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ID:2284123 SECTION:2

**ENZYME**

**PURPOSE:**

The purpose of the experiment is that understand the connection between optimal condition and enzyme activity. Also, the change of pH, temperature, substrate and enzyme concentration is how affect is.

**INTRODUCTION:**

The building block of enzyme is protein. Enzyme work as catalyst. It reduces the time which is reactions occur. If enzyme is not found, reactions won’t occur slowly. While it binds to substrate, enzymes make reaction happen faster. The binding substrate and enzyme is known lock-key model because both are compatible with each other. Each lock has one type of key, so it is special for the substrate. Enzymes have multiple regions that can be activated by co-factors which are generally vitamins to turn them on and off.

Temperature and enzyme activity: When the temperature is increased, the collision between all molecule will increase. Therefore, there will be less time between collision, and more molecules reaches the activation energy, so the reaction rate is increased (Santhosh, 2018). Due to enzyme has protein structure, changing of temperature is affect the reaction rate. Each enzyme has optimum temperature. Enzyme is work very effective in that temperature. For example, the optimum temperature of enzymes which is in the human body is 37oC. When temperature is increase or vice versa, the reaction rate changed, and its rate is decreased. When the temperature is too high, the enzyme will be denatured. Denaturing enzyme is irreversible.

pH and enzyme activity: Because of most enzymes are protein, changing hydrogen ion(pH) is affected the enzyme’s work. Each enzyme has specific optimum pH. For example, the pH of the pepsin enzyme which active in the stomach is 2.0(18.6: Enzyme Activity, 2020). If the pH level is outside optimal value, the enzyme slows down and may even stop working.

Substrate concentration and enzyme activity: While substrate concentration is increased, rate of an enzymatic reaction is increased. However, when the limiting rate is reached, rate of the enzymatic activity stops. Because there is certain the amount of the enzyme.

Enzyme concentration and enzyme activity: The reaction continues as long as the substrate is present in the environment. The only point which stop the reaction is the point which finished the substrate. If the amount of enzyme is increased, the reaction continues rapidly.

**MATERIAL\METHOD:**

Effect of pH on enzyme activity:

* Iodine drops are added the all spots.
* Starch and amylase are mixed in a text tube.
* One or two drops of mix are added in the spots periodically via helping Pasteur pipette.
* If the sample turn out black, the means that starch is found in the sample. Therefore, hydrolysis of starch does not complete. Test again until the color of the sample is lighter.
* If the sample color is iodine color, the experiment is over. The elapsed time should be calculated.
* Then, table is drawn, and graph.

Effect of temperature on enzyme activity:

* The beaker which contains 15 ml of %1 starch solution and 3 ml %1 NaCl solution is taken.
* Then the solution is divided equally in three test tubes and label them as A, B, C.
* The temperature of beaker which contain ice cubes at 5oC protect from the loose their temperature, keep on the table.
* Two beaker which is contain water is taken and then, it heats over the Bunsen burner.
* Tube A is transferred into the beaker which is contain ice cubes. Then tube B transferred the beaker which contain water at 37oC, and tube C is transferred the beaker which contain water at 50oC.
* 1 ml saliva solution is taken with dropper and is transferred to tube A, B, C.
* Few drops from tube A and transfer this into the first series of test tubes having iodine. And similarly, the procedure is continuing from tube B to second series of test tube and from tube C to third series of test tube.
* Note the time which is read at zero minute.
* After two-minute interval, again take a few drop solution tube A, B, C. Then transferred to iodine solutions. Note the change in color of iodine.
* Repeat this procedure two-minute intervals and note the changing in color of iodine until the color of iodine doesn’t change.
* Note the time in the table.

Effect of enzyme concentration on enzyme activity:

* Test tubes are labelled as A, B, C.
* 3 ml of water is transferred to each tube.
* Amylase solution is mix again.
* 3 ml amylase solution is transferred to tube A.
* The amylase and water solution are mixed.
* Take 3 ml of tube A and transferred to tube B. and vortex.
* Take 3 ml of tube B and transferred to test tube C and vortex.
* Determine the concentration of each tube and again labelled the different tube as 1, 2, 3.
* Add 1.5 ml at pH 6.8 solution to each tube.
* 2 ml solution is transferred from tube A to tube, from tube B to tube 2, from tube C to tube 3. In this procedure, before and after all the solution is vortex.
* One drop of IKI solution is put to each spot.
* 1 ml of starch solution is transferred to tube 1. Mix, the start the timer. Every ten seconds one drop of solution is put the spot until the color does not change. Then stop the timer.
* Repeat the procedure for remain tubes and notes the result.

Effect on the substrate concentration on enzyme activity:

* 5 test tube is taken and labelled.
* Solution which have pH 3 is transferred to each tube. Then, substrate which have different amounts is added each tube at 37oC.
* The table is found in the computer screen.
* Then, experiment is reset and then, solution which have pH 5 is transferred to each tube at 37oC.
* Then, the substrate which have different amounts is added to each tube.
* The table which contain the pH 3 and pH 5 and number of molecules of products formed per minute(x106) is found in the computer screen.

