Kinetics analysis of **β-fructofuranosidase** enzyme

1-Effect of Time Incubation On The Rate Of An Enzymatic Reaction

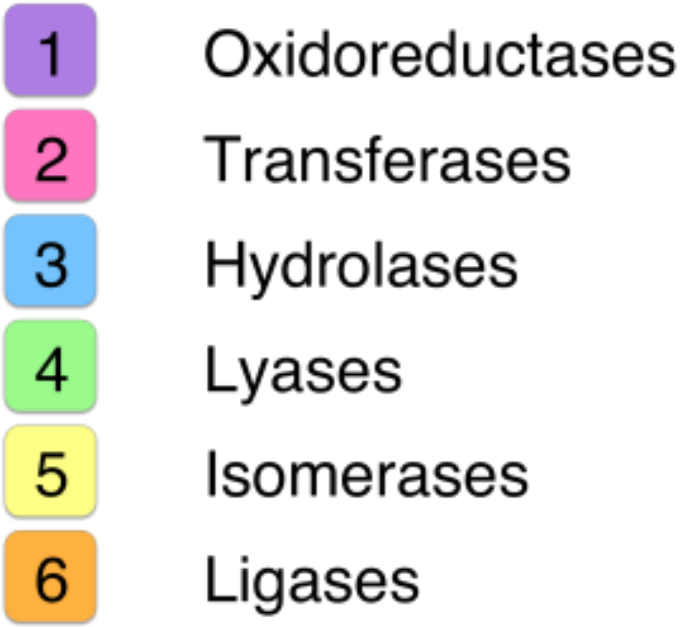
Enzyme kinetics

It is the study of the chemical reactions that are catalyzed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction is investigated.

Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

β-fructofuranosidase enzyme

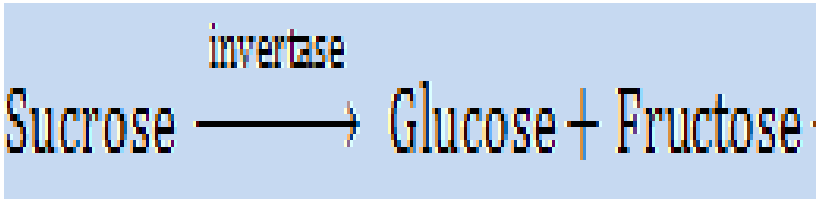
(EC 3.2.1.26)Knowing also in other names *saccharase*, *beta-fructosidase*, Invertase , *sucrase*, and the systematic name: **β**-fructofuranosidase)



Invertase

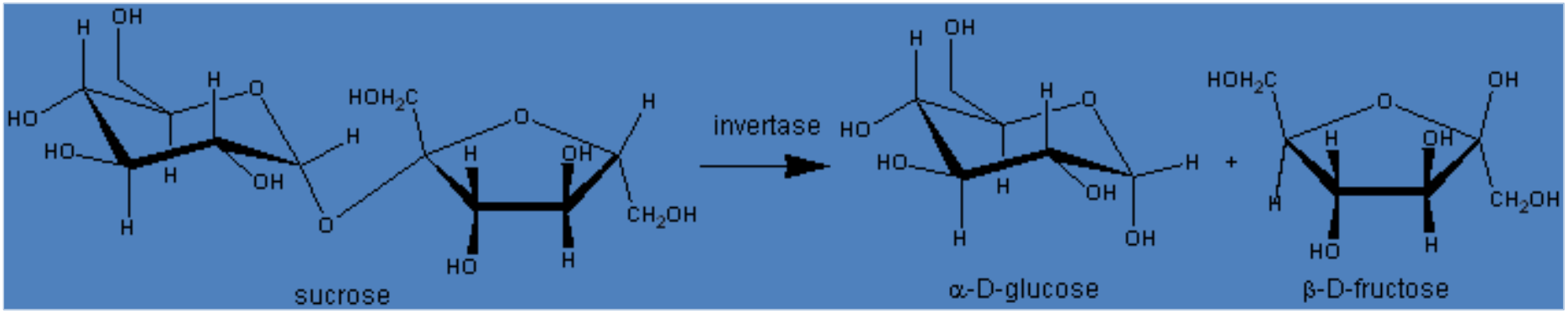
Systematic names and numbers

β-Fructofuranosidase (EC 3.2.1.26)



