The Effects of Enzyme Concentration on the Rate of an Enzyme Catalyzed Reaction.

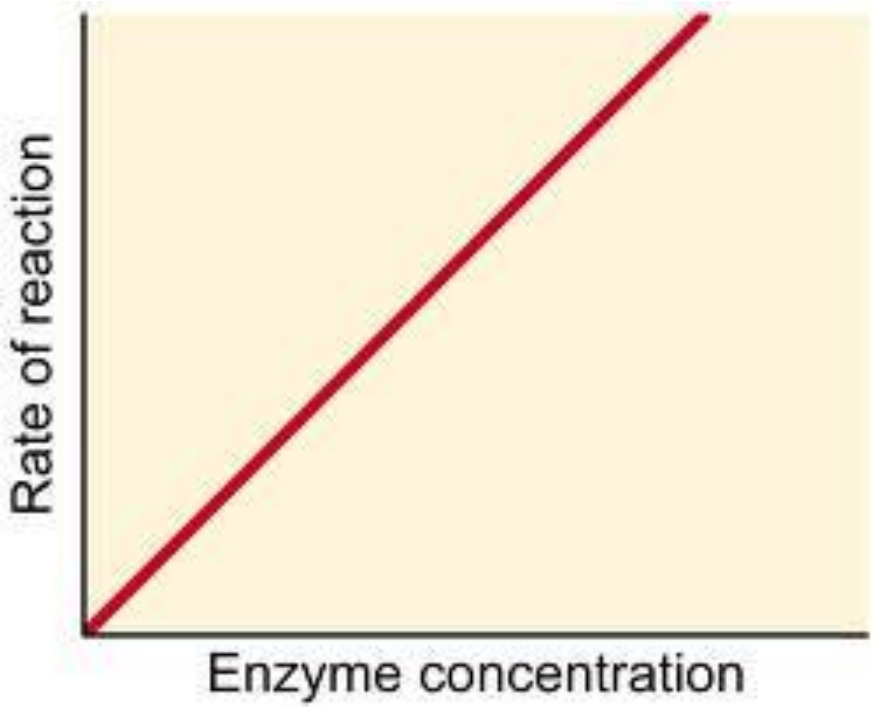
Enzymology

Principle

- A series of 5-minutes assays, will performed in which **a different enzyme concentration is added** each time the reaction is initiated.
- Provided that substrate remains in excess, the rate of an enzyme catalyzed reaction is **directly proportional to increasing enzyme concentration**.
- This relationship is shown in the figure. The results should indicate the range of enzyme concentrations that yield a linear response

Effects of enzyme conc. on rate of reaction.

The rate of reaction is directly proportional to increasing enzyme concentration



Objectives:

To establish the relationship between enzyme concentration and the rate of an enzyme catalyzed reaction

Materials:

- 1.0M Sodium acetate buffer
- 0.1M Magnesium chloride
- 0.05M p-nitrophenyl phosphate
- 0.5M Potassium hydroxide
- Stock solution of crude/ purified wheat germ Acid Phosphatase

Method:

1) Label 7 test tubes (A, B, C, D, E, F, and G) and blank.

2) Pipette the following solutions as indicated in the following table:

Tube no.	Buffer pH 5.7(ml)	MgCl2 (ml)	Substrate (ml)	Dis. Water (ml)
Blank	0.5	0.5	0.5	5.5
A	0.5	0.5	0.5	5.3
B	0.5	0.5	0.5	5.2
C	0.5	0.5	0.5	5.1
D	0.5	0.5	0.5	5.0
E	0.5	0.5	0.5	4.9
F	0.5	0.5	0.5	4.7
G	0.5	0.5	0.5	4.5

3) Place the tubes in the water bath at 37 ºC for 5 minutes.

4) Start the reaction by adding the enzyme at 2 minutes intervals as in the following table:

Tube no.	Enzyme (ml)	Start the reaction (min.) by Enzyme	Stop the reaction (min.) by add KOH 0.5 ml
Blank	0	0 min	0 min
A	0.2	0 min	5 min
B	0.3	2 min	7 min
C	0.4	4 min	9min
D	0.5	6 min	11 min
E	0.6	8 min	13 min
F	0.8	10 min	15 min
G	1	12 min	17 min

5) Stop the reaction by adding 0.5 ml KOH after 5 min. as indicated in the previous table.

6) Read the absorbance at 405 nm against the blank.

Result

Tube no.	Volume of Enzyme (ml)	Absorbance at 405nm	Velocity (µ moles of P-NP/minute)
Blank			
A			
B			
C			
D			
E			
F			
G			

-Use the extinction coefficient for p-NP to determine the micromoles of product produced in 5 min at each of the enzyme concentrations.

- Plot velocity against enzyme concentration (units/ml). Describe the shape of this curve and discuss the reasons for its shape.