5.1.1 Enzyme experiments: turning times into rates, and explaining the curve

Name: _____ Class: _____ Date: _____ **Total: 36 marks**

KEY WORDS enzyme • temperature • rate • investigation • catalyst

Objective

Use enzyme-investigation data to calculate relative rates, describe temperature and pH curves, explain their shapes and identify variables. This sheet applies the enzyme action taught in 5.1. Keep the amount of substrate and the endpoint comparable when interpreting reaction times.

1 Key knowledge and worked examples

■ Enzyme action and the required explanation

An **enzyme** is a protein that acts as a biological **catalyst**: it speeds a reaction without being used up. Enzymes make metabolic reactions fast enough to support life. A **substrate** fits a complementary **active site**, forming an enzyme-substrate complex. Products leave and the enzyme can act again. Specificity depends on the matching shapes. **Denaturation** changes the active-site shape so the substrate no longer fits; an enzyme is not alive and cannot be "killed".

Turn a fixed-endpoint time into a relative rate

time falls ↓

time / s	rate = 1000÷ time
100	$1000 \div 100 = 10$
50	$1000 \div 50 = 20$
40	$1000 \div 40 = 25$

↓ rate rises

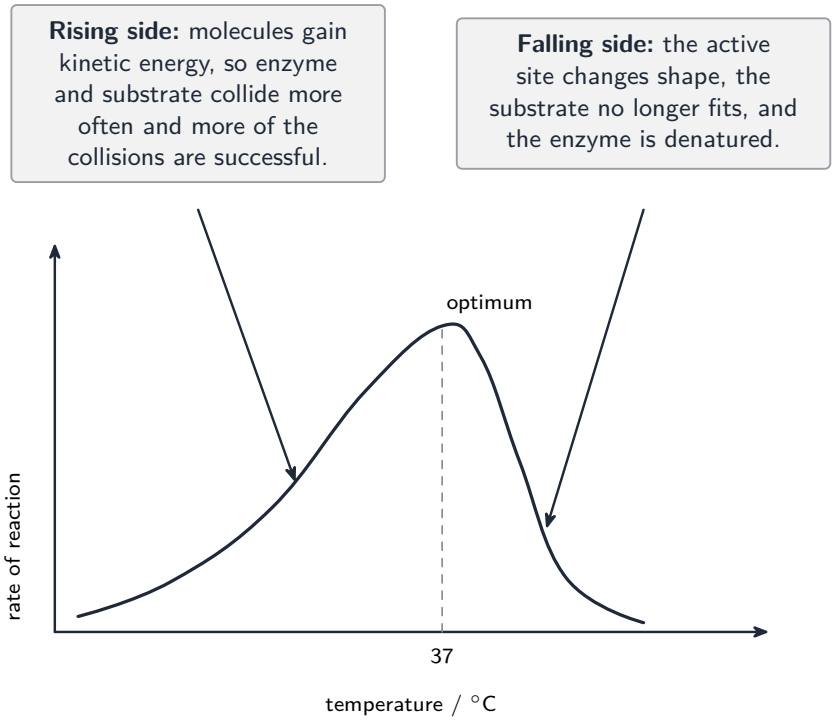
A shorter time always means a faster rate.
Never compare the times themselves.

For the same amount of reaction and the same endpoint, relative rate is proportional to $1/t$. This sheet scales it by 1000 for convenient numbers; it is not a universal rate unit.

$$\text{relative rate} = \frac{1000}{\text{time in s}}$$

Worked example: times of 100 s and 40 s give relative rates $1000/100 = 10$ and $1000/40 = 25$. The rate ratio is $25/10 = 2.5$. Equivalently, divide the longer time by the shorter, $100/40 = 2.5$. Subtracting gives a time difference, not a rate ratio.

