PROTOCOL

INVESTIGATING CATALASE ACTIVITY

Using a gas syringe to measure the effect of substrate concentration on catalase activity

Catalase is an enzyme found in a wide range of organisms, including yeast, catalysing the degradation of hydrogen peroxide to oxygen and water. There are a variety of methodologies to measure catalase activity. In this protocol, a gas syringe is used to capture and measure the oxygen gas evolved as a result of enzyme activity. The independent variable in this particular protocol is substrate concentration; however, a range of options are available including the effect of pH (by introducing buffers), effect of inhibitors (e.g. copper sulfate), effect of enzyme concentration and the effect of temperature.

Curriculum links:

The use of a gas syringe makes this protocol more relevant to Advanced Higher Biology learners. This protocol could be used to explore the following key areas:

- Cells & Proteins:
  - Key Area 1a: Laboratory techniques for biologists - Health and safety
  - Key Area 1b: Laboratory techniques for biologists - Linear dilutions
  - Key Area 1b: Laboratory techniques for biologists - Use of buffers to control pH
- Investigative Biology:
  - Key Area 1a: Scientific principles and process - Scientific method
  - Key Area 2: Experimentation

Assessment Task: Project

AH Advanced Higher Course Specification

SQA

Advanced Higher Biology

BACKGROUND

In this investigation, a suspension of *S. cerevisiae* is prepared and combined with various concentrations of hydrogen peroxide in a 1-armed conical flask with continuous mixing. A delivery tube connected to a gas syringe allows the capture and measurement of oxygen gas evolved during the degradation of hydrogen peroxide. The crystal structure of catalase from *S. cerevisiae* has been determined (Figure 1), revealing that it exists as a tetramer (quaternary structure) with strong similarity to catalases from distantly related organisms.

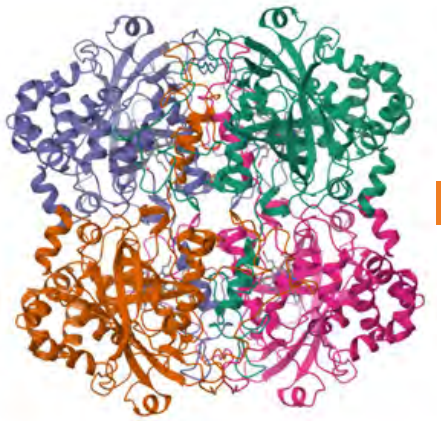


Figure 1. The crystal structure of catalase from *S. cerevisiae*, published on the RCSB Protein Data Bank (entry 1A4E).

AIM

To investigate the effect of substrate concentration on catalase activity

Null hypothesis: changing substrate concentration will have no effect on catalase activity.

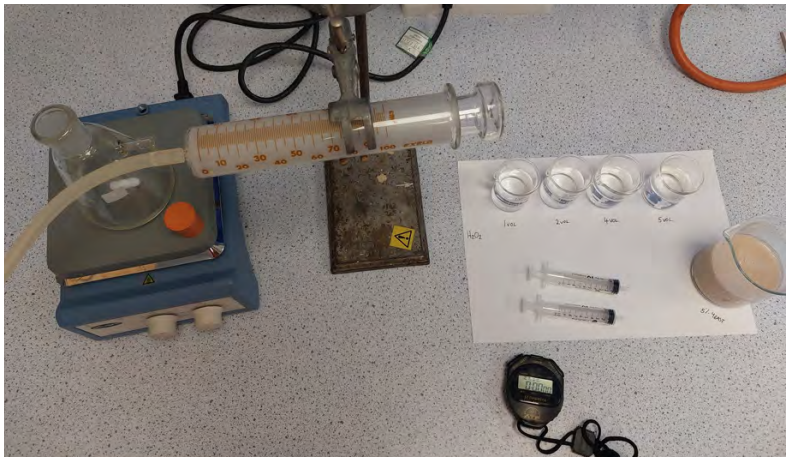
HEALTH AND SAFETY

When starting an experiment with a class, you must ensure you are comfortable and familiar with the risk assessment. What are the hazards, the level of risk and control measures put in place? Is the current risk assessment appropriate for your class? Do you need to make adjustments. This page of the SSERC website provides a Risk Assessment template and information about Dynamic Risk Assessments.
Click the download icon to view our model risk assessment for this activity.

PREPARATION OF MATERIALS

Materials required per group (for triplicate readings):

- 120 cm^3 5% yeast suspension
- 24 cm^3 20 vol H_2O_2
- Retort stand with clamp
- 100 cm^3 gas syringe (e.g Timstar, product SY14858)
- Magnetic stirrer and flea
- 2x 10 cm^3 plastic syringes
- 250 cm^3 side-armed conical flask
- rubber bung to fit conical flask
- silicone tubing to connect flask and gas syringe
- stopwatch



Preparation of 5% yeast:

Each group will require 120 cm^3 - prepare 150 cm^3 to allow for errors.
Combine 7.5 g Baker's yeast with 150 cm^3 water and mix to form a suspension. A magnetic stirrer works well to achieve a suspension.

