**The Breakdown of Starch by Diastase**

**Pupil materials**

**Do Barley seeds Contain enzymes?**

Diastase is an enzyme which breaks down large insoluble starch molecules to smaller, soluble molecules of a sugar called maltose.

 ***diastase***

 ***starch maltose***

We know that enzymes control chemical reactions in living organisms. This experiment shows that the enzyme we are using occurs in *living cells*.

**Materials**

Halved germinating barley grains

Halved germinating barley grains, boiled for 15 minutes

 diastase solution

3 Petri dishes containing starch agar

1 plastic pipette

cork borer

forceps

iodine solution (for use in the second part of the experiment)

**Method**

1. Label the Petri dish lids A,B and C.
2. Use the cork borer and forceps to create a ‘well’ in dish A.
3. Carefully put 4 drops of diastase solution into the well.
4. Use the forceps to place 10 germinating barley grains evenly on the surface of the starch-agar in dish B. Carefully press each grain into the agar.
5. Repeat step 4 with boiled germinating barley grains in dish C.
6. The Petri dishes should now be left for 24 hours at room temperature.

***Petri dishes containing starch-agar, left for 24 hours and flooded with***

***iodine solution.***

  

A*. diastase solution B. germinating barley- C. boiled germinating*

 *grains grains*

1. After 24 hours, flood the dishes with iodine solution. Gently swirl the plate to cover all the starch-agar with iodine solution.
2. Carefully pour the iodine solution into a sink and rinse the surface of the agar with gently running cold water.
3. The dishes can now be placed on a white surface and examined.
4. Record the appearance of the dishes.

**![C:\Users\Kate\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.IE5\VINNZWGT\MC900078711[1].wmf]()Conclusions**

1. The iodine solution has turned blue black in some areas. What is therefore present in these areas?
2. Explain why some areas are clear.
3. Look at dish A and dish B. What do these two results tell you about the enzyme diastase?
4. What two conclusions can you draw from the result in dish C?

**The Effect of Temperature on Diastase**

In this experiment we will use a similar method to investigate how temperature affects diastase. We will use diastase solution for this experiment.

![C:\Users\Kate\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.IE5\VINNZWGT\MC900078711[1].wmf]() Why do think we will use the enzyme solution instead of barley grains to compare the activity of the enzyme at different temperatures?

**Materials**

diastase solution

boiled diastase solution

4 Petri dishes containing starch-agar

A small beaker of distilled water

 2 plastic pipettes (use one for the enzyme solution and one for the water)

cork borer

forceps

iodine solution (for use in the second part of the experiment)

water-proof pen

**Method**

1. Using the water-proof pen put a large dot at the top edge of each Petri dish lid. Carefully turn over the Petri dish so that you do not disturb the agar and put a corresponding dot at the edge of the base of the dish.
2. When you replace the lid the two dots should correspond. This will tell you where the top of the dish is when the lid has been removed.

|  |
| --- |
| *dot on lid indicates the top of the dish and matches the dot on the base* |

1. Replace the lids and position them so that the dots are at the top.
2. Using the cork borer and forceps, cut two wells in each dish. Place the wells half way down, not too close to the edge of the dish (see diagram below).
3. Replace the lids, matching the dots. Write the letter ‘W’ (for water) on the lid over the left hand well and the letter ‘E’ (for enzyme) over the right hand well.
4. Label the lids: 00C; 200C; 350C and ‘boiled enzyme’.
5. Using a dropping pipette, put 4 drops of enzyme solution into the right hand well in each dish.
6. Use a clean pipette to put 4 drops of water into the left hand well in each dish.
7. Carefully replace each lid matching the dot on the lid with the dot on the base.
8. Leave each dish in the appropriate place (fridge, oven, room), according to the temperature on the, lid for 24 hours.

 0oC 20oC

 water enzyme water enzyme

 35oC

 water enzyme water boiled enzyme

11. After 24 hours remove each lid and place the lid of each dish beside its corresponding base. Be careful not to get them mixed up.

1. Flood each dish with iodine solution. Gently swirl the dish to cover all of the starch-agar with iodine solution.
2. Carefully pour the iodine solution into a sink and rinse the surface of the agar with gently running cold water. Put each dish back beside its lid.
3. Set the dishes out on a white surface in the order:

**0oC 20oC 35oC boiled enzyme**

1. Examine the dishes and record their appearance.
2. Measure the diameter of the clear zone in each dish and record the measurements in a table with suitable headings. Which is the dependent variable, and which is the independent variable?

![C:\Users\Kate\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.IE5\VINNZWGT\MC900078711[1].wmf]() **Conclusions**

1. In each Petri dish, one well is filled with enzyme solution and the other well is filled with distilled water. Why is this done?
2. Using data from your table, plot a graph of enzyme activity against temperature.
3. From your results, what conclusions can you draw about the effect of temperature on the activity of the enzyme diastase?

**The Effect of pH on Diastase**

In this experiment we will again use Petri dishes containing starch-agar. This time diastase is set up in a series of buffer solutions of different pH in wells cut in the agar.

A ***buffer solution*** is a solution which maintains a constant pH and resists any tendency for the pH to change.

|  |  |  |
| --- | --- | --- |
|  | Remember! |  |
|  | pH = 7 solution is neutral |  |
|  | pH < 7 solution is acidic |  |
|  | pH >7 solution is alkaline |  |

**Materials**

1 Petri dish containing starch agar

cork borer

forceps

water-proof pen

iodine solution (for the second part of the experiment)

pH 4 buffer solution

pH 7 buffer solution

pH 10 buffer solution

pH 4 buffer solution + diastase

pH 7 buffer solution + diastase

pH10 buffer solution + diastase

**Method**

1. Using the water-proof pen put a large dot at the top edge of the Petri dish lid. Carefully turn over the Petri dish so that you do not disturb the agar and put a corresponding dot at the edge of the base of the dish.
2. When you replace the lid the two dots should correspond. This will tell you where the top of the dish is when the lid has been removed.
3. Position the lids so that the dots are at the top.
4. Using the cork borer and forceps create 6 wells in the dish as shown in the diagram below.
5. Position the lids so that the dots are at the top.
6. Draw a straight line from the top to the bottom of the Petri dish lid as shown below .

*buffer solution only buffer soluton + diastase*

 *pH4*

 *pH 7*

 *pH 10*

1. Using a fresh pipette each time, put 4 drops of the appropriate buffer solution into the wells on the left side of the dish.
2. Repeat step 8 adding the appropriate buffer solution + diastase to the wells on the right side of the plate.
3. Store the dish for 24 hours at 35oC.
4. After 24 hours flood the dish with iodine solution. Gently swirl the dish to cover all of the starch-agar with iodine solution.
5. Carefully pour the iodine solution into a sink and rinse the surface of the agar with gently running cold water.
6. Place the dish on a white surface.
7. Examine the dish and then record the appearance of the dish.

**Conclusions**

1. What do the results tell you about the effect of pH on the activity of diastase.
2. What is the purpose of having three wells filled with buffer solution only?

**Further study and discussion**

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* How would you improve the design of the experiment you set up to show how temperature affects the activity of the enzyme?
* Most experiments can be done in more than one way. For example, the effect of temperature on the activity of diastase can be investigated using a set of test tubes set up as follows:

 *starch suspension + diastase solution*

The test tubes can be placed in a series of water baths set at different temperatures.

How might you use this design to show that temperature affects the activity of the enzyme?

* Germinating barley seed can be ground up with water creating a ‘mush’ which contains diastase. How might you use this to investigate the effect of pH on enzyme activity?