How does Temperature Affect Catalase Function in Hydrogen Peroxide?

Background Information: Enzymes are organic catalysts made of proteins*,***,*****. The substrates, which are the substances an enzyme work on, bind to the enzyme's active site*. Increasing kinetic energy in the form of heat allows more successful collisions with substrates*, and the rate of reaction would increase to an optimum temperature. After the optimal functioning temperature is reached, catalase denatures*,***, *****, and its active site changes shape. The substrate can no longer bind to the active site. Catalase is an enzyme that breaks down hydrogen peroxide (H_2O_2) into oxygen and water to prevent it from accumulating in cells and damaging its organelles*.

Research Question: What is the optimum functioning temperature of catalase in hydrogen peroxide? In this experiment, the aim is to determine the optimum functioning temperature for catalase in H_2O_2, where the rate of reaction is at its highest. Catalase has been known to oxidise toxins** other than H_2O_2 as well. Finding the optimum functioning temperature is of importance as applications of catalase in medicine requires the fastest response in breaking down toxins like methanol and formic acid**.

Hypothesis: If incubation temperature increases, the time taken for the filter paper disks to rise would decrease to a minimum at catalase's optimum functioning temperature, then increase.

Variables
Independent variable: Incubation temperature (°C)
Dependent variable: Time taken for filter paper disks to rise (seconds)
Controlled variables: Same pH, same volume of substrate (H_2O_2), same period of incubation time.

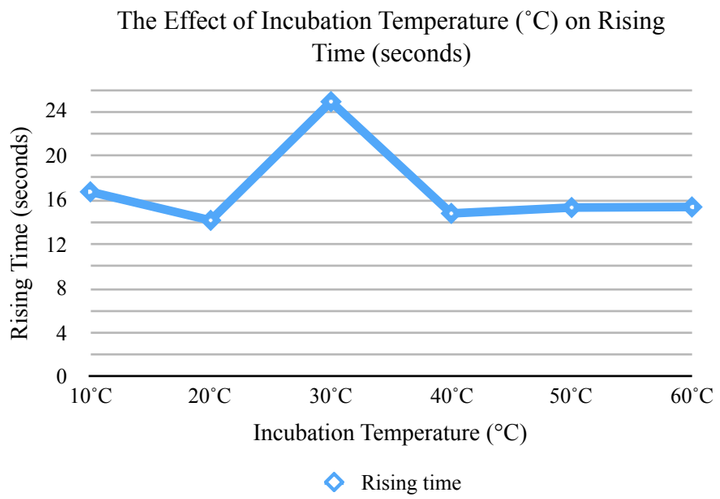
Safety Considerations: Wear protective clothing i.e. goggles, labcoat, gloves (to prevent catalase acting on skin), Not standing too close/touching water baths at high temperatures

Materials:
1. 24 test tubes
2. 18 filter paper disks
3. 360mL of hydrogen peroxide
4. Forceps
5. Stirring rod
6. 12mL of catalase solution
7. Water baths (10˚C, 20˚C, 30˚C, 40˚C, 50˚C, 60˚C)

Method
1. 2mLs of catalase solutions were collected in a test tube
2. The test tubes were incubated at each incubation temperature for 10 minutes.
3. The catalase solution incubated at 10˚C was poured into a petri dish and 3 filter paper disks were dipped in the solution.
4. 1 disk was dropped into 20mLs of H_2O_2 solution in a test tube.
5. Once the disk reached the top of the solution time time was recorded.
6. Steps 4 and 5 were repeated 2 more times.
7. Steps 3-6 were repeated for each remaining incubation temperature (20˚C, 30˚C, 40˚C, 50˚C, 60˚C)

Raw Data Table for Filter Paper Disk Rise Time

	10˚C	20˚C	30˚C	40˚C	50˚C	60˚C
Trial 1 (sec)	20.94	23.09	23.56	15.85	18.83	20.18
Trial 2 (sec)	18.96	8.43	26.22	21.62	13.55	10.56
Trial 3 (sec)	10.33	10.96	(Invalid)	6.89	13.57	(Invalid)
$\bar{x}$	(20.94+18.96+10.33)/3 = 16.74	(23.09+8.43+10.96)/3 =14.16	(23.56+26.22)/2 = 24.89	(15.85+21.62+6.89)/3 =14.79	(18.83+13.55+13.57)/3 = 15.32	(20.18+10.56)/2 = 15.37



Discussion
What is the trend observed: The general trend observed is that as incubation temperature increases from 10°C to 20°C, average rising time decreased from 16.74 seconds to 14.16 seconds. The value for 30˚C (24.89 seconds) was considered anomalous, as discussed below. As the temperature continues to increase from 40˚C to 60˚C, the average rise time increased from 14.79 seconds to 15.37 seconds.
Why was the trend observed: As incubation temperature increases, increased kinetic energy allows for more successful collisions between catalase and hydrogen peroxide therefore the rate of reaction increases*, ***,*****. This is seen as a decrease in rising time from 10˚C (16.74 seconds) to 20˚C (14.16 seconds), the lowest rise time out of all 6 incubation temperatures. At 30˚C, the anomalous data could be explained by room temperature fluctuations and/or uneven distribution of catalase enzyme. From 40˚C to 60˚C, the 0.58 seconds increase (from 14.79 seconds to 15.37 seconds) in rise time could be explained by catalase denaturing due to heat. Its tertiary structure is being denatured, and the active site can no longer bind to H_2O_2 molecules effectively due to its changed shape (no longer complementary) *,***,*****.
Are there anomalies observed: Yes, at 30°C, the average rise time was 24.89 seconds, where it is expected that the rise time would decrease from 14.16 seconds for 20°C due to increasing kinetic energy. Also, at 60˚C, it was expected that the rise time would be higher than the rise time at 10˚C because the catalase's tertiary structure is being denatured by heat beyond its optimum functioning temperature*,***,*****, decreasing the rate of reaction and raising the rise time. However, at 60˚C the rise time is 15.37 seconds compared to 16.74 seconds at 10˚C.
What are potential sources of error that caused the anomalies, what are their effects and how to minimise their effect on results: The incubated catalase solutions, once taken out of the water baths, was left to sit at room temperature at different amounts of time before they were used in the trials. This may have impacted the results for 10˚C and 20˚C, as room temperature was higher than the incubation temperature, causing rate of reaction to increase due to increased kinetic energy and causing the disk rise times to decrease.

When the disks were dipped into the catalase solution and patted dry with paper towels, the time elapsed wasn't uniform across all temperatures. This caused some disks to have more catalase soaked from them than others, which causes rate of reaction to increase or decrease as number of available catalase active site increases or decreases. To minimise the effects, it is advisable to time how long the disks have been left on the paper towel.

Although theoretically catalase's optimum functioning temperature is 40˚C and will start to denature after 60˚C, the rise time didn't increase sharply. As the catalase used in the experiment came from fungi****, their tolerance range might be wider than the tolerance range for humans, and the rate of reaction won't decrease as sharply as expected. To make the effects of denaturation due temperature more obvious, it is advisable to utilise mammalian catalase.

Conclusion
The hypothesis that rise time would decrease to a minimum value at the optimum temperature for catalase, then increase again as incubation temperature increases was partially supported. The optimum temperature for catalase function was determined to be 20˚C, when the average time taken for the catalase-soaked disk to rise is at 14.16 seconds. At 30°C, an anomalous result was observed (24.89 seconds), possibly caused by fluctuations in room temperature and/or uneven distribution of catalase on the filter paper disks. Sources of error were identified, and improvements such as uniform timing were suggested to minimise their effects on experimental results.

Bibliography
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