Exercise 27

Aim: To study the effect of temperature on the activity of salivary amylase.

Principle: Most of the enzymes are proteinaceous in nature. Over a limited range of temperatures, the rate of enzyme catalyzed reactions increases as temperature rises. The rate of many biologic reactions roughly doubles with a 10°C rise in temperature and is halved if the temperature is decreased by 10°C. There is an optimal temperature at which the reaction is most rapid. Above and below this, the reaction rate decreases sharply.

Requirement: Glass wares: test tubes, cavity block, beakers, dropper, funnel, test-tube stand, test-tube holder; Chemicals: NaCl, Na_2HPO_4, KH_2PO_4, Iodine crystals, potassium iodide, Prepared Reagents as in Experiment 26; Equipments: water bath or incubator, thermometers; Miscellaneous - cotton, rubber, distilled water,

Procedure

The first four initial steps are the same as in previous Experiment 26.

- In a beaker, take 15 mL of starch solution, 3 mL of 1% NaCl solution and 3 mL of buffer solution. Mix them thoroughly. Divide the solution into three test tubes and mark them as A, B and C. All three test tubes are experimental tubes.
- Now transfer experimental tube A into a beaker containing ice and a thermometer for recording temperature. Temperature of this beaker is to be maintained at 5°C. Transfer the second experimental tube B into water bath set at 37°C and the third experimental tube (C) into the beaker maintained at 70°C.
- Without taking them out add to the three experimental tubes (A, B,and C), 1 mL of saliva solution.
- Take a drop from each of the experimental tubes with the help of a dropper and add it to the corresponding indicator tubes containing iodine solution. Note this time as zero minute reading.
- At intervals of every 2 minutes keep on repeating the above step and note the change in colour of iodine solution. Continue this till the colour of iodine does not change.
- Note the time taken for different experimental tubes till they do not give any colour with iodine.

Observation

To digest completely 5 mL of 1% starch solution, 1 mL of diluted enzyme takes ------- minutes at 5^{0}C, ------- minutes at 37^{0}C and -----minutes at 70^{0}C.

Time minute	Reaction with iodine from experimental tube A	Reaction with iodine from experimental tube B	Reaction with iodine from experimental tube C
0 2 4 6 8 10 12 ____ ____ ____	Blue colour	Blue colour	Blue colour

Discussion

On the basis of the following questions draw your conclusion:

- At which temperature the reaction is optimum?
- Did all three sets of tubes reach achromatic point? If not, why so?
- What inference do you draw about enzyme activity from your experiment?

23-04-2018