**2.2.3 – 2.2.7 Enzymes**

**At the end of this section you should be able to ……**

|  |  |  |
| --- | --- | --- |
| Define enzymes |  |  |
| Refer to their protein nature, folded shape, |  |  |
| Refer to their roles in plants and animals. |  |  |
| Refer to their role in metabolism. |  |  |
| Describe the effect of pH and temperature range on enzyme activity. |  |  |
| Use the Active Site Theory to explain enzyme function and specificity. |  |  |
| Explanation of the term Optimum activity under specific conditions as applied to pH range. |  |
| Describe heat denaturation of protein |  |  |

**Contemporary Issue**

* Bioprocessing with immobilised enzymes – procedure, advantages, and use in bioreactors

**Mandatory Activities**

* Investigate the effect of pH on the rate of one of the following: amylase, pepsin, or catalase activity.
* Investigate the effect of temperature on the rate of one of the following: amylase, pepsin or catalase activity.
* Prepare one enzyme immobilisation and examine its application.

**2.2.7 H.L. Enzymes**

* **Use the active site theory to explain enzyme function, its flexibility, 3d molecules with variable domains and specificity.**
* **Describe how enzymes are proteins whose activity is affected by environmental pH, temperature.**
* **Explain the term optimal activity under specific conditions applied to pH range.**
* **Heat denaturation of protein**

**Mandatory Activity: Investigate the effect of heat denaturation on the activity of one enzyme**

Key words

enzyme, domain, specificity, active site, denaturation, immobilised, bioprocessing, substrate, induced fit

**Enzymes**

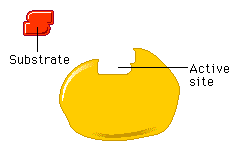
* **Biological catalysts** – **A catalyst speeds up or slows down a chemical reaction without being used up itself**
* **Made of protein**: Long chains of amino acids called polypeptide chains.
* **Folded shape**: The polypeptide chains are twisted, folded and coiled into a molecule of unique 3D shape.

**Function: In metabolic activities i.e. all the chemical reactions in a cell**

* Plants: Catalyse anabolic reactions e.g. photosynthesis and catabolic reactions e.g. respiration
* Animals: Catalyse anabolic reactions e.g. protein synthesis and catabolic reactions e.g. respiration

**Active Site**

* The substance the enzyme works on is called its **substrate**.
* An enzyme works by combining with its substrate and converting it to a **product(s).**
* Only a specific region on the surface of the enzyme binds to the substrate. This region is called the **active site (domain)**. The domain varies from enzyme to enzyme.
* Enzymes are **substrate specific.** This means only certain substrates with a shape complimentary to the active site of the enzyme can combine with the enzyme.

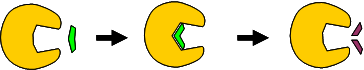


enzyme_formula

**Induce fit theory**

The shape of the active site is flexible. As the substrate enters the active site, it causes (induces) a slight change in its shape so that the active site fits even more snugly around the substrate.

**Substrate induces a change in shape of active site**

****

**Enzyme substrate complex formed**

**formed**

**Product formed**

**Enzyme remains unchanged**

**Substrate**

**Enzyme**

**Active site**

**Factors which affect enzyme activity**

**Enzymes are proteins whose activity are affected by**

1. pH
2. Temperature

**Effect of pH on the rate of enzyme activity**

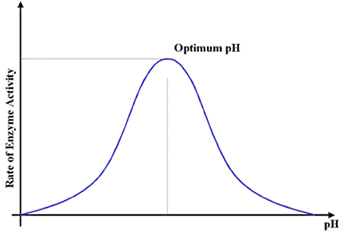
**Generally**

Enzymes are inactive at low pH

Each enzyme has an optimum pH at which it is most active

An enzymes activity decreases at high pH

Enzymes are denatured at very low or high pH



1. **INVESTIGATE THE EFFECT OF pH ON THE RATE OF CATALASE ACTIVITY.**

**MATERIALS:** Enzyme source (Yeast) , Hydrogen peroxide (substrate) Range of buffer solutions (vary pH), Water bath (temp constant), Enzyme: catalase

**Boiling Tube**

H2O2 - substrate

**Water bath:** to keep temperature constant

**Graduated Cylinder**

**Buffer** – to vary pH

**Yeast** – enzyme source

Water

**Thermometer**

**PROCEDURE**

1. Add yeast and water and one of the buffers to the cylinder.
2. Add hydrogen peroxide to a boiling tube.
3. Stand the cylinder and boiling tube in the beaker of water at 250C
4. Add the hydrogen peroxide to the cylinder.
5. Note the volume in the cylinder immediately and record.
6. Read the volume again after a set time and record.
7. Calculate the height of foam (activity of enzyme).
8. Repeat the procedure for different pH buffers.
9. Record results

.**Control:** Used boiled yeast

**Hot Plate**

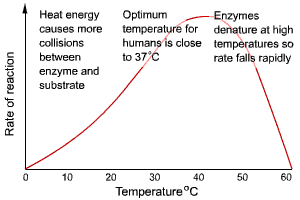
Example of a catabolic reaction

catalase

2 H2O2 2 H2O + O2

2 H2O2 2 H2O + O2

**Effect of Temperature on the rate of enzyme activity**



**Generally**

At very low temperatures, enzymes are inactive.

