Investigating Enzyme Activity

Learning Outcome

- *5.1.5* – **Investigate** and **describe** the effect of changes in temperature and pH on enzyme activity with reference to optimum temperature and denaturation

Scientific investigation - Review

When conducting a biological investigation, we seek to answer a Research Question (RQ) which typically is framed as:

How does the **Independent Variable** (IV) impact / affect the **Dependent Variable** (DV) in **study species**?

- Independent Variable → variable being manipulated; includes control group and test groups
- Dependent Variable → variable being measured; includes unit of measure
- study species → the organism being studied; written as species name and (common)

ex RQ: How does the concentration of saltwater (0%, 1%, 2%, 3%, 3.5%) affect the growth of *Allium tuberosum* (garlic chives) as measured by change in height (cm) over 4 weeks?

- ✓ RQ specific and detailed
- ✓ Clear what is being investigated

* In addition to the IV and DV, **Control Variables** need to be considered

→ Variables other than IV that can impact DV and thus need to be accounted for

ex Controls: what else can impact plant growth? ✓ Temperature ✓ soil composition and volume ✓ light availability ✓ amount of water provided ✓ humidity ✓ condition of plant } all need to be kept constant to ensure only IV impacts DV

Measuring rates of chemical reactions

reactant (s) —enzyme→ product (s)

larger change = faster rate

measure how much reactant is being consumed over a given time period $\frac{\Delta \text{reactant}}{\text{time}} = \frac{\text{final} - \text{initial}}{\text{time}}$

measurement options for reactants:

→ % change in mass of reactant (if its a solid)

$\frac{\text{final mass} - \text{initial mass}}{\text{initial mass}} \times 100$

∴ how quickly mass changed used as rate

→ colour change using food tests:

as reactant is converted to product the reaction with a reagent will produce different colour

∴ how quickly colour change used as rate

measure how much product is being made over a given time period $\frac{\Delta \text{product}}{\text{time}} = \frac{\text{final} - \text{initial}}{\text{time}}$

measurement options for products

→ counting bubble formation (if product is gas)

∴ how many bubbles formed over time used as rate

→ water displacement (if product is gas)

∴ how much water displaced over time used as rate

→ change in pressure in sealed container (if product is gas):

pressure can be measured directly or

Δ pressure can cause a liquid to move (↑P to ↓P) and how quickly it moves can be used as rate

Data collection

In order to minimize random error and increase precision, more trials are conducted. (5 minimum)

→ more data collected per experimental groups reduces impact of potential outliers

ex: dataset 1: (8, 9, 0) $\bar{x} = 5.7$ — outlier reduced mean alot

dataset 2: (8, 9, 0, 7, 8, 9) $\bar{x} = 6.8$ — outlier reduced mean less

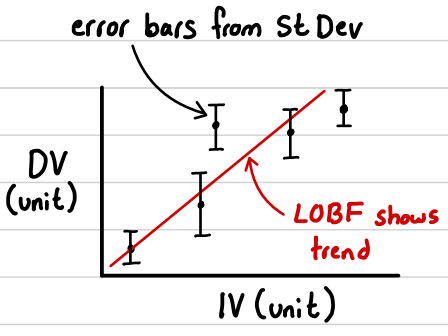
* In Biology, Standard deviation (St D) often used to assess variability

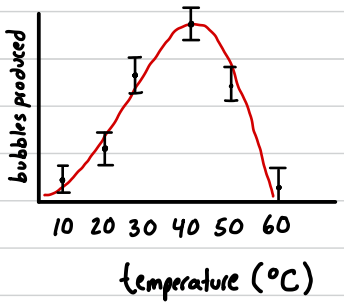
Data presentation

DV will be a continuous measure

IV will be what we are testing (i.e. temperature, pH)

