The effect of pH on the time taken by amylase to hydrolyse starch

Introduction

Amylase is an enzyme that catalyses the hydrolysis of starch. You will investigate the effect of pH on the rate of hydrolysis. The rate of reaction will be determined from how long it takes for starch to disappear from a reaction mixture. You will investigate the rate of reaction at pH 5.0, 6.0, 6.4, 7.0 and 8.0.

Materials

You are provided with the following:

- amylase solution
- starch solution
- buffer solutions at pH 5.0, 6.0, 6.4, 7.0 and 8.0
- iodine solution in a dropper bottle
- large beaker to use as a water bath
- thermometer
- 2 spotting tiles
- timer
- 10 test tubes
- 2 test tube racks
- syringes or pipettes measuring up to 2 cm³
- marker pen or labels
- dropping pipette
- eye protection
- source of hot water.

You may ask your teacher for any other apparatus you require.
Method

Read these instructions carefully before you start your investigation.

Wear eye protection at all times.

1. Set up a water bath at 30 ºC.
2. Label a test tube, pH 5.
3. Add 2 cm³ of amylase solution to this tube.
4. Add 1 cm³ of the pH 5 buffer solution to this amylase solution.
5. Add 2 cm³ starch solution to a second tube.
6. Put both test tubes into your water bath for 3 minutes.
7. Place a single drop of iodine solution into each well on the spotting tiles.
8. After 3 minutes, add the starch solution to the amylase and buffer solution and mix thoroughly. Immediately return the tube containing the mixture to the water bath and start the timer.
9. After 20 seconds, use the dropping pipette to place one drop of the mixture onto the first drop of iodine solution on the spotting tile. If starch is still present the mixture should turn blue-black. Return any untested mixture back into the test tube. Leave the pipette in the mixture in the test tube.
10. Wait another 20 seconds and remove a second drop of the mixture and test as you did in step 9.
11. Repeat step 10 until the blue-black colour fails to appear. Count how many wells of iodine solution have been used. Each one equals 20 seconds of reaction time. If blue-black colour is still present when all wells on both spotting tiles have been used, record the time and move on to the next pH.
12. Repeat steps 1 – 11 with buffers at pH 6.0, 6.4, 7.0 and 8.0. Make sure you rinse out the dropping pipette before you start your next pH.

You will need to decide for yourself:

- when the blue-black colour fails to appear.
ISA BIO3T/P13 Candidate Results Sheet: Stage 1

The effect of pH on the time taken by amylase to hydrolyse starch

Centre Number

Candidate Number

Candidate Name

Record your data in a table in the space below. (3 marks)

Hand in this sheet at the end of each practical session.
## ISA BIO3T/P13 Candidate Results Sheet: Stage 2

The effect of pH on the time taken by amylase to hydrolyse starch

<table>
<thead>
<tr>
<th>Centre Number</th>
<th>Candidate Number</th>
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<tbody>
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Candidate Name ...................................................................................................................................

Use the space below to calculate the value of 1000 divided by time in seconds for each of your results in Stage 1. This produces values that are proportional to rates of reaction and are easy to plot.
Use graph paper to plot an appropriate graph of your processed data.

(6 marks)

Hand in this sheet at the end of each practical session.