Enzymology

Practical Manual
BIOC231

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Computer No.:
Section:
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<td>36</td>
</tr>
</tbody>
</table>
Experiment 1: Effect of Amylase activity on Starch

Definition of enzymes: enzymes are biological catalysts. They greatly enhance the rate of specific chemical reactions that would occur very slowly.

Starch which is the storage form of glucose in plant. Starch consist of

1- Amylose

1-4α- glycosidic linkage

2- Amylopectin

1-6 α- glycosidic linkage
Contents of Saliva:

In animals, saliva is produced in and secreted from the salivary glands. It is a fluid containing:

- Electrolytes: (2-21 mmol/L sodium, 10-36 mmol/L potassium, 1.2-2.8 mmol/L calcium, 0.08-0.5 mmol/L magnesium, 5-40 mmol/L chloride, 2-13 mmol/L bicarbonate, 1.4-39 mmol/L phosphate)

- Mucus. Mucus in saliva mainly consists of mucopolysaccharides and glycoproteins;

- Antibacterial compounds (thiocyanate, hydrogen peroxide, and secretory immunoglobulin A)

- various enzymes. The major enzymes found in human saliva are alpha-amylase, lysozyme, and lingual lipase. Amylase starts the digestion of starch before the food is even swallowed. It has pH optima of 6.7-7.4. Human saliva contains also salivary acid phosphatases A+B, N-acetylmuramyl-L-alanine amidase, NAD(P)H dehydrogenase-quinone, salivary lactoperoxidase, superoxide dismutase, glutathione transferase, glucose-6-phosphate isomerase, and tissue protein. The presence of these things causes saliva to sometimes have a foul odor.

Healthy people produce about 1.5 L of saliva per day.

Amylase:

found in two forms:

1. \( \alpha \)-amylase (in saliva and pancreatic juice) which is endoglycosidase that attack starch randomly. Inactivated by the acidity of the stomach.

2. \( \beta \)-amylase (from plant origin) which is exoglycosidase cleaves maltose from the non-reducing end to produce \( \beta \)-maltose
**Principle:**

When we want to measure enzyme activity either we measure the decrease in the substrate concentration or the increase in the product concentration.

\[
[S] \xrightarrow{E} [ES] \xrightarrow{\text{Amylase}} \text{Maltose} \xrightarrow{\text{pH 6.4-7.2 Cl}^+} \text{reducing sugar} \xrightarrow{\text{Indicator I}_2} \text{Indicator Fehling} \xrightarrow{\text{Blue color}} \text{Red copper oxide ppt}
\]

**Other uses of amylase in industry:**

It is used in clarification of fruit juices. The turbidity present in natural beverages is due primly to the presence of starch and cellulose molecules too large to be completely soluble. Amylase hydrolysis these molecules to glucose which are more water soluble.

**Reagents:**

- Starch 1% solution in 0.3% aqueous sodium chloride
- Freshly prepared; iodinated potassium iodide solution.
- Amylase
Procedure:

Prepare 2 test tubes which contain the following:

<table>
<thead>
<tr>
<th>Test tube</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>-</td>
<td>1 ml</td>
</tr>
<tr>
<td>Starch</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

Allow the tubes to stand for 30 min in water bath (37°C - 40°C)

| Iodine solution | 1-2 drops | 1-2 drops |

References:

# Results Sheet

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Observation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Starch only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (Starch + Amylase)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Experiment 2: Determination of α-amylase activity

History:

α-Amylases (EC 3.2.1.1) is an enzyme of glycoside hydrolases mainly produced in the salivary glands and pancreas, play a well-known role in hydrolyzing a-1,4-glucosidic bonds between glucose in starch (consists of two types of polysaccharide amylose, amylopectin) and maltose is release. Elevated level of α-Amylases in serum can be used as markers for clinical diagnosis of diseases, e.g. Pancreatitis. When the pancreas is diseased or inflamed, amylase releases into the blood.