Describe both sides, then explain them

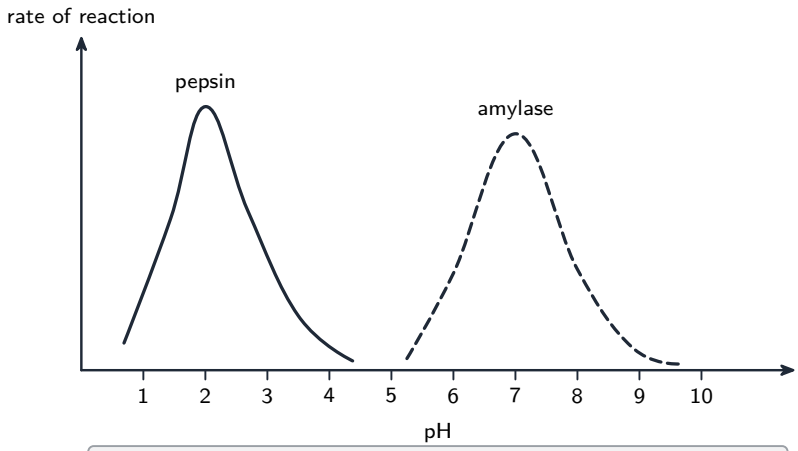


Describe: quote the axes and peak: the rate rises to its maximum near 37 °C in this graph, then falls towards zero near 60 °C.

Explain below the optimum: molecules gain kinetic energy, increasing successful enzyme-substrate collisions and complex formation.

Explain above the optimum: high temperature changes the active-site shape; the substrate no longer fits and the enzyme is denatured. The optimum is the temperature giving the highest measured rate; read it from the supplied graph.

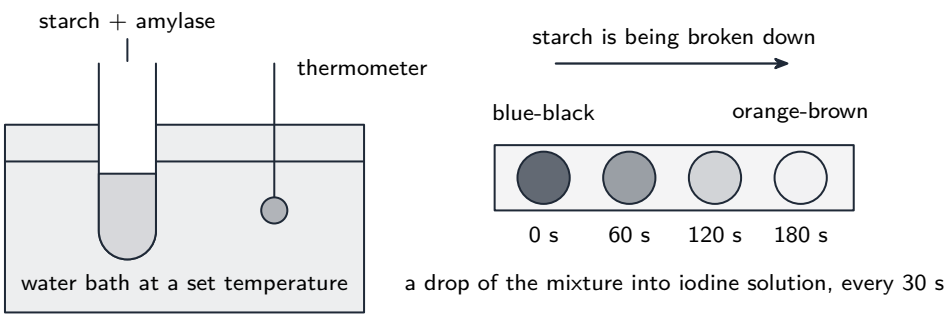
pH optima differ between enzymes



Each enzyme has its OWN optimum pH.
Away from it, on either side, the active site changes shape and the enzyme is denatured.

Pepsin in the stomach has an optimum near pH 2; amylase has an optimum near pH 7. Read each peak rather than assuming every enzyme prefers neutral conditions. Moving away from the optimum can affect active-site shape and fit; sufficiently extreme pH can denature the enzyme. Do not use the temperature explanation of increased kinetic energy to explain a pH change.

Plan a fair investigation



What you measure is a TIME: how long until iodine solution stays orange-brown, which is the moment the last of the starch has gone.

- **Independent variable** : deliberately changed, such as temperature or pH.
- **Dependent variable** : measured, such as time to an endpoint or product volume in a fixed time.
- **Control variables** : enzyme/substrate volumes and concentrations, and whichever of temperature or pH is not changed. Use a water bath to maintain temperature.

- Repeat measurements and calculate a mean. Repeats support reliability; they do not by themselves remove a systematic error.

reaction	measurement
amylase and starch	time until iodine stays orange-brown: starch is no longer detected
catalase and hydrogen peroxide	oxygen volume in a fixed time
pectinase and crushed apple	juice volume after a stated reaction time
protease and cloudy milk	time until the milk clears

2 Practice

Now use the methods above.

2.1 A student timed how long amylase took to break down all the starch at three temperatures.

temperature / °C	time taken / s
20	100
30	50
40	40

Calculate the rate of reaction at each temperature, using rate = 1000 ÷ time. [3]

2.2 State which temperature gave the fastest reaction, and explain how the times show this. [2]

2.3 Name the independent variable and the dependent variable in this investigation. [2]

2.4 State **two** variables the student must keep the same. [2]

2.5 Explain why the reaction was faster at 40 °C than at 20 °C. Use the words *kinetic energy* and *collisions*. [3]

2.6 The student repeated the experiment at 70 °C. After 10 minutes the iodine solution was still blue-black. Explain this result. [3]

3 Exam-style questions

3.1 An enzyme breaks down its substrate in 250 s at 15 °C and in 50 s at 35 °C. How many times faster is the reaction at 35 °C? [1]

- ☐ **A** 0.2
- **B** 5
- **C** 200
- **D** 300

3.2 Pepsin digests protein in the stomach. A student timed how long pepsin took to digest a piece of cooked egg white at four pH values.

pH	time taken / s
1	120
2	60
4	200
7	no change after 600

(a) Calculate the rate of reaction at pH 2 and at pH 4, using rate = 1000 ÷ time. Give each answer to 2 significant figures. [2]

(b) State the optimum pH of pepsin, and give the evidence from the table. [2]

(c) Explain why no protein was digested at pH 7. [2]

3.3 A food company uses the enzyme **pectinase** to get more juice out of crushed apples. A student investigated how temperature affects pectinase.