The activity of enzymes increases when the temperature increases.

Each enzyme has a specific temperature that they are most active. This is **the optimum temperature** for that enzyme.

When the temperature is too high (>60oC), the enzymes are **denatured.** The shape of the active site is altered and they can no longer function.

1. **INVESTIGATE THE EFFECT OF TEMPERATURE ON THE RATE OF CATALASE ACTIVITY.**

**MATERIALS:** Enzyme source (yeast), Hydrogen peroxide ,(substrate),Buffer pH 9 (constant pH), Water bath (vary temp) , Enzyme (catalase)

**Thermometer**

– to monitor temperature

**Hot Plate**: to vary temperature

**PROCEDURE**

1. Add yeast, water and pH 9 buffer to the cylinder.
2. Add hydrogen peroxide to a boiling tube.
3. Stand the cylinder and boiling tube in an ice-cold water bath until the desired temperature (00C) is reached.
4. Add the hydrogen peroxide into the cylinder.
5. Note the volume in the cylinder immediately and record.
6. Read the volume again after 2 minutes and record.
7. Calculate the height of foam (activity of enzyme).
8. Repeat the procedure for other temperatures.
9. Record results

**Control:** Used boiled yeast

**Boiling Tube**

H2O2 - substrate

**Water bath:** to vary temperature

**Graduated Cylinder**

**Buffer** –to keep pH constant

**Yeast** – enzyme source

Water

catalase

2 H2O2 2 H2O + O2

2 H2O2 2 H2O + O2

As with all proteins the shape of the enzyme determines its function. Changes in pH and temperature disrupt the 3 dimensional shapes of enzymes altering the shape of its active site so it can no longer bind the substrate. The enzyme is denatured – loss of shape and function

1. **H.L. INVESTIGATE THE EFFECT OF HEAT DENATURATION ON THE RATE OF CATALASE ACTIVITY**

**Materials**: Enzyme source (yeast), Hydrogen peroxide (substrate),Buffer pH 9 (constant pH), Water bath, Enzyme (catalase)

**PROCEDURE**

1. Place yeast in a boiling tube and place into the

**Thermometer**

water bath at 1000C.

1. Add the heated yeast and the buffer to the graduated cylinder.

**Graduated Cylinder**

**Buffer** –to keep pH constant

**Boiled Yeast** + **water** – enzyme source

1. Add hydrogen peroxide to a boiling tube.
2. Stand the cylinder and boiling tube in the water bath

until the desired temperature (250C) is reached.

1. Add the hydrogen peroxide into the cylinder.
2. Note the presence or absence of foam formation and record.
3. Repeat the procedure using an unheated yeast sample.

**Water bath**

**Boiling Tube**

H2O2 - substrate

**Immobilised enzymes**

**Immobilised enzymes** : Enzymes which are attached to an inert substance e.g. sodium alginate

**Advantages**: reused, recovered, pure product, more efficient, more stable, longer lasting

**Bioreactor:** A vessel or container in which products are made by cells

**Uses:** Conversion of sucrose to glucose, Clarification of juices, Meat tenderisation

1. **PREPARE ONE (i) ENZYME IMMOBILISATION AND (ii) EXAMINE ITS APPLICATION**

**MATERIALS:** Yeast, Sodium alginate**,** Calcium chloride, Sucrose

Sodium alginate and yeast

Calcium chloride – **forms beads**

Bead containing immobilised yeast

(i) **Prepare enzyme immobilisation**

Sodium alginate **traps enzyme**

**PROCEDURE**

**(i) Prepare enzyme immobilisation**

1. Mix sodium alginate and water and yeast in a beaker.
2. Draw the mixture into a syringe.
3. Release the mixture from the syringe, one drop at a time,

into the calcium chloride solution. Beads containing yeast cells will form.

1. Leave the beads to harden.
2. Filter the beads through a sieve and rinse with distilled water.

**(ii) Application of the immobilised enzyme – production of glucose from sucrose**



(ii) **Application of the immobilised enzyme**

1. Mix yeast and water and pour into a separating funnel (Free yeast).
2. Place the beads into another separating funnel (Immobilised yeast).
3. Pour sucrose solution into each of the separating funnels.
4. Using Clinistix, immediately test samples from each funnel for glucose.
5. Repeat the test at intervals until glucose appears in both.
6. Record result.
7. Run off the remaining product from each funnel into the beakers.

Immobilised yeast

+ sucrose

Free yeast + sucrose

1. Compare the turbidity of the solutions from both funnels.