Principle:

The α-amylase activity is measured using a colorimetric method with 3,5-dinitrosalicylic acid (DNS) reagent. In this method, starch by α-amylase is converted into maltose. Maltose released from starch is measured by the reduction of 3,5-dinitrosalicylic acid.

\[
\text{Starch} + \text{H}_2\text{O} \xrightarrow{\alpha-\text{Amylase}} \text{Maltose (reducing agent)}
\]
Maltose reduces the pale yellow coloured alkaline 3, 5-Dinitro salicylic acid (DNS) to the orange-red colored. The intensity of the color is proportional to the concentration of maltose present in the sample.

This intensity change in color is measured using a spectrophotometer as the absorbance at 540nm wavelength. Wave length is set to 540 nm because it is the region where orange-red color absorbs.

This procedure applies to all products that have a specification for α-amylase

**Reagents:**

- 0.02 M Sodium phosphate buffer
- 1% Starch
- 2 N Sodium hydroxide
- Sodium potassium tartrate tetrahydrate
- Dinitrosalicylic acid color reagent
- Standard Maltose Stock Solution
- Amylase enzyme

Procedure:

Adjust spectrophotometer at 540 nm and 25°C.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Test</th>
<th>Blank</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Distill water</td>
<td>-</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>-</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>

Mix well and incubate at 25°C for 3 minutes

Dinitrosalicylic acid color reagent

Mix well and read the increase in optical density at 540 nm against blank

Determine micromoles maltose released from standard or standard curve

Calculation

Enzyme activity = \( \text{OD (test)} \times \text{concentration of St (µmoles)} \times \text{dilution of enzyme} \)

\[ \text{OD (st)} \times 3 \text{ min} \]

\[ \text{Enzyme Unit} = \frac{\text{µmoles maltose formed}}{\text{min/0.5 ml (x 2)}} \]

\[ = \frac{\text{µmoles maltose formed}}{\text{min/ml}} \]
Results Sheet
Experiment 3: Effect of pH on amylase activity

- The effect of pH on α-amylase activity will be studied.
- Enzymes are affected by changes in pH. The optimum pH value is defined as the pH at which the enzyme rate of reaction (enzyme activity) reaches the maximum activity (V_max).
- Deviation in pH from the optimum causes a decrease in enzyme catalytic activity.
- Extremely high or low pH values generally result in complete loss of activity for most enzymes.
- The enzyme stability is dependent on the optimum pH. Each enzyme has a region of optimum pH for stability.
- The optimum pH can be determined by incubating the enzyme in different incubation media containing different pH buffer ranges from 1.5–10. The enzyme activity will be calculated at each pH at which the enzyme will be incubated. Plot a curve of enzyme rate of reaction (enzyme activity) against the different pH at which the enzyme catalytic reactions are incubated.
- From the curve, the optimum pH which gives the maximum activity of the enzyme will be determined.
Procedure:

<table>
<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Blank</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch (PH=1.5)</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
<td>0.5 ml</td>
<td>-</td>
</tr>
<tr>
<td>Starch (PH=6.9)</td>
<td>-</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch (pH=10)</td>
<td>-</td>
<td>-</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Distill water</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>

Mix well and incubate at 25°C for 3 minutes

<table>
<thead>
<tr>
<th>Dinitrosalicylic acid color reagent</th>
<th>1 ml</th>
<th>1 ml</th>
<th>1 ml</th>
<th>1 ml</th>
<th>1 ml</th>
</tr>
</thead>
</table>

Incubate all tubes in a boiling water bath for 5 minutes. Cool to room temperature

<table>
<thead>
<tr>
<th>Distill water</th>
<th>8 ml</th>
<th>8 ml</th>
<th>8 ml</th>
<th>8 ml</th>
<th>8 ml</th>
</tr>
</thead>
</table>

