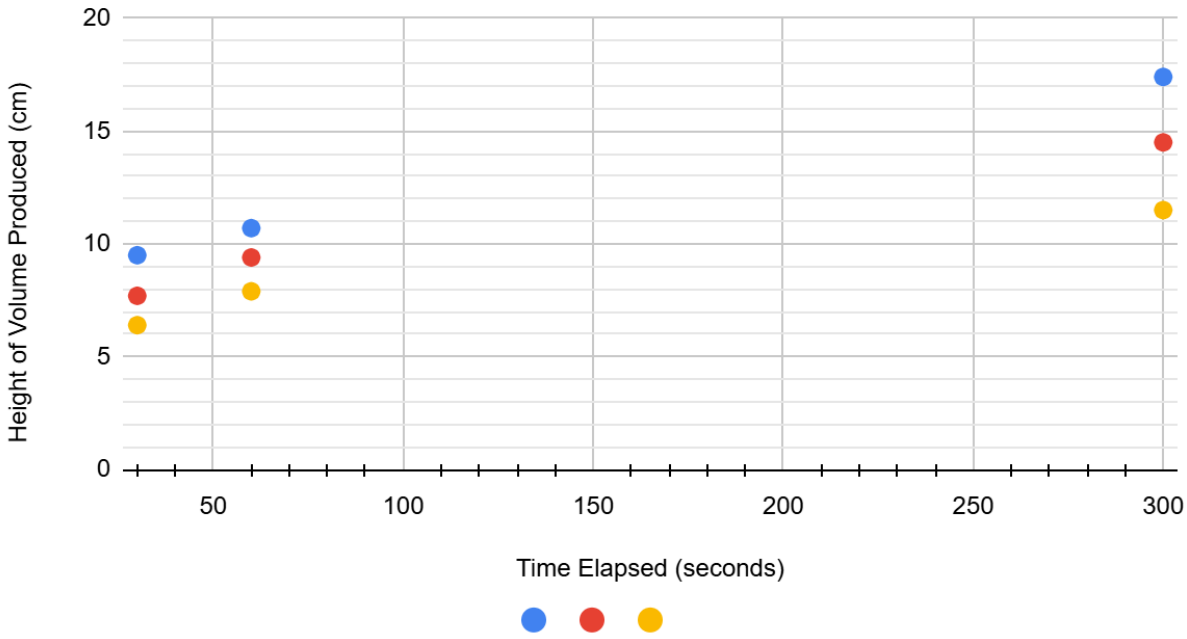
pH 7 Group: Volume Produced by Catalase over 300 seconds



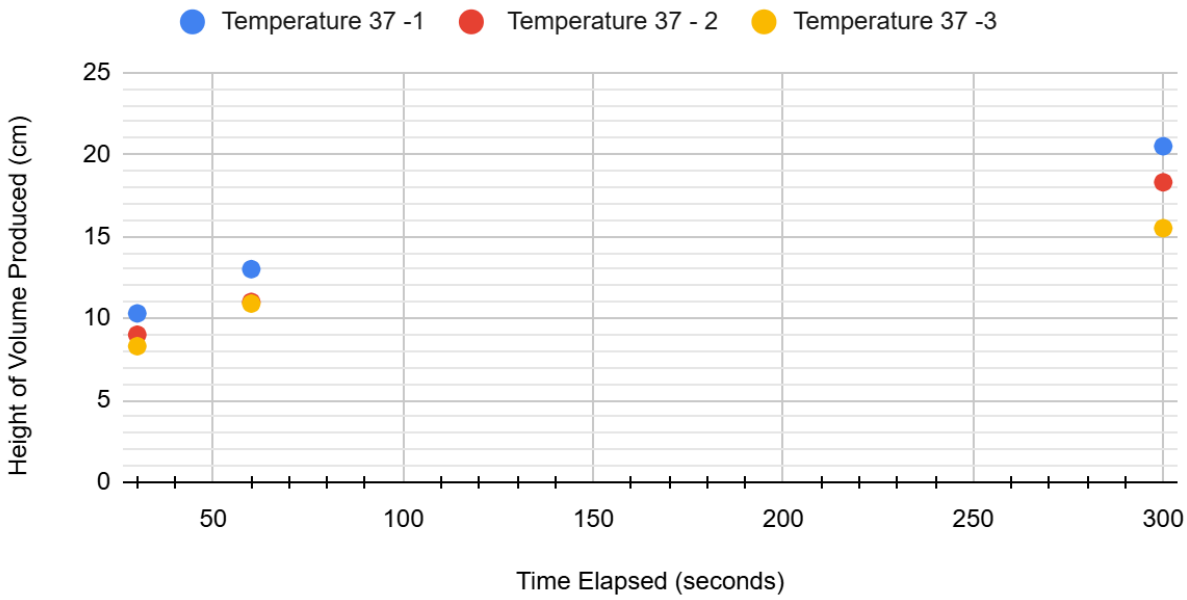
pH 4 Group: Volume Produced by Catalase over 300 seconds



pH 10 Group: Volume Produced by Catalase over 300 seconds



Temperature 37°C Group: Volume Produced by Catalase over 300 seconds



Temperature 60 Group: Volume Produced by Catalase over 300 seconds.