Experiment No. 5

Determination of Deactivation Kinetics of Soluble Enzymes

Objective:

To determine the kinetic constant of deactivation of a soluble enzyme, α - amylase.

Introduction:

An important criterion when using enzymes in technical processes for material conversion or in analytical procedures is the stability of the enzyme during the process. The stability of the enzyme dictates the length of time for which the enzyme is active under the reaction conditions. Depending on the type of enzyme, its purity, enzyme pretreatment and reaction conditions, the enzyme activity can change detectably over periods of time ranging from minutes to years. The main factors affecting enzyme stability are: temperature, pH, concentrations of substrate and product, chemical effects (inhibitors, heavy metals, impurities) breakdown caused by microbes.

Temperature dependency of the catalytic activity and stability of an enzyme

If the enzyme reaction follows a Michaelis - Menten relation

$$r = r_{\max} \frac{C_S}{K_S + C_S}$$

then the maximum velocity of the reaction increases initially with temperature according to the Arrhenius equation

$$r_{\max} = AC_E \exp\left(-\frac{E_A}{RT}\right)$$

where A is the pre-exponential factor, C_E is the enzyme concentration, EA is the activation energy and R is the universal gas constant. At higher temperatures the conformation of the enzyme is altered because of stronger rotational forces and movement of the molecules and this in turn has an adverse effect on the ability of the enzyme to catalyse the reaction. The optimal reaction temperature for technical enzymes lies between 200^{o}C and 60^{o}C. If this temperature is exceeded only minimally then the conformational changes are reversible and the deactivation can be described thermodynamically.

Irreversible deactivation processes are described by kinetic expressions. Enzyme deactivation is a reaction of the first order:

$$E_N \rightarrow E_D$$

where E_N is native enzyme and E_D is deactivated enzyme with the rate equation

$$\frac{da}{dt} = -k_d a$$

where a is the activity and k_d is rate of deactivation. Integration results:

$$a = a_0 e^{-k_d t}$$

deactivation is extremely temperature dependent according to the equation:

$$k_d = k_{d_0} e^{-E_{A,d}/RT}$$

List of Reagents and Instruments

A. Equipment: Flasks, Spectrophotometer, Sample tubes, Micropipette

B. Reagents

- **Reagent A: A1.** Dissolve 67. 5 gm of Sodium potassium tartrate in 100 ml of water

  A2: Dissolve 1.56 gms of Phenol in 10% Sodium Hydroxide (4.375 gm of Sodium Hydroxide in 43.75 ml of water)

  Mix A1 and A2 and keep in dark bottle

- **Reagent B (DNS reagent):** Dissolve 3, 5- DinitroSalicylic acid 1 gm in 100 ml of Water

C. Enzyme and Chemicals Required

- **Enzyme**: α - amylase.
- **Chemicals:** Starch

Acetate Buffer composition

Chemical name	Composition
Acetic Acid	1 M
Sodium acetate	1 M

Procedure:

Enzyme Assay:

- Chemicals/Reagents required: 0.25 ml starch 2 %; 0.10 ml enzyme;
- Incubate at 75^{o}C for 10 min.
- Perform glucose analysis of the sample by DNS method
- Enzyme activity is defined as the amount of enzyme required for the formation of 1 μmol. of glucose per minute per ml of enzyme.

Enzyme Deactivation Kinetics:

For studying the deactivation kinetics of the enzyme, upon incubation under conditions shown in the following table , the enzyme assay is performed as above.

Table: Plan for testing α-amylase activity

No.	**T (^{o}C)**	**Activity (a) after**	
		H	**U**
1	65	1	
2		2	
3		3	
4		4	
5	75	1	
6		2	
7		3	
8		4	
9	85	1	
10		2	
11		3	
12		4	
13	95	1	
14		2	
15		3	
16		4	

From the measurements of enzyme activity at various times and different temperatures the order of the deactivation kinetic, the rate constant of deactivation and the activation energy of the deactivation will be calculated.

A reaction is of the first order if the plot of ln (a/a_0) against time (t) gives a straight line:

$$\ln\left(a / a_0\right) = -k_d t$$

where a_0 is the activity of the native enzyme and k_d is the slope of the straight line.

If $\log_n$ of the various deactivation rate constants $k_d(T)$ is plotted against the reciprocal of the absolute temperature, the slopes of the straight lines obtained give the negative quotient of the deactivation energy (E_A) and the gas constant ($R = 8.314$ J mol^{-1} K^{-1}).

Task Required

Determine the activity of the enzyme at various temperatures and time of incubation

Calculate the order of reaction for the deactivation kinetics at a specific temperature

Calculate the rate constant for the deactivation reaction

Calculate the energy of deactivation.