Reactions catalysed:

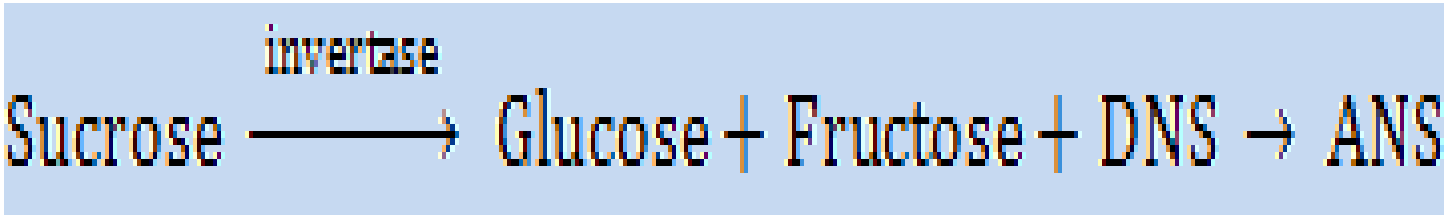
It hydrolyses sucrose to yield glucose and fructose

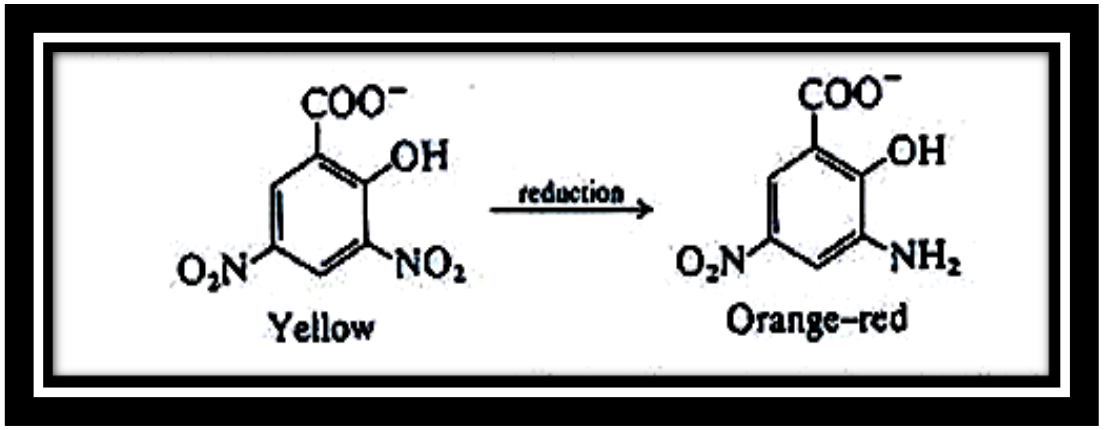


How measure the enzyme activity

- In today's lab, we will demonstrate how enzyme activity is dependent on certain environmental factors and conditions.
- 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.

