THE EFFECT OF INCUBATION TIME ON THE RATE OF AN ENZYME CATALYZED REACTION

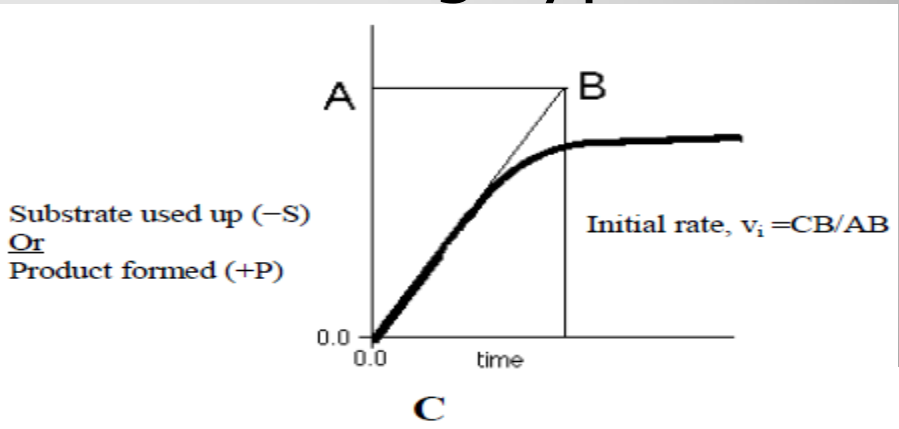
The progress of an enzyme catalyzed reaction may be followed by:

Measuring either the quantity of substrate used up

OR

the quantity of product formed and plotting against time.

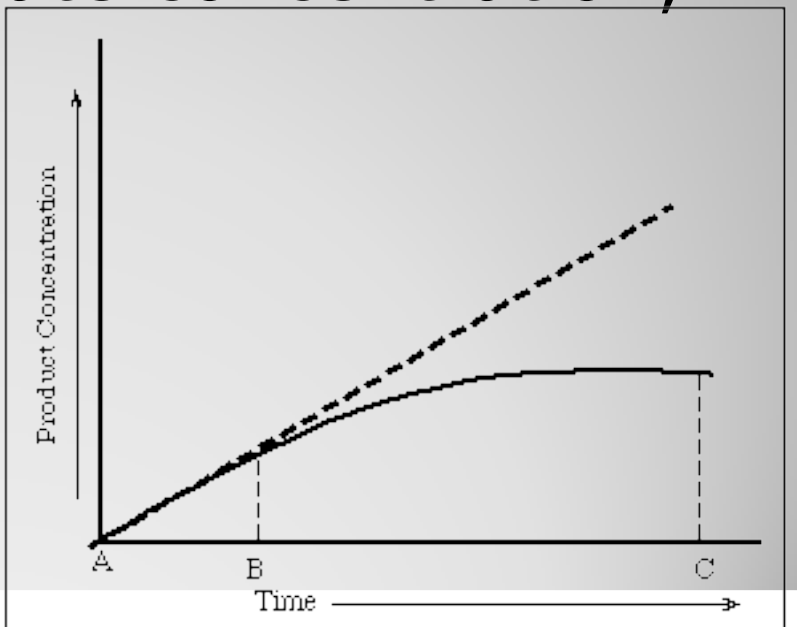
Typically, a curve of the following type is obtained



The rate of the reaction is highest at time zero and decreases with increasing time, eventually falling to zero itself, **where the above curve reaches a plateau**.

This usually occurs either **when all the substrate is used up or when equilibrium is reached**.

- The initial rate of reaction **vi**, measured as the **tangent to the above curve at the origin (time= 0),** is used in the study of enzyme kinetics and is affected by factors which include enzyme concentration, substrate concentration, temperature and pH.



Principle

Under acid conditions, the enzyme catalyzes the hydrolysis of p-nitrophenyl phosphate (pNPP) to inorganic phosphate and p-nitrophenol.

If base is added to the mixture after the completion of the reaction, the p-nitrophenol is converted to a colored form which absorbs lights at 405 nm.

- Assuming a path length of 1.0 cm and an extinction coefficient o f18.8 x 10^3 liter mol-1 cm-1, you can use the absorbance at 405 nm to calculate the number of micromoles of p-nitrophenol released. Since this is a fixed-time assay that is stopped after 5 minutes, the velocity of the reaction (µmoles of p-nitrophenol/minute) can be easily computed.

Set up a series of identical enzyme reaction tubes each of which is allowed to incubate for a different period of time (zero through 30 minutes).
The results should indicate how long the reaction is linear under the given conditions of substrate and enzyme concentration.

Objectives

1-To demonstrate the validity of the assumption that the relationship between product yield and time has been linear throughout.

2- To Calculate the initial velocity of reaction, vi.

Materials

Chemicals:

- Sodium acetate buffer
- Magnesium chloride
- p-nitrophenyl phosphate
- Potassium hydroxide
- Stock solution of crude wheat germ Acid Phosphatase

Equipments:

- Water bath
- Spectrophotometer
- Test tubes Pipettes

Method

- Prepare a series of seven reaction tubes labeled 0 through 30 minutes at 5-minute intervals (0, 5, 10 … minutes).
- To each of these tubes add:

0.5 ml of sodium acetate buffer (pH 5.7),

0.5 ml of $MgCl_2$,

0.5 ml of p-nitrophenyl phosphate, **(Except blank)**

5 ml of distilled water.

- Place all the tubes in a test rack situated in a water bath at 37 ºC and let the temperature equilibrate for 5 minutes.

Add 0.5ml of the enzyme to each tube, mix, start the stopwatch, and let the reaction proceed for 5 minutes before adding the KOH to terminate the reaction.

Total incubation time (min)	Clock time (min)	
	Start reaction (add enzyme)	Stop reaction (add KOH)
0	0	0
5	0	5
10	2	12
15	4	19
20	6	26
25	8	33
30	10	40

Results

Incubation time(min)	Absorbance at 405nm
0 (blank)	
5	
10	
15	
20	
25	
30	

- Velocity= A X $\mathbf{10^6}$ / ε X Time

Use the extinction coefficient (18.8 x 10^3 liter mol^{-1} cm^{-1}) for p-nitrophenol to calculate the micromoles of product released at each time point. Prepare a graph, plotting μmoles of p-nitrophenol released against time.

THANK YOU