She put 20 g of crushed apple and 2 cm^3 of pectinase into each of six test tubes. She kept each tube at a different temperature for 20 minutes, then filtered the mixture and measured the volume of juice collected.

temperature / °C	volume of juice / cm^3
10	3.0
20	5.5
30	9.0
40	12.5
50	6.0
60	0.5

(a) Name the independent variable and the dependent variable. [2]

(b) State **two** variables that were kept the same. [2]

(c) Describe the results shown in the table. [3]

(d) Explain the results between 10 °C and 40 °C. [3]

(e) Explain why almost no juice was collected at 60 °C. [3]

(f) Suggest one change the student could make so that her results are more reliable. [1]

Solutions

Each answer shows the steps, then the **result**.

2.1

- divide 1000 by each time

$$\frac{1000}{100} = \mathbf{10} \qquad \frac{1000}{50} = \mathbf{20} \qquad \frac{1000}{40} = \mathbf{25}$$

- the units are "arbitrary units" here, because 1000 was only chosen to keep the numbers tidy

2.2

- **40 °C**
- it took the **shortest time** (40 s), and a shorter time means a faster rate

2.3

- independent variable: the **temperature**
- dependent variable: the **time taken for the starch to be broken down**

2.4

- any **two** of: the volume or concentration of amylase; the volume or concentration of starch; the pH; the volume of iodine solution used for each test

2.5

- at 40 °C the molecules have more **kinetic energy**, so they move faster
- enzyme and substrate molecules **collide more often**, and more of those collisions are successful
- so more enzyme-substrate complexes form each second and the starch is broken down faster

2.6

- 70 °C is far **above the optimum temperature** for amylase
- the heat has **changed the shape of the active site**, so starch no longer fits it
- the enzyme is **denatured**, and the change is permanent, so no starch was broken down and the iodine stayed blue-black

3.1 B

- turn each time into a rate, or divide the longer time by the shorter one

$$\text{how many times faster} = \frac{\text{longer time}}{\text{shorter time}} = \frac{250\ \text{s}}{50\ \text{s}} = \mathbf{5}\ \text{times faster}$$

- **A** is the division upside down, $50 \div 250$; **C** is the difference $250 - 50$; **D** is the sum $250 + 50$. None of those compares two rates.

3.2

(a)

- divide 1000 by each time

$$\text{pH 2: } \frac{1000}{60} = \mathbf{17} \qquad \text{pH 4: } \frac{1000}{200} = \mathbf{5.0}$$

(b)

- **pH 2**
- it gave the **shortest time**, 60 s, so the fastest rate; the times get longer on both sides of it

(c)

- pH 7 is far from pepsin's optimum pH, so the **shape of the active site has changed** and the protein no longer fits
- the enzyme is **denatured**, so no enzyme-substrate complexes can form

3.3

(a)

- independent variable: the **temperature**
- dependent variable: the **volume of juice collected**

(b)

- any **two** of: the mass of crushed apple (20 g); the volume of pectinase (2 cm^3); the time the tubes were left (20 minutes); the same apple variety; the same filter paper

(c)

- the volume of juice **rises** as the temperature rises from 10 °C to 40 °C, from 3.0 cm^3 to 12.5 cm^3
- the **maximum** volume is at **40 °C**
- above 40 °C the volume **falls sharply**, to only 0.5 cm^3 at 60 °C
- a description quotes figures off the table; it gives no reasons

(d)

- as the temperature rises the molecules gain **kinetic energy** and move faster
- pectinase and its substrate **collide more often**, and more of the collisions are successful
- so more of the pectin is broken down in the 20 minutes, releasing more juice

(e)

- 60 °C is far **above the optimum temperature** of pectinase
- the heat has **changed the shape of the active site**, so the substrate no longer fits
- the enzyme is **denatured**, so almost no pectin was broken down and almost no extra juice was released

(f)

- **repeat** each temperature at least three times and take a **mean** volume