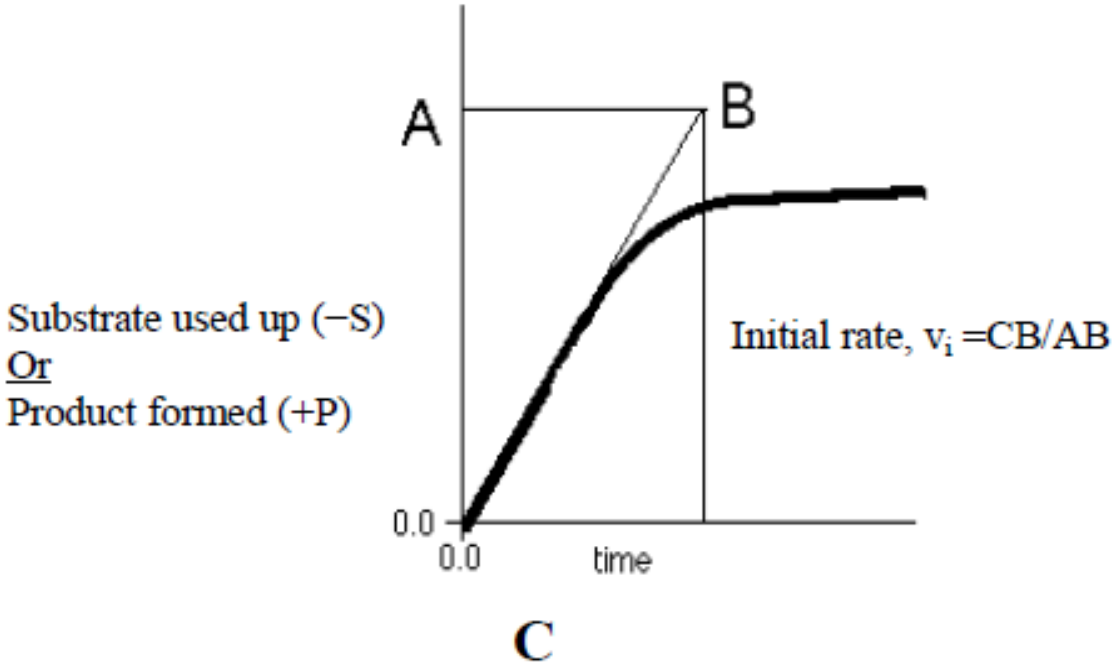
- **Sucrose (substrate)**, commonly known as table sugar, is a disaccharide composed of an alpha-D-glucose molecule and a beta-D-fructose molecule linked by an alpha-1,4-glycosidic bond.
- When this bond is cleaved in a hydrolysis reaction, **an equamolar** mixture of glucose and fructose is generated.

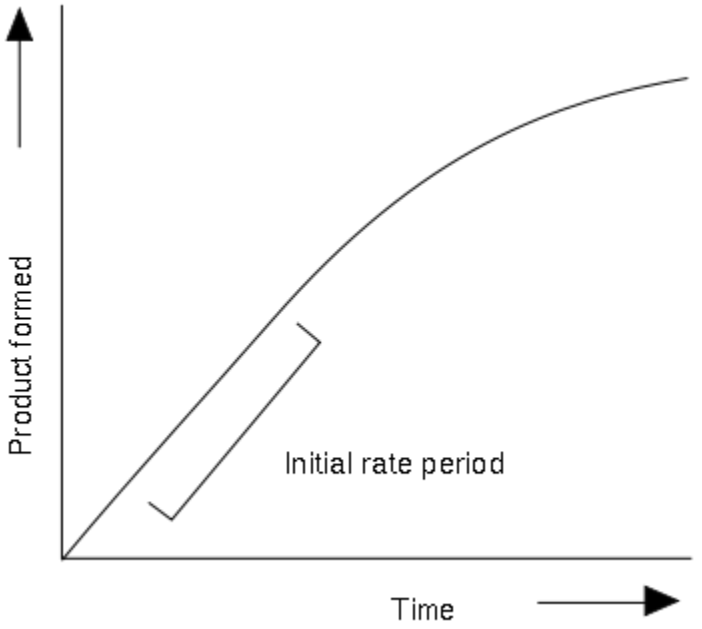

invertase
Sucrose
Glucose + Fructose + DNS → ANS


COO⁻
OH
O₂N
NO₂
reduction
COO⁻
OH
O₂N
NH₂
Yellow
Orange-red

Effect of Time Incubation On The Rate Of An Enzymatic 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 beginning and being slower with increasing time, where the above curve reaches a plateau. This usually occurs either when all the substrate is used up or when equilibrium is reached.