Preparation of hydrogen peroxide solutions:

Each group will require 30 cm^3 of each concentration. Prepare 40 cm^3 of each to allow for pipetting errors. For each working solution, a stock of 20 vol hydrogen peroxide is used. Example calculation below.

For 5 vol: Add 10 cm^3 stock + 30 cm^3 water
For 4 vol: Add 8 cm^3 stock + 32 cm^3 water
For 2 vol: Add 4 cm^3 stock + 36 cm^3 water
For 1 vol: Add 2 cm^3 stock + 38 cm^3 water

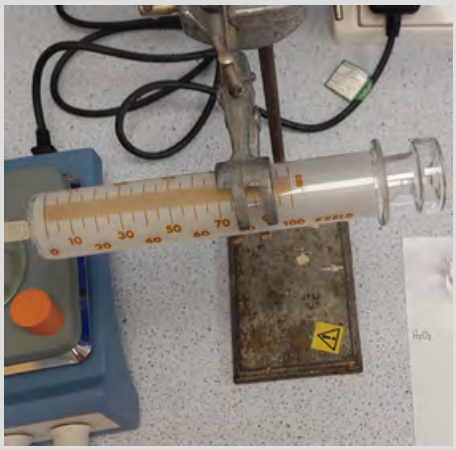
20 Vol stock → 5 vol final
4× dilution
×10 [1 part stock + 3 parts water = 4
10 cm^3 stock + 30 cm^3 water = 40 cm^3 required] ×10
→ Gives 40 cm^3 5 vol H_2O_2

Note about range of substrate concentrations

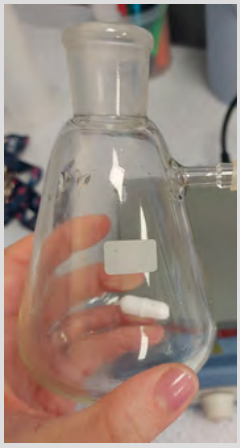
The range of hydrogen peroxide concentrations from 1 vol to 5 vol are recommended if using a 100 cm^3 gas syringe. Concentrations above this can yield volumes of oxygen greater than the capacity of the gas syringe.

METHOD

1. Mount the gas syringe in the retort stand.

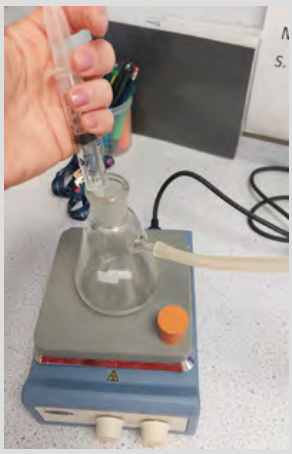


2. Add a magnetic flea to the conical flask and mount the flask on the magnetic stirrer.



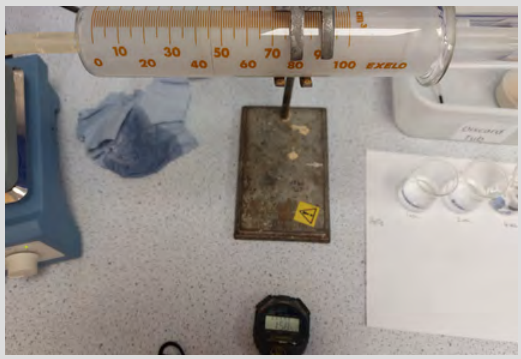
3. Attach the silicone tubing to the conical flask and gas syringe.

4. Using a 10 cm^3 syringe, add 10 cm^3 1 vol H_2O_2 to the conical flask. Set the stirrer to a slow speed.



5. Reset the stopwatch. Use the second 10 cm^3 syringe to add 10 cm^3 5% yeast to the conical flask. Immediately insert the bung into the neck of the conical flask and start the stopwatch.

6. After 2 minutes, record the volume of oxygen collected into the gas syringe. Remove the rubber bung and rinse out the contents of the conical flask down the sink. Repeat the experiment with the remaining substrate concentrations.



A quick video guide on how to carry out this full protocol is available by clicking on the video button.

RESULTS

Record the volume of oxygen gas collected in a table similar to the one below. The concentration range of hydrogen peroxide is recommended based on a 100 cm^3 gas syringe. Concentrations greater than 5 vol may yield volumes of oxygen greater than the capacity of the syringe.

Concentration of H_2O_2 (vol)	Volume of oxygen gas collected (cm^3)			
	1	2	3	Average
1				
2				
4				
5				

SAMPLE RESULTS

The following results were obtained for this experiment. The mean values were plotted as a line graph.

Concentration of H_2O_2 (vol)	Volume of oxygen gas collected (cm^3)			
	1	2	3	Average
1	12	14	13.5	13.2
2	23.5	23	24.5	23.6
4	46	48	46.5	46.8
5	64	62.5	64	64