Mix well and read the increase in optical density at 540 nm against blank

Determine micromoles maltose released from standard or standard curve

Calculation

Enzyme activity = \(\text{OD (test) } \times \text{ concentration of St (µmoles)} \times \text{ dilution of enzyme} \)

\[\text{OD (st) } \times \text{ incubation time (3 min)}\]

Enzyme Unit = \(\frac{µ\text{moles maltose formed / min}}{0.5 \text{ ml enzyme (x 2)}}\)

\[= \frac{µ\text{moles maltose formed / min}}{\text{ ml enzyme}}\]
Experiment 4: Investigation effect of temperature on the activity of lipase

This practical gives you a chance to:

- investigate how lipase activity changes with temperature
- consider how indicators can help us to follow chemical reactions.

Procedure

1- Label a test tube with the temperature (25°C- 40°C- 70°C).
2- Add 5 drops of phenolphthalein to the test tube.
3- Measure out 5 ml of milk using a measuring cylinder (or syringe) and add this to the test tube.
4- Measure out 7 ml of sodium carbonate solution using another measuring cylinder (or syringe) and add this to the test tube. The solution should now be pink.
5- Place a thermometer in the test tube. Take care as the equipment could topple over.
6- Place the test tube in a water bath and leave until the contents reach the same temperature as the water bath.
7- Remove the thermometer from test tube and replace it with a glass rod.
8- Use the 2 ml syringe to measure out 1 ml of lipase from the beaker in the water bath for the temperature you are investigating.
9- Add the lipase to the test tube and start the stop clock/stopwatch.
10- Stir the contents of the test tube until the solution loses its pink color.
11- Stop the clock/watch and note the time in a suitable table of results.

Reference:
http://www.nuffieldfoundation.org
## Results Sheet

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Observation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (at 25°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (at 40°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (at 70°C)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
QUESTIONS

1- When fat breaks down, what is produced?
2- Use this information to explain why the phenolphthalein changes colour?
3- What is the effect of temperature on the time taken for lipase to break down the fat in milk?
4- Why does the temperature affect the action of lipase in this way?
5- What is the difference between a ‘time taken’ and a ‘rate of reaction’ curve for this investigation?
ANSWERS
Experiment 5: The hydrolysis of sucrose by yeast β-Fructofuranosidase

Principle:
β-Fructofuranosidase is a glycosidase found in yeast. It catalyses the hydrolysis of sucrose to glucose and fructose. The enzyme is also known as invertase or sucrase, but these names are no longer used. The substrate sucrose is a non-reducing sugar, whereas the products formed are both reducing sugar. Therefore the reaction can be followed by the estimation of the quantity of reducing sugar formed. Between the several methods which can be used for such estimation, Benedict quantitative method was utilized.

Benedict quantitative reagent is composed of:
1- Copper sulphate: to provide the oxidizing Cu+2 ions.
2- Sodium carbonate: to provide the alkaline medium necessary for the formation of the highly reactive reducing sugar 1-2 endediol.
3- Sodium citrate: combines with Cu carbonate to prevent its precipitation by forming a slightly soluble complex with cupric ions (Cu+2 ions). This complex dissociates slowly to give a sufficient supply of Cu+2 ions.
4- Potassium thiocyanate (KSCN): reacts with cupric ions to give Cu(SCN)2
5- Potassium ferrocyanide (K4Fe(CN)6): prevents the re-oxidation of the formed cuprous thiocyanate (CuSCN) to cupric thiocyanate.
The reaction takes place as fallow:

1- The enolization of reducing sugar in alkaline medium to give a highly reactive reducing compound, which is 1-2 enediol

\[
\text{Reducing sugar} + \text{Na}_2\text{CO}_3 \rightarrow \text{1,2 enediol}
\]

2- Formation of cupric carbonate

\[
\text{Na}_2\text{CO}_3 + \text{CuSO}_4 \rightarrow \text{CO}_3 + \text{Na}_2\text{SO}_4
\]