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.

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.

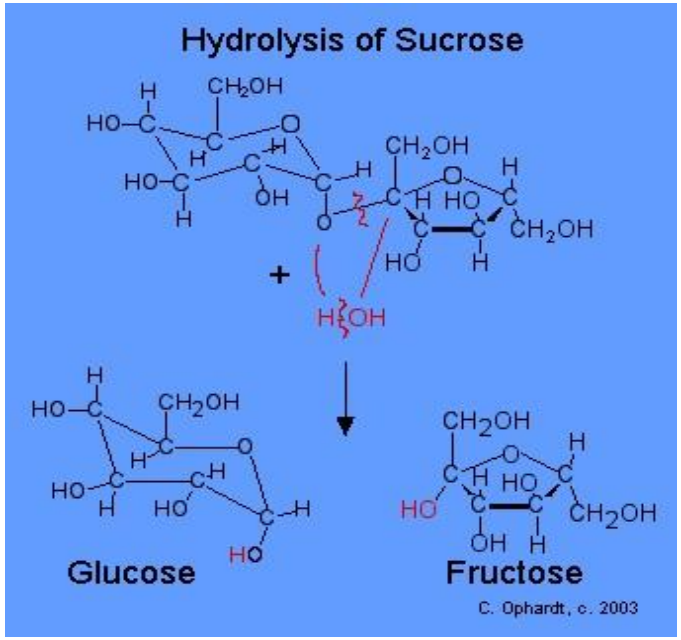
Objective:

1-to study the effect of time on rate of enzyme catalyzed reaction.

2- Calculate the initial velocity of reaction, vi.

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 .



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 .

Part I :Preparation Of Calibration Curve For Reducing Sugars

Method :

- Prepare 7 test tubes in the following manner , table (1):

Tube	0.005M Standard reducing sugars (ml)	Water (ml)	Acetate buffer (ml)	DNS reagent .(ml)
Blank	-	2.0	1.0	2.0
A	0.2	1.8	1.0	2.0
B	0.4	1.6	1.0	2.0
C	0.8	1.2	1.0	2.0
D	1.0	1.0	1.0	2.0
E	1.5	0.5	1.0	2.0
F	2.0	-	1.0	2.0

Part I :Preparation Of Calibration Curve For Reducing Sugars

2- Mix each tube properly then , cover each tube with aluminum foil and place in a boiling water bath for 5 min .

3- Remove the tubes from the water bath , cool under tap water , then add 20ml of water to each tube and mix properly .

4- Measure the absorbance of each tube against the blank at 540nm , then record the absorbance in the following table (2) .

5- Construct the calibration curve by plotting the absorbance at 540nm against the Concentration of reducing sugars in micro Molar .

