Exercise 26

Aim: To study the enzymatic action of salivary amylase on starch.

Principle: An enzyme is a biological catalyst. The enzyme salivary amylase or ptyalin present in saliva initiates the breakdown of starch, which hydrolyzes it into disaccharide maltose, isomaltose and short chain glucose polymers called - dextrins. Enzymatic activity of salivary amylase can be tested by detecting the presence or absence of starch.

Requirement: *Glass wares:* Test tubes, cavity blocks, beakers, dropper, and funnel; *Chemicals:* NaCl, Na_2HPO_4, KH_2PO_4, Iodine crystals, potassium iodide; *Equipments:* water bath or incubator thermometer; *Miscellaneous* - cotton, rubber, distilled water, test-tube stand, test-tube holder.

Preparation of reagents

(i) 1% starch solution: Add 1 g of soluble starch to 10ml of distilled water and mix them. Boil 90 ml of distilled water and to it add 10 mL of starch solution already prepared by stirring. Leave the solution overnight and then filter to get 1% starch solution.

(ii) 1% NaCl solution: Dissolve 1 g of NaCl in 100 mL of distilled water.

(iii) Iodine solution (Lugol's): Dissolve 1 g of iodine crystals and 2g of potassium iodide in 100 mL of distilled water.

(iv) Preparation of buffer solution at pH 6.8: Buffer solution can be prepared by dissolving one buffer tablet of 6.8 or 7 pH in 100 ml distilled water or prepare M/15 Na_2HPO_4 solution (9.67g Na_2HPO_4 in 1000 ml of distilled water) and M/15 KH_2PO_4 solution (9.06g of KH_2PO_4 dissolved in 1000 ml of distilled water). Mix equal volume as per requirement to get buffer solution.

Procedure

- Take cotton soaked in distilled water. Remove the excess water by pressing and then spread the moistened cotton over the mouth of a funnel in such a way that it acts as a filter. After cleaning mouth, chew a piece of rubber/cotton and pour the saliva into the funnel. Saliva filtered through wet cotton will be collected in the test tube. Avoid using filter paper for filtering saliva. Take 1 ml of saliva and add 19 ml of distilled water to get saliva solution.
- Take two sets of test tubes (8-10 test tubes in each set) in two separate test tube stand each containing 1 mL of iodine solution to

act as indicator tubes. Mark them 1, 2, 3 . . .. in both test tube stands.

- Switch on the electric water bath or oven. Set the temperature at 37°C. Maintain uniform temperature (37°C) of the water in water bath or water in a beaker inside incubator throughout the experiment.
- In a test tube, take 10 ml of starch solution, 2 ml of 1% NaCl solution and 2 ml of buffer solution. Mix them well and transfer half of the solution into another test tube. Mark one test tube as experimental tube and the other as a control tube.
- Now transfer both experimental and control test tubes to the water bath or keep them in the beaker containing water inside incubator for about 10 minutes so that temperature of solutions reaches 37°C.
- Add 1 ml of saliva solution to the experimental tube and 1ml of distilled water to control tube. Keep both tubes in water bath/ incubator throughout experiment.
- With the help of a dropper, take a drop each from experimental and control tubes and pour it into two separate indicator tubes (marked 1) containing iodine (from two series of indicator tubes - one for experimental and other for control). Record the time of mixing as zero minute reading and note the change in colour of iodine in both tubes.
- After two minutes, again transfer a drop each from experimental and control tubes to indicator tubes (marked 2) and note the colour of iodine. Repeat the step at interval of every two minutes till the colour of iodine solution does not change any further (achromatic point). Always take the same amount of solution throughout the experiment to be added to iodine tubes.
- Compare the series of experimental tubes with the control iodine tubes.

Time (Min) Indicator tube	**Control Indicator tube**	**Experimental**
0	Blue colour	Blue colour
2	_____	_____
4	_____	_____
6	_____	_____
___	_____	_____
___	_____	_____
___	_____	_____

Observation

(i) Note the colour in control indicator tubes.

(ii) Note the colour change in both test tubes and the time taken for the change.

(iii) Perform the Benedict's test for confirmation of the presence of reducing sugar.

Discussion

On the basis of following questions draw your conclusion:

- Did the colour change occur in both sets?
- Which set showed colour change and why?
- Which set did not show colour change and why?
- Which set showed positive Benedict's reaction and what does it confirm?
- How much time did it take to reach the achromatic point (no change in the colour of the indicator)?

23-04-2018