3- Formation of sodium cupric citrate complex:
4- Ionization of sodium cupric complex

5- Reaction of KSCN with Cu²⁺ ions:

\[
\text{Cu}^{2+} + \text{KSCN} \rightarrow \text{Cu(SCN)}_2
\]

Cupric thiocynate (blue)

6- Reduction of Cu(SCN)₂ by 1,2 enediol to cuprous thiocynate:

\[
\text{Cu(SCN)}_2 \rightarrow \text{CuSCN} \quad \text{boil}
\]
Procedure:
A- Hydrolysis of sucrose by yeast β-Fructofuranosidase

Prepare five tubes containing the following mixtures:

<table>
<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sucrose 0.3M</strong></td>
<td>10 ml</td>
<td>8 ml</td>
<td>6 ml</td>
<td>4 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td><strong>D.W</strong></td>
<td>0</td>
<td>2 ml</td>
<td>4 ml</td>
<td>6 ml</td>
<td>8 ml</td>
</tr>
<tr>
<td><strong>Buffer pH 4.5</strong></td>
<td>6 ml</td>
<td>6 ml</td>
<td>6 ml</td>
<td>6 ml</td>
<td>6 ml</td>
</tr>
</tbody>
</table>

Pre-incubate at 37°C for 5 min

<table>
<thead>
<tr>
<th>Yeast suspension</th>
<th>4 ml</th>
<th>4 ml</th>
<th>4 ml</th>
<th>4 ml</th>
</tr>
</thead>
</table>

Incubate for 15 min

<table>
<thead>
<tr>
<th>1% NaOH</th>
<th>2 ml</th>
<th>2 ml</th>
<th>2 ml</th>
<th>2 ml</th>
</tr>
</thead>
</table>

Final conc. Of sucrose

|                | 150  | 120  | 90   | 60   | 30   |

Note:
The yeast must be added to each tube at a constant time intervals, i.e. tube 1 at time 0, tube 2 at 2min etc. This will enable the incubation time to be measured exactly and ensures that each tube is incubated for the same time. Incubate each tube for exactly 15 min. Stop the reaction by the addition of 2ml of 1% sodium hydroxide. This will be at 15 min for tube 1, 17 min for tube 2 and so on.
Experiment 6: Determination of the hydrolyzed sucrose solution by Benedict method

1- Place the sugar solution of hydrolyzed sucrose from the previous experiment in burette.

2- Measure 5ml of Benedict quantitative reagent into 100 ml conical flask add approximately 1g of anhydrous sodium carbonate and few pieces of porcelain. Heat the mixture vigorously.

3- Run in the sugar solution slowly from the burette until a bulky white precipitate is formed. Continue the titration by adding the sugar solution drop by drop until the last trace of blue or green has disappeared.

4- Record the volume of the sugar required to titrate 5 ml of benedict reagent. This volume will be your titer number.

Note:

1- The end point must be determined while the mixture is still boiling. When the mixture is not boiling atmospheric oxidation occurs and the green color returns.

2- The addition of sodium carbonate to the titration mixture results in the liberation of CO₂, which prevents atmospheric oxidation.

3- If the mixture bumps or it becomes too concentrated during titration, remove it from the heater, boil 10 ml water in a test tube and add it to the reaction mixture. Heats the mixture until it boils again and continues the titration.

4- The tip of the burette must be over the mouth of the flask while mixture is titrated.
**Calculation:**

The concentration of the sugar in each tube can be calculated from the following sugar equivalent equation

\[ M \times V = M' \times V' \]

The equivalents for a number of sugars are given as follow, but they only applied if the above conditions are strictly adhered to.

