## **Required Practical Review**



# Biology Practical - Investigate the effect of pH on the rate of an enzyme-controlled reaction

Free science lessons: <u>https://www.youtube.com/watch?v=JyXXoevEWc8</u> GCSEpod: <u>https://members.gcsepod.com/shared/podcasts/title/11567</u>

### Know it

- 1. Place one drop of iodine solution into each depression on the spotting tile.
- 2. Place labelled test tubes containing the buffered pH solutions, amylase solution and starch solutions in to the water bath
- 3. Allow the solutions to reach 30 °C
- 4. Add  $2cm^3$  of one of the buffered solutions to a test tube.
- 5. Use the syringe to place 2 cm<sup>3</sup> of amylase into the buffered pH solution.
- 6. Use another syringe to add  $2 \text{ cm}^3$  of starch to the amylase/buffer solution.
- 7. Immediately start the stop clock and leave it on throughout the test.
- 8. Mix using a glass rod.
- 9. After 10 seconds, remove one drop of the mixture with a glass rod. Place this drop on the first depression of the spotting tile with the iodine solution. The iodine solution should turn blue-black.



10. Use the glass rod to remove one drop of the mixture every 10 seconds. Put each drop onto the iodine solution in the **next** depression on the spotting tile.

Rinse the glass rod with water after each drop

Continue until the iodine solution and the amylase/buffer/starch mixture remain orange.

- 11. Repeat the procedure with solutions of other pHs
- 12. Record your results in a table such as the one here.

pH of solution	Time taken for amylase to completely break down the starch in seconds (s)

### 13. Plot a graph with:

- 'Time taken to break down starch (s)' on the y-axis
- 'pH of solution' on the x-axis

or

- 14. Calculate the rate of reaction and plot a graph with:
  - 'Rate of reaction' on the y-axis
  - 'pH of the solution' on the x-axis

### **Review it**

Complete the tasks below into your book.

### Up to grade 4

- 1. What is the independent variable in this reaction?
- 2. What is the dependent variable in this reaction?
- 3. What are the control variables? What must the students keep the same in order for this to be a fair test?

### Grade 5-7

- 4. What does the amylase break the starch down into?
- 5. Describe the lock and key theory.
- 6. How would the rate of this reaction alter if the solution was maintained at 15°C instead of 30°C?

### Grade 7+

- 7. Explain (in terms of bonds and enzyme structure) why altering the pH affects the rate of the enzyme activity.
- 8. Explain the term limiting factor.
- 9. Sketch a graph showing the rate of enzyme activity for the following: 1) increasing temperature 2) increasing substrate concentration. Explain why these trends occur.

### Test it

Answer the exam questions below into your book.

### Q1.

Catalase is an enzyme found in many different tissues in plants and animals. It speeds up the rate of the following reaction.

hydrogen peroxide <u>catalase</u> water + oxygen

Figure 1 shows a 25-day-old broad bean seedling.



Some students investigated whether different parts of bean seedlings contained different amounts of catalase.

The students:

- put hydrogen peroxide into five test tubes
- added a different part of a bean seedling to each tube
- recorded the results after half a minute.

If there was catalase in part of the seedling, oxygen gas was given off. When oxygen gas is given off, foam is produced in the tubes.

Figure 2 shows the results.



The students made the following conclusions:

- most parts of a bean seedling contain catalase
- the seed contains a lot of catalase
- stems and roots have quite a lot of catalase
- the leaves have a little bit of catalase
- the seed coat has hardly any catalase.

The students' teacher said that the students needed to improve their investigation in order to make valid conclusions.

(a) Describe how you would carry out an investigation to compare the amounts of catalase in different parts of bean seedlings.

You should include details of how you would make sure your results give a valid comparison of the amounts of catalase.

(6)

(b) Scientists investigated the effect of pH on the activity of the enzyme catalase in a fungus.

The table below shows the scientists' results.

рН	Enzyme activity in arbitrary units						
	Test 1	Test 2	Test 3	Test 4	Test 5	Mean	
3.0	0	0	0	0	0	0	
4.0	6	5	8	4	7	6	
5.0	38	65	41	42	39		
5.5	80	86	82	84	88	84	
6.0	100	99	96	103	102	100	
6.5	94	92	90	93	91	92	
7.0	61	63	61	62	63	62	
8.0	22	22	21	24	21	22	

(i) Calculate the mean enzyme activity at pH 5.0.

- (ii) On a **separate** piece of graph paper, copy and finish off the graph as below. Remember to:
  - add a label to the vertical axis
  - plot the mean values of enzyme activity
  - draw a line of best fit.



- (iii) At what pH does the enzyme work best?
- (iv) Predict the activity of the enzyme at pH 9.0.

(v) Suggest why the enzyme's activity at pH 3.0 is zero.

(1) (Total 15 marks)

arbitrary units

(1)

(1)

### Mark it

### Q1.

(a) Marks awarded for this answer will be determined by the Quality of Communication (QC) as well as the standard of the scientific response. Examiners should also refer to the information in the Marking guidance and apply a 'best-fit' approach to the marking.

### 0 marks

No relevant content.

### Level 1 (1-2 marks)

The method described is weak and could not be used to collect valid results, however does show some understanding of the sequence of an investigation.

#### Level 2 (3-4 marks)

The method described could be followed and would enable some valid results to be collected, but lacks detail.

### Level 3 (5-6 marks)

The method described could be easily followed and would enable valid results to be collected.

### Examples of the points made in the response:

- bean seedlings of same age
- cut material from same part of each organ (for repeats) e.g. top 1 cm of stem / a whole cotyledon / seed
- equal mass of each organ
- accept weight for mass
- grind / homogenise
- in equal amounts of water / buffer
- equal volumes of hydrogen peroxide solution
- equal concentrations of hydrogen peroxide solution
- same temperature
- temperature maintained in water bath
- quantitative measure of gas production eg height of foam in mm / collect gas in graduated syringe in cm<sup>3</sup>
- for same time period
- repetitions (3+ times)
- calculate mean for each.

#### (b) (i) correct answer: 40

1 mark for 45 as the anomalous result has been included in the calculation

#### or

#### (<u>38 + 41 + 42 + 39</u>) r <u>4</u>

**1** mark for

<u>160</u> or 4 6

<ul><li>(ii) vertical axis correctly labelled:</li><li>'Enzyme activity in arbitrary units'</li></ul>	
allow ecf from (b)(i)	1
points plotted correctly ±1 mm	
deduct <b>1</b> mark for each incorrect plot	2
suitable line of best fit	
not feathery, not point to point	1
(iii) 6.0 / 6	
allow $\pm 0.1$	
if 6.0 not given, allow correct for candidate's graph $\pm$ 0.1	1
(iv) in range 0 to 14 units	
allow correct for candidate's graph	1
<ul> <li>(v) enzyme denatured / enzyme (active site) shape changed</li> <li>allow substrate no longer fits (active site)</li> <li>ignore reference to temperature</li> </ul>	
do not allow enzyme dies	1

[15]