KINETICS ANALYSIS OF **B-FRUCTOFURANOSIDASE** ENZYME

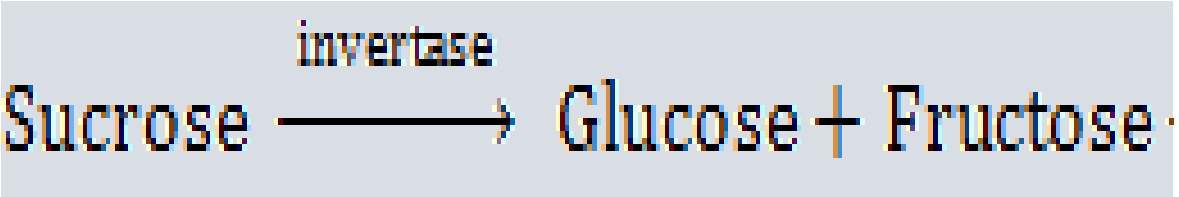
2-The effects of enzyme concentration on the rate of an enzyme catalyzed reaction.

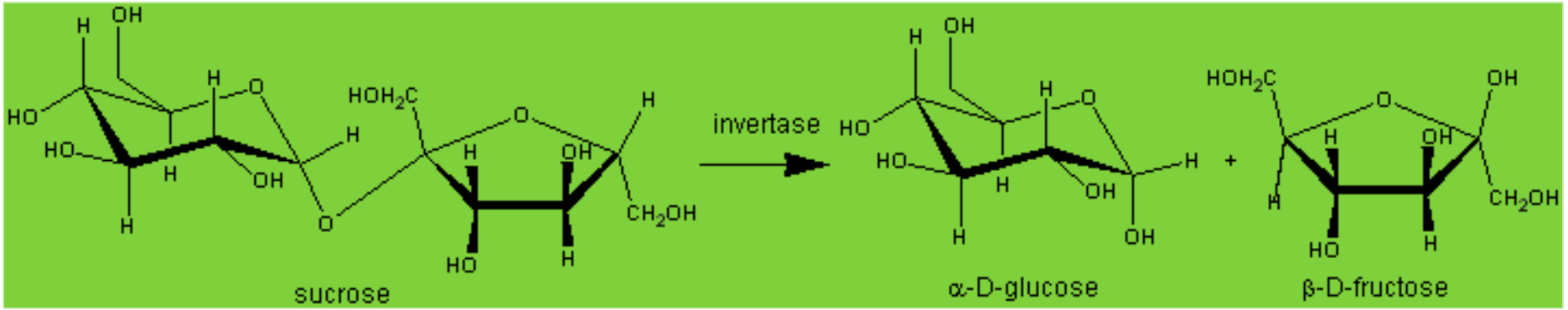
Systematic names and numbers

β-Fructofuranosidase (EC 3.2.1.26)

Reactions catalysed:

It hydrolyses sucrose to yield glucose and fructose



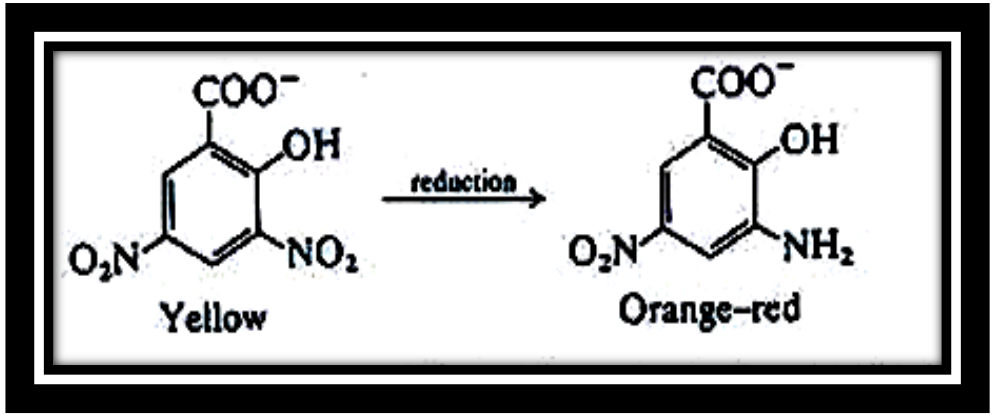


- The activity of the enzyme can be detected by using a reagent that can detect **reducing sugars** (*glucose* and *fructose*).
- One reagent commonly used to measure **invertase** activity in industrial procedures is dinitrosalicylate (DNS).
- Reducing sugars are produced by the action of invertase on sucrose; these reducing sugars reduce DNS to aminonitrosalicylate (ANS).
- The reduction of DNS to ANS results in an observable color change from a [yellow/orange] and Absorbance is determined at 540 nm.

DNS is added to the mixture after the completion of the reaction, the mixture is converted to a colored form which absorbs lights at 540 nm.

the velocity of the reaction (μ moles of reducing sugar/minute) can be easily calculated.

$$\text{Sucrose} \xrightarrow{\text{invertase}} \text{Glucose} + \text{Fructose} + \text{DNS} \rightarrow \text{ANS}$$