continuous vs continuous = scatter plot





Investigating Catalase Action

Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen gas

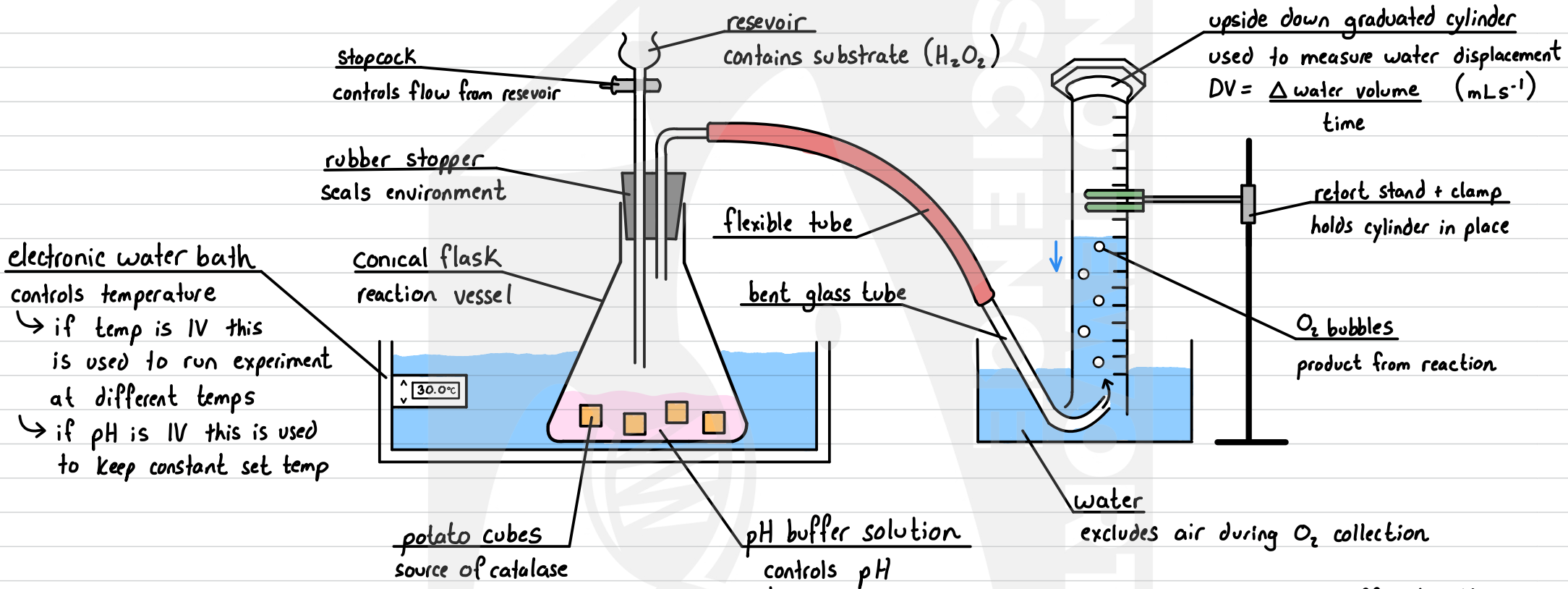
$$2\,H_2O_{2\,(l)} \xrightarrow{\text{catalase}} 2\,H_2O_{(l)} + O_{2\,(g)}$$

background: catalase is present in most cells as its role is to protect them from oxidative damage which can can cause toxic byproducts, like H_2O_2 to be formed and can harm cells

↳ a ready source of catalase is in potatoes

research question: How does pH (3, 5, 7, 9, 11) or temperature (20, 30, 40, 50 °C) impact catalase activity in potatoes as measured by water displacement from oxygen production over time?

experimental setup:



Method:

Part A: setup

1- measure 10mL of pH 7 buffer solution using graduated cylinder and pour into flask
2- cut a potato into several equal cubes using scalpel / knife (size is up to you, ex: 5mm³)
3- place 5 potato cubes into the flask
4- place rubber stopper snugly on flask
5- measure 5mL of H_2O_2 using graduated cylinder and pour into resevoir *ensure stopcock is closed
6- place flask into water bath set at 30°C *wait for temperature to reach this
7- fill container with water and place graduated cylinder as shown in setup above using retort stand and clamps
8- connect flask to cylinder using glass tubes and a rubber hose as shown above

Part B: data collection

1- record the water level on the graduated cylinder - this is 'initial volume'
2- start a timer (s)
3- open stopcock to allow H_2O_2 to enter flask *should see bubbles
4- after a set period of time (length up to you, ex: 180s), record the water level on the graduated cylinder - this is 'final volume'
5- repeat steps 1-4 several more times and calculate mean change in volume / time (mLs^{-1})
6- repeat Parts A & B but alter the IV → for pH, use a different pH buffer
→ for temperature, set water bath to another temperature

Assessment Tasks

After the investigation, complete the following:

*recommended program is Excel

(1) Write the data you collected into a data table. Data tables typically take this format:

Table 1: write caption here.

IV	DV (± uncertainty)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
A					
B ...					

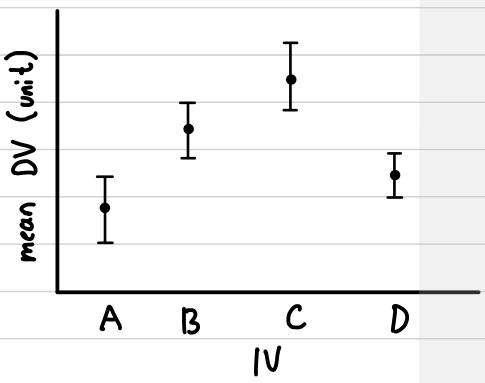
(2) Calculate the mean and standard deviation for your groups and present as a processed data table like below:

Table 2: write caption here.

IV	mean DV (unit)	St. Dev
A		
B...		

(3) Graph your data from your processed data table using a scatter plot. It may look something like this:

Graph 1: write caption here.



error bars are calculated from st. dev

(4) Analyze your data. what patterns do you see. Any overal trend? Any outliers?

(5) Discuss strengths and weaknesses/limitations for your investigation. How could it have been improved?