25 ml of Benedict’s reagent is equivalent to 50 mg of glucose

- 53 mg of fructose
- 68 mg of lactose
- 74 mg of maltose
- 49 mg of hydrolyzed sugar

Since we used 5ml of Benedict reagent which is equivalent to 9.8 mg of hydrolyzed sugar

5ml benedict = 9.8 mg

5ml benedict = Titer no. ml

9.8 mg of sugar = Titer no. ml

\[ X \text{ mg of sugar} = 1 \text{ ml} \]

\[ X \text{ mg of sugar/ml} = 1 \times 9.8 / \text{titer No.} \times \text{dilution factor} \]

Dilution factor= Final volume / Initial volume

**References:**

2. Harvey, R and Champe, P. Lippincott biochemistry, london. 2005
Results Sheet

Fill the table below and plot a relationship between the substrate and product concentration.

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>[S] mM</th>
<th>Titer number ml</th>
<th>[P] mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Experiment 7: Estimation of lipase activity

Lipase is a pancreatic enzyme secreted into the small intestine. It catalyses the hydrolysis of triacylglycerols to free fatty acids and glycerol as follow:

\[
\begin{align*}
\text{Triacylglycerol} & \quad \xrightarrow{\text{Lipase}} \quad \text{Free fatty acids} \\
\end{align*}
\]

The release of fatty acids in the solution will cause decrease in the pH and the rate of the reaction may be followed by:

1- Noting the change of pH with time.
2- Titration the liberated free fatty acids with standard alkali using a suitable indicator
3- By continues titration using an automatic apparatus, (pH-state) which keeps the pH constant and at the same time plots a curve of titer number against time.

Method 2 has been adapted for this experiment. The liberated free fatty acids at different enzyme concentrations will be titrated with 0.05 N NaOH. Since we are using oils as substrates CaCl\(_2\) is used as emulsifying agent for two reasons:

1- to increase the surface area
2- To decrease the surface tension, thus the oil drop is effectively attacked with the enzyme.
Materials:
1- Lipase (1g%)
2- Chloroform (10%)
3- Fresh oil as the substrate
4- Calcium chloride
5- Sodium hydroxide 0.05 N

Procedure
Prepare 6 tubs which contain the following:

<table>
<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil Substrate (ml)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D.W</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Lipase (ml)</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix well

Incubate in a water bath 37°C

| Enzyme concentration (µg) | 0.02 | 0.04 | 0.06 | 0.08 | 0.1 | - |

Titrte the liberated fatty acids with NaOH noting the time of the titration should not exceed 10 min.

References:
2- Harvey, R and Champe, P. Lippincott biochemistry, london. 2005
Draw a graph of enzyme concentration against ml of NaOH has taken. Is your curve hyperbolic or linear, comment?

<table>
<thead>
<tr>
<th>Enzyme concentration</th>
<th>ml of NaOH (titer no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results Sheet
**Experiment 8: Indirect estimation of lactate dehydrogenase**

Lactic acid produced during anaerobic glycolysis can be converted to pyruvic acid with the aid of the enzyme lactate dehydrogenase when oxygen becomes available. The hydrogen acceptor NAD$^+$ accepts the hydrogen atoms from the lactic acid and the pyruvic acid molecule results. Part of the produced pyruvic acid enters the citric acid cycle after being converted to acetyl CoA. The remainder of the pyruvic acid is converted into glycogen.

\[
\text{CH}_3\text{CHOHCOOH} \rightarrow \text{CH}_3\text{COCOOH} \rightarrow \text{Acetyl CoA} \rightarrow \text{Citric acid cycle} \\
\text{Pyruvic acid} \rightarrow \text{Glycogen}
\]

In this experiment, yeast will be used as a source of lactate dehydrogenase. The reaction will be followed by allowing methylene blue dye to function in place of the natural hydrogen acceptor NAD+. As methylene blue is reduced it becomes colorless.