**RESULT:**

Effect on pH on enzyme activity:

|  |  |
| --- | --- |
| pH range | Hydrolysis time |
| 5 | 170 sec |
| 6 | 100 sec |
| 7 | 25 sec |
| 8 | 100 sec |

Effect of temperature on enzyme activity:

|  |  |
| --- | --- |
| Temperature | Hydrolysis time |
| 5oC | 14 min |
| 37oC | 4 min |
| 50oC | 10 min |

Effect of the enzyme concentration on enzyme activity:

|  |  |
| --- | --- |
| Concentration of enzyme (%) | Hydrolysis time |
| 5 | 150 sec |
| 2.5 | 300 sec |
| 1.25 | 550 sec |

Effect of substrate concentration on enzyme activity:

|  |  |  |
| --- | --- | --- |
| Amount of substrate | Number of molecules of products formed per minute(x106) at pH 3 | Number of molecules of products formed per minute(x106) at pH 5 |
| 0.5 g | 19 | 39 |
| 1.0 gr | 39 | 81 |
| 2.0 gr | 82 | 168 |
| 4.0 gr | 96 | 198 |
| 8.0 gr | 96 | 198 |

**DISCUSSION:**

Effect on pH on enzyme activity:

When we observe the table and graph, we realize that all reaction is occur but fast one’s pH is 7. Reaction which is occur fast is the reaction which have high rate. If the reaction is occurred fast and easily at different special value such as temperature, pH etc., the value is the optimum value. In this experiment, the optimum pH is 7 because reaction is occurred fast in that value. Optimum pH is the pH which show the maximum activity. The salivary amylase’s optimum pH is 6.8 (Action of Salivary Amylase on Starch (Theory): Class 12: Biology: Amrita Online Lab, 2020). Therefore, the closest pH value to pH 6.8 is pH 7. pH which have maximum activity is equal 7. Therefore, the result is expected.

Test tubes must be found water bath about 30 degree. It is important for the protect the constant temperature. When the tubes are taken the outside of water-bath, the temperatures of tubes change but it should be constant. If it changes, the experiment gives us wrong result. Each experiment has constant value and variable. If these constant value and variables change gather, the experiment will give us wrong result. Constants must be stable; variables should be changed to understand the aim of the experiment.

Effect on temperature on enzyme activity:

The optimum value provides the maximum activity. While the activity is increased, the hydrolysis time will be decreased. The light of this information, when we observe the table and graph, we realize that optimum temperature is 37oC. Optimum temperature of the saliva solution (salivary amylase) s’ range is 32-37 (Action of Salivary Amylase on Starch (Theory): Class 12: Biology: Amrita Online Lab, 2020). The closest temperature to optimum temperature is 37oC. Therefore, we realize the result what we expected.

The temperature of A, B, C should be equal to first measurement. If the temperatures are different each other such as compare the initial A and final A, the result would be wrong. Because the temperature may be far away to optimal value, so the experiment gives us wrong result. If the temperature doesn’t fix to particular value, temperature of tubes wants to be equal to outside temperature, so it changes. Therefore, the temperature of A, B, and C stays as constant.

The enzyme concentration on enzyme activity:

Enzyme which is the protein provide for faster reaction. The enzymes work as long as there is substrate in the environment. The rate of an enzyme-catalyzed reaction is directly dependent on the enzyme concentration (18.6: Enzyme Activity - Chemistry LibreTexts, 2020). The reaction rate increases as the concentration of catalyst is increased (18.6: Enzyme Activity - Chemistry LibreTexts, 2020). When the amount of enzyme is increased, the reaction will continue as fast. Because each enzyme can bind the substrate. Therefore, all substrates are reached in less time. When we compare the result of experiment and our information, we realize that the result is expected. We expect that the reaction which have more enzyme is the fastest reaction.

Serial dilution is a sequential dilution that are performed to convert a dense solution into a more usable concentration (Sapkota, 2020). When using serial dilution, dilution factor doesn’t change because the matter is the same, we compare the first and last tube. It is easier than individual dilution and margin of error is decreased.

The substrate concentration of enzyme activity:

We use the solutions which are pH 3, pH 5. When we observe the table, we realize that the enzyme is work well at pH 5 so the optimum pH value of this enzyme is 5. Amount of enzyme which is transferred the tubes is enough for 4 gr substrate. When we have 20.0 gr substrate and the same amount of enzyme which is equal in that experiment, the reaction stops after 4 gr because enzyme just enough for 4 gr. If we add more enzyme to the tubes, more substrate turns into product. Enzyme bind the substrate and a reaction is occurred. Then, formed product is the result of the solution.

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