SEMESTER IV

17BTU414B ENZYMOLOGY PRACTICAL 3H - 1C

Total hours/week: L:0 T:0 P:3 Marks: Internal: 40 External: 60 Total: 100

Practical

1. Purification of an enzyme from any natural resource
2. Quantitative estimation of proteins by Bradford/Lowry's method.
3. Perform assay for the purified enzyme.
4. Calculation of kinetic parameters such as Km, V_{max}, K_{cat}

References

1. Lubert Stryer, (2006). *Biochemistry* (6th ed.). WH Freeman.
2. Robert Murray, K., David Bender, A., Kathleen Botham, M., Peter Kennelly, J., Victor Rodwell, W., Anthony Weil, P. (2009). *Harper's illustrated Biochemistry* (28th ed.). McGrawHill.
3. Nicholas Price, & Lewis Stevens. (1999). *Fundamentals of Enzymology*. Oxford University Press.
4. Athel Cornish-Bowden, (2004). *Fundamentals of Enzyme Kinetics* (3rd ed.). Portland Press.
5. Hans Bisswanger, (2004). *Practical Enzymology*. Wiley–VCH.
6. Richard, B. (2002). *The Organic Chemistry of Enzyme-catalyzed Reactions*. Silverman Academic Press.

EX. NO-1 Purification of an enzyme from any natural resource

Aim: To identify the enzymes present in different solutions

Principle: Different enzymes will identify using following reactions

Starch —*Amylase*→ Simple sugars

Sucrose —*Invertase*→ Glucose +fructose

H_2O_2 —*Catalase*→ water +oxygen

Sources: Dry leaves, fresh leaves, raw potatoes, Boiled potatoes, Sprouted seeds, apple, banana and yeast.

Apparatus required: Glass wares, Pestle and Mortar, Water bath, Centrifuge, Testubes, Beaker.

Reagents Required: 2% solution of Glucose, Maltose , Starch and Sucrose, Benedicts reagent and Iodine solution

Control preparation

- Take 3 test tubes
- Add 2.5ml of 2% solution of starch to 1 test tube maltose to the second and glucose to third test tube
- Add 1 ml of 2% iodine solution to each test tube.
- Add 1 ml of Benedicts reagent to all test tubes and vertex
- Use this as reference for the color change in the sample.

Procedure

Sample preparation for Amylase and Catalase Assay

- Take 5 g of each sample and homogenize using distilled water in Pestle and Mortar
- Transfer homogenate to centrifuge tubes and centrifuge for 3 min.
- Collect the supernatant in test tubes

- Mark the test tubes accordingly to the sources.

Amylase assay

- Take 2.5 ml of different samples in different test tubes.
- Add 2.5ml of 2% solution of starch,1 ml of 2% iodine solution and 1 ml of Benedicts reagent to all test tubes and vertex
- Place the test tubes in boiling water bath for few minutes.
- Take out the test tubes from water bath and compare the color with control.

Invertase assay

- Take 2.5 ml of different samples in different test tubes.
- Add 2.5ml of 2% solution of sucrose and add 1 ml of Benedicts reagent to all test tubes and vertex.
- Place the test tubes in boiling water bath for few minutes.
- Take out the test tubes from water bath and compare the color with control.

Catalase assay

- Take 5 ml of H_2O_2 in different test tubes
- Crush different samples and add it to the different test tubes
- Observe the test tubes for effervescence

Observation table

Source	**Amylase**	**Invertase**	**Catalase**

EX. NO-2 Quantitative estimation of proteins by Bradford/Lowry's method

Aim: To determine Specific activity of œ Amylase from different source

Principle: Specific activity is calculated by determining amount of proteins present in I mg in 1 ml of enzyme source and dividing it by the enzyme activity.

Materials and Reagents: Lowry's reagent, Folin's reagent, BSA standard solution

Procedure

1. Take 7 test tubes.
2. Pipette 0,0.2,0.4,0.6,0.8 and 1ml of working BSA solution to 6 test tubes and number it from 1-6.
3. Make the volume as 1 ml in each test tube by adding water
4. Add 1ml of diluted enzyme to 7 th test tube.
5. Add 5 ml of Lowry's reagent to all test tubes.
6. Incubate the test tubes at room temperature for 15 min.
7. After incubation add 0.5 ml of FC reagent to all test tubes
8. Keep the test tubes in dark at room temperature for 30 minutes.
9. Measure the OD and calculate the concentration of protein in 1ml of enzyme.

Tabular column

Sl.no	Vol of BSA (ml)	Vol of water (ml)	Concentration of protein in µg	Lowry's reagent	Incubation at room temperature for 15min	FC reagent	Incubation at room temperature for 15min	OD at 660nm
1				5ml		0.5 ml		
2								
3								
4								
5								
6								
7								

Calculations:

Enzyme activity of œ Amylase =…….mmole/min

1ml of 1:25ml diluted enzyme consists of…… µg of protein

1ml of undiluted enzyme has ……..mg of protein

Specific activity=enzyme activity/mg of proteins= ……..µmol/min/mg

Result: Specific activity of enzyme is ……

EX. NO-4 Calculation of kinetic parameters such as Km, Vmax, Kcat

Aim: to determine Km and Vmax of œ Amylase

Reagents required :Citrate buffer (pH 5.3),Enzyme extract, Starch solution, DNS reagent.

Procedure:

1. Clean and dry 10 testubes
2. Mark test tubes as C1 T1 to C10 T10 depending on substrate concentration.(c- control without enzyme).
3. Add o.5 ml of diluted (1:5) enzyme to test tubes marked as T
4. Add substrate (in the range of 0.1-1) to different test tubes
5. Add buffer to make the volume as 2ml
6. Vertex the test tubes and incubate at room temperature for 15 min
7. Add 1ml of DNS and keep it in boiling water bath for 5 min.
8. Cool all test tubes and add 4 ml of distilled water to all test tubes.
9. Vertex the contents in test tube and read the absorbance at 540nm.
10. Calculate the activity for each test tube.
11. Plot a graph and determine the constants by using Michalis-Menton plot and Line-weaver Burk plot.

Tabular column:

Sl.no	Test tube	Vol of enzyme (ml)	Vol of substrate (ml)	Vol of buffer(ml)	Incubate at room temperature for 15 min	Vol of DNS	Keep it in boiling water bath for 5 min .Add 4 ml of distilled water after 5 min	OD at 540nm	Activity	I/V	[S]	I/[S]
1	C1 T1											
2	C1 T1											
3	C1 T1											
4	C1 T1											
5	C1 T1											
6	C1 T1											
7	C1 T1											
8	C1 T1											
9	C1 T1											
10	C1 T1											