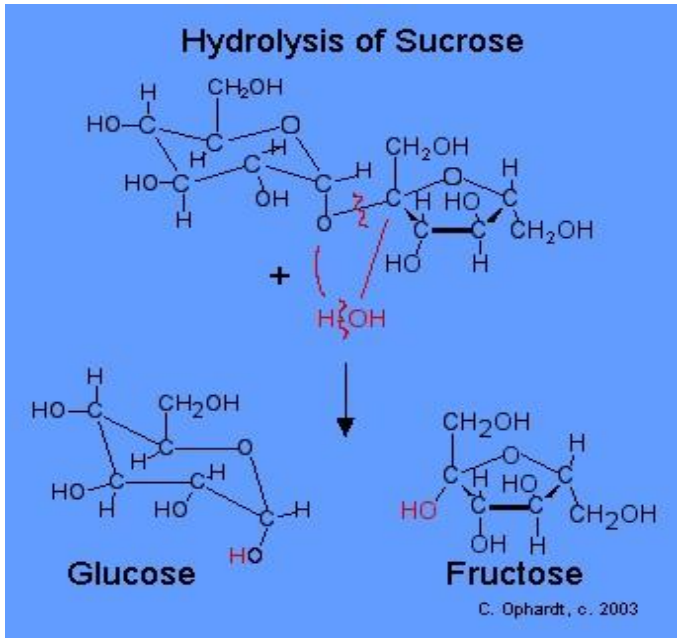
EFFECT OF TIME INCUBATION ON THE RATE OF AN ENZYMATIC REACTION

Objective

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

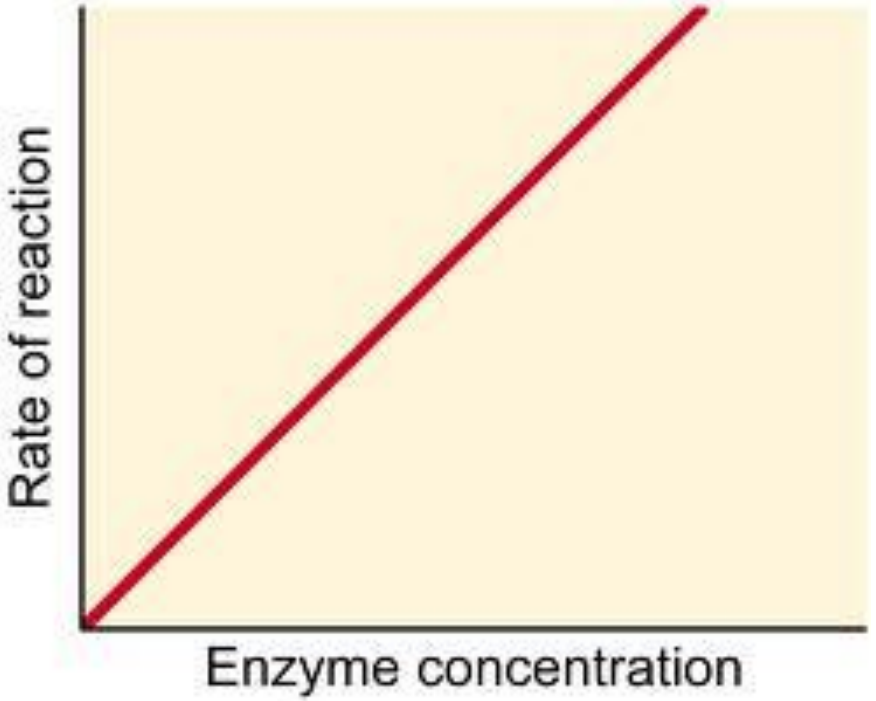
Principle

Within acidic environment using acetate buffer (PH= 4.7) **β-fructofuranosidase** enzyme cleavage its substrate (Sucrose) non reducing sugar to mixture of reducing sugar glucose and fructose, using 3,5,dinitrocylislic acid .



The rate of reaction is directly proportional to increasing enzyme concentration

a series of 10 -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.**
The results should indicate the range of enzyme concentrations that yield a linear response



Material

Solutions :-

- 0.05M Sodium Acetate buffer , pH 4.7 .
- 0.18 M Sucrose ,
- Reducing sugar (0.005M glucose + 0.005M fructose)
- Beta Fructofuranosidase (Invertase) enzyme extract from yeast.
- DNS (dinitrosalicylicacid)Reagent .
- Sodium Bicarbonate .

Method:

1- Prepare 8 tubes in the following manner table (1) :

Tube	Acetate buffer (ml)	0.18M Sucrose (ml)
Blank	1.0	2.0
A	1.0	2.0
B	1.0	2.0
C	1.0	2.0
D	1.0	2.0
E	1.0	2.0
F	1.0	2.0

2- Mix each tube then add 0.05ml of diluted enzyme according to the following table (2) , **EXCEPT FOR THE BLANK ADD 0.05ml OF DISTILLED WATER INSTEAD** , mix and start the stop clock immediately , incubate each tube for 10 min , then stop the reaction by adding 2.0ml of the DNS reagent to each tube.

Note : Mix each tube frequently during the incubation time .

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Tube	Enzyme Solution	Enzyme concentration x 10-3
Blank	---	0
A	E1	8.0X
B	E2	10X
C	E3	15X
D	E4	20X
E	E5	30X
F	E6	60X

Table (2).

Method:

Tube	Start (min) By adding 0.05ml E	Stop By adding 2 ml DNS (min)
Blank	0.0	0.0
A	1.0	11.0
B	2.0	12.0
C	3.0	13.0
D	4.0	14.0
F	5.0	15.0
G	6.0	16.0

Table(3).

Method:

- 3- Mix properly , cover each tube by aluminum foil and place in a boiling water bath for 5min to allow the color to develop .
- 4- Then remove from water bath cool under tap water , add 20ml of distilled water to each tube , mix properly then measure the absorbance at 540nm .
- 5- Record the absorbance of each test tube in the following table (4),
- 6- Convert the Absorbance reading obtained to micromoles of sucrose hydrolyzed making use of the standard reducing sugars calibration curve , then divide by 10 to obtain the number of micromoles of sucrose hydrolyzed /min (v_i).
- 7 – Draw a graph between the (v_i) the micromoles of sucrose hydrolyzed /min and enzyme concentration .

Result

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

Tube	Absorbance 540nm	µmoles of sucrose hydrolyzed	µmoles /min (v_i)
A			
B			
C			
D			
E			
F			

Table (4)

Discussion

Comment on the curve shape and conclude the relationship between enzyme concentration and the rate of an enzyme catalyzed reaction.

Thank You