

Experiment-7: Effect of pH, temperature and substrate concentration on enzyme action

Aim: To determine the effect of pH temperature and substrate concentration on enzyme action

Principle:

Enzyme reaction rates are influenced by several factors such as pH, temperature and the concentration of reactants/substrate concentration. For all enzymes there is an optimum temperature and pH for the formation of enzyme-substrate complexes. As the temperature increases there is more movement of molecules and therefore more collisions between enzymes and substrates – so the enzyme activity increases. However, there is a limit to which enzyme activity can increase because at a certain temperature an enzyme will denature.

When the pH is too acidic or too basic for an enzyme, its hydrogen bonds begin to break, causing its active site to change its shape. An altered active site can't bind with its substrate so enzyme activity decreases. If the pH is too unfavorable then covalent bonds can break, causing the enzyme to denature.

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum.

Materials:

pH meter, Water baths-2, ice, 500 mL beaker, test tubes, droppers, white spot plates, starch, iodine.

Procedure:

Effect of pH:

- Switch on the water bath and keep it at 40°C.
- Take 3 test tubes (16x125mm) and name them 1 2 3.
- Prepare solutions as per the table 5.1.
- Place test tubes in water bath at 40°C for 30 min.
- Then place 3 drops from each test tube separately on white spot plate and add 2 drops of iodine solution to each. Observe the change in the colour which corresponds to the extent of hydrolysis by colour of test.

Table 5.1: Amylase enzyme activity with different pH buffers

Test tube Number	1% starch Solution (mL)	2mL buffer solution of pH	Amylose or saliva
1	2	2	2
2	2	7	2
3	2	12	2

Effect of Temperature:

1. Switch on the water bath and keep it at 40°C.
2. Take 3 test tubes (13x100mm) and name them 1 2 3. Take 6 mL of your own saliva (which will have salivary amylase) in a 10 mL graduated cylinder.
3. Take 2 mL of saliva in each tube and add 2 mL of 2% starch solution to each tube.
4. Place tube 1 in ice bath (10°C), 2 in water bath with 40°C, and 3 in water bath with 100°C for 30 minutes.
5. Then place 3 drops from each test tube separately on white spot plate and add 2 drops of iodine solution to each. Observe the change in the colour which corresponds to the extent of hydrolysis by colour of test.

Table 5.2: Enzyme activity at different Temperatures

Test tube Number	1% starch Solution (mL)	Amylose or saliva	Temperature
1	2	2	10 °C
2	2	2	40 °C
3	2	2	100 °C

Effect of Substrate:

1. Switch on the water bath and keep it at 40°C.
2. Take 3 test tubes (13x100mm) and name them 1 2 3
3. Prepare solutions as per the table 5.3.
4. Add 1 mL of 3 M HCl to each test tube. Place test tubes in water bath at 40°C. Observe precipitate formation in the test tube. Note the time in seconds and observe which test tube forms the precipitate first. Note down the time for remaining two test tubes also.

Table 5.3: Enzyme activity with different substrates

Test tube Number	2 % Renin Solution (mL)	Distilled Water (mL)	Milk (mL)
1	4	0	3
2	2	2	3
3	1	3	3

Result:

Reference:

- 1) Sadasivam S and Balasubramanian T (1985). Practical Manual (Undergraduate), Tamil Nadu Agriculture University, Coimbatore, p.2.
- 2) Sadasivam S, Manickam A (2008). Biochemical Methods, New Age International Publishers, 3rd Edition, ISBN: 978-81-224-2140-8.