Part I :Preparation Of Calibration Curve For Reducing Sugars

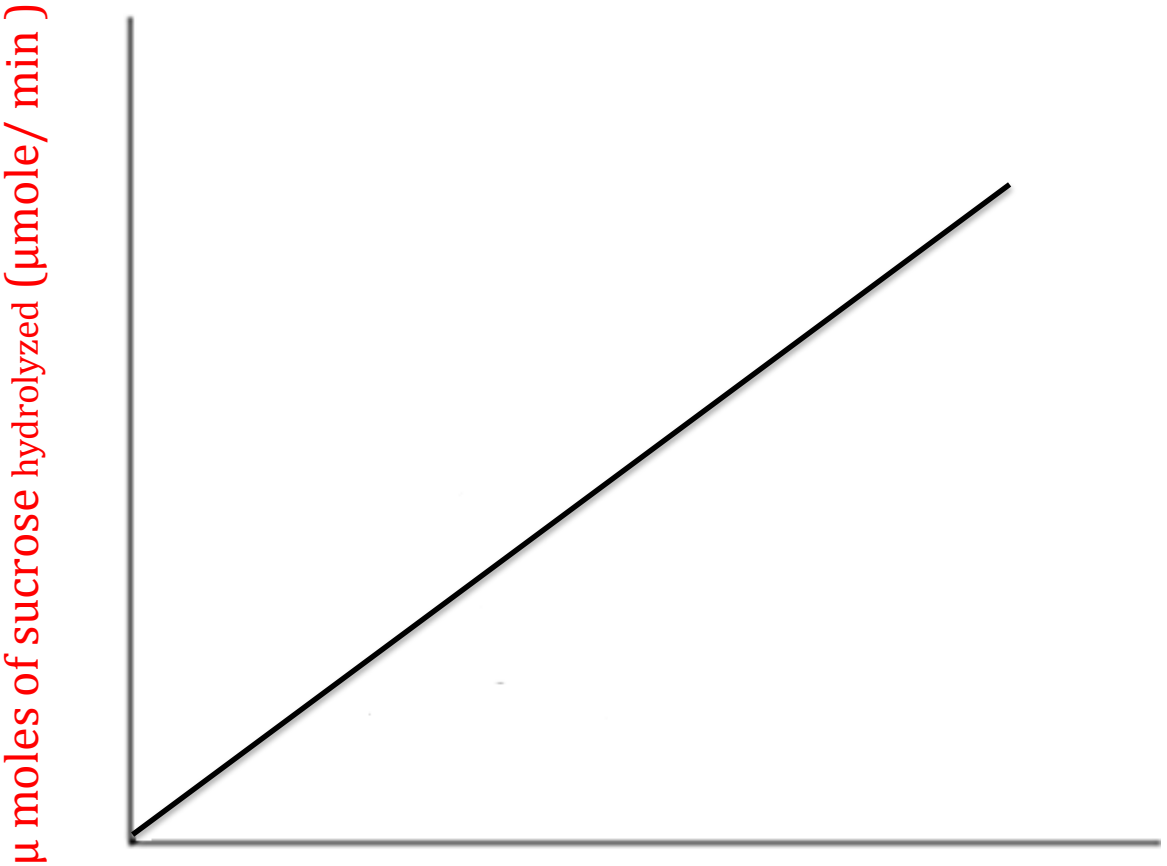
Results:

table (2)

Tube	Absorbance 540nm	Concentration of reducing sugars in morality (M) .	Concentration of reducing sugars in micro Molar (µM)
A		(0.2÷2)× 0.005= 0.0005	0.0005×10 ^6= 500µ M
B		(0.4÷2)× 0.005= 0.001	0.001×10 ^6= 1000µ M
C		(0.8÷2)× 0.005= 0.002	0.002×10 ^6= 2000µ M
D		(1÷2)× 0.005= 0.0025	0.0025×10 ^6= 2500µ M
E		(1.5÷2)× 0.005= 0.00375	0.00375×10 ^6= 3750µ M
F		?	؟

Part II: Effect of Time Incubation On The Rate Of An Enzymatic Reaction .

Results:



Part II: Effect of Time Incubation On The Rate Of An Enzymatic Reaction .

Method:

1- Prepare 8 tubes in the following manner table (3) :

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

Part II: Effect of Time Incubation On The Rate Of An Enzymatic Reaction .

2- Add the following at exact time manner Table 4 .

Table (4).

Tube		Start Time (min) By adding 0.05 ml of Enzyme	Stop Time (min) By adding 2.0ml DNS . (min)
Blank	Total reaction time	0	0
A	10 min	1	11
B	20 min	2	22
C	30 min	3	33
D	40 min	4	44
E	50 min	5	55
F	60 min	6	66

Part II: Effect of Time Incubation On The Rate Of An Enzymatic Reaction .

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 (5).

6- Convert the Absorbance reading obtained to micromoles of sucrose hydrolyzed making use of the standard reducing sugars calibration curve .

7- Obtain the relationship between the micromoles of sucrose hydrolyzed and Time in min . Then determine the initial velocity of the reaction

