**Investigating the effect of pH on the activity of catalase**

**Skills - A03.1 Using techniques, apparatus and materials - A03.3 Observing, measuring and recording - A03.4 Interpreting and evaluating observations and data**

* Wear eye protection if available.
* Hydrogen peroxide is a powerful bleach.
* Wash it off with plenty of water if you get it on your skin.
* Catalase is a common enzyme which is the catalyst in the breakdown of hydrogen peroxide, **H2O2**. Catalase round in almost every kind of living cell. Hydrogen peroxide is a toxic substance formed in cells.
* The breakdown reaction is as follows:

**2H2O2 ------------► 2H2O + O2**

* The rate of the reaction can be determined from the rate of oxygen production.
* One indirect but simple way to measure rate of oxygen production is to soak up a catalase solution onto a little square of filter paper and then drop it into a beaker containing a solution of **H2O2**. The paper sinks at first, but as the reaction proceeds, bubbles of oxygen collect on its surface and it floats up.  
  (The time between placing the paper in the beaker and it floating to the surface is a measure of the rate of the reaction.

In this investigation, you will test this hypothesis:

**Catalase works best at a pH of 7 (neutral).**

1. Label five 50cm3 beakers pH 5.6, 6.2, 6.8, 7.4, 8.0.
2. Measure 5cm3 of 3% hydrogen peroxide solution into each beaker.
3. Add 10 cm3 of the correct buffer solution to each beaker. (A buffer solution keeps the pH constant at a particular value.)
4. Cut out 20 squares of filter paper exactly 5 mm x 5 mm. Alternatively, use a hole punch to cut out circles of filter paper all exactly the same size. Avoid handling the paper with your fingers, as you may get grease onto it. Use forceps (tweezers) instead.
5. Prepare a leaf extract by grinding the leaves in a pestle and mortar. Add 25 cm3 of water and stir well.
6. Allow the remains of the leaves to settle and then pour the fluid into a beaker. This fluid contains catalase.
7. Prepare a results table like the one below.
8. Pick up a filter paper square with forceps and dip it into the leaf extract.
9. Make sure you are ready to start timing. Then place the filter paper square at the bottom of the beaker containing H2O, and pH 5.6 buffer solution. (Do not let it fall near the side of the beaker.) As you put the square into the beaker, start a stopwatch. Stop the watch when the paper floats horizontally at the surface.
10. Record the time in your table and repeat steps 8 and 9 twice more.
11. Follow steps 8-10 for each of the other pHs.
12. Pour some of the remaining leaf extract into a test tube and boil for 2 minutes. Cool under a tap.
13. Repeat steps 8-10, using the boiled extract.
14. Calculate the mean (average) time taken at each pH and enter it into your table.
15. Draw a graph to show time taken for flotation plotted against pH and compare with Figure 5.5.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Time taken for paper to float in seconds** | | | | |
| pH | 5.6 | 6.2 | 6.8 | 7.4 | 8.0 |
| Tests 1 |  |  |  |  |  |
| Tests 2 |  |  |  |  |  |
| Tests 3 |  |  |  |  |  |
| Mean |  |  |  |  |  |
| Boiled Extract |  |  |  |  |  |

**Questions**

**A1** Does the enzyme have an optimum pH? If it does, what do your results suggest it to be?  
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**A2** Do your results support the hypothesis you were testing, or do they disprove it? Explain your answer.  
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**A3** What is the effect of boiling the extract?  
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**A4** Why do the filter paper squares have to be exactly the same size?  
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**A5** In most experiments in biology, we can never be quite sure that we would get exactly the same results if we did it again. There are always some limitations on the reliability of the data that we collect. Can you think of any reasons why the results you got in your experiment might not be absolutely reliable? For example:  
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* Might there have been any variables that were not controlled and that might have affected the results?  
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* Were you able to measure the volumes and times as accurately as you would have liked?  
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