\[
\text{CH}_3\text{CHOHCOOH} \rightarrow \text{CH}_3\text{COCOOH} \rightarrow \text{Methylene Blue (blue)} \rightarrow \text{Methylene Blue (colorless)}
\]
Materials:
- yeast suspension
- 5% sodium lactate solution
- 0.1% methylene blue
- Water bath 37°C
- Boiling water bath

Procedure:
- Label three clean test tubes as a, b and c as followed
- Make sure that you shake the bottle of yeast suspension before removing your sample

<table>
<thead>
<tr>
<th>Test tube</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast suspension</td>
<td>2ml</td>
<td>2ml</td>
<td>2ml Yeast suspension</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre heated for 10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>in boiling water bath</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and cooled to 37°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>before being used</td>
</tr>
<tr>
<td>Sodium lactate</td>
<td></td>
<td>10 drops</td>
<td>10 drops</td>
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<tr>
<td>Methylene blue</td>
<td>1 drop</td>
<td>1 drop</td>
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</table>

Continue to add methylene blue drop wise (mixing after each drop) until each solution becomes a uniform light blue in color

Mix and place in water bath 37°C

Observe the tubes after 10 min. Note any color changes and record your observations

References:
2. Harvey, R and Champe, P. Lippincott biochemistry, London. 2005
## Results Sheet

<table>
<thead>
<tr>
<th>Experiment</th>
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<td>B</td>
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**Experiment 9: Detection of enzymes**

**Pepsin:**
- It is protease found in the digestive system of many vertebrates.
- The pancreas secretes pepsinogen (proenzyme).
- Activated when chief cells in the stomach release it into HCL which activates it.
- It degrades food proteins into peptides which can be readily absorbed by the intestine.
- Optimum pH = 5

**Detection of pepsin:**

W.B (37-40°C)  
1 ml milk + 1 ml buffer soln. (pH 5) + 0.5 ml pepsin \(\rightarrow\) Coagulation occurs (10-20 min)

**Lipase**
- It is a pancreatic enzyme secreted into the small intestine.
- Catalyses the hydrolysis of triacylglycerols to free fatty acids and glycerol.
- Optimum pH = 8

**Detection of lipase:**

1 ml milk + 2 drops ph.ph + drops of NaOH (0.1 N) till pink color appear W.B (37-40°C)  
+ 0.5 ml lipase \(\rightarrow\) Turns colorless (10-20 min)

**Amylase**
- glycosidase that attack starch randomly. Inactivated by the acidity of the stomach.
- Optimum pH = 7
Detection of amylase:

1 ml starch + 1 ml amylase $\xrightarrow{W.B \ (37-40^\circ C) \ for \ 30\ min}$ Take 1 ml of the solution every 5 min, and test the presence of starch by iodine solution.

Urease

- Urease is found in bacteria, yeast, and several higher plants.
- Catalyzes the hydrolysis of urea into carbon dioxide and ammonia
- $(\text{NH}_2\text{CO} + \text{H}_2\text{O}) \rightarrow \text{CO}_2 + 2\text{NH}_3$
- Optimum ph = 7.4

Detection of Urease:

2.5 ml urea + drops Na$_2$CO$_3$ + drops phenol red (Red color), then add acetic acid drop by drop until the color change to (Yellow) + filter paper soaked in urease $\xrightarrow{W.B \ (37-40^\circ C)}$ red color returns $(10-20 \ min)$
## Results Sheet

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Experiment 10: Detection of Enzyme mixture

Detection of enzyme mixture scheme

1-Pepsin Detection
- Coagulation
  - +ve
  - -ve
- no Coagulation
  - +ve
  - -ve

2-Lipase Detection
- Colorless
  - +ve
  - -ve
- Pink color
  - +ve
  - -ve

3-Amylase Detection
- Yellow iodine color
  - +ve
  - -ve
- Blue color
  - +ve
  - -ve

4-Urease Detection
- Red color
  - +ve
  - -ve
- Yellow color
  - +ve
  - -ve
## Results Sheet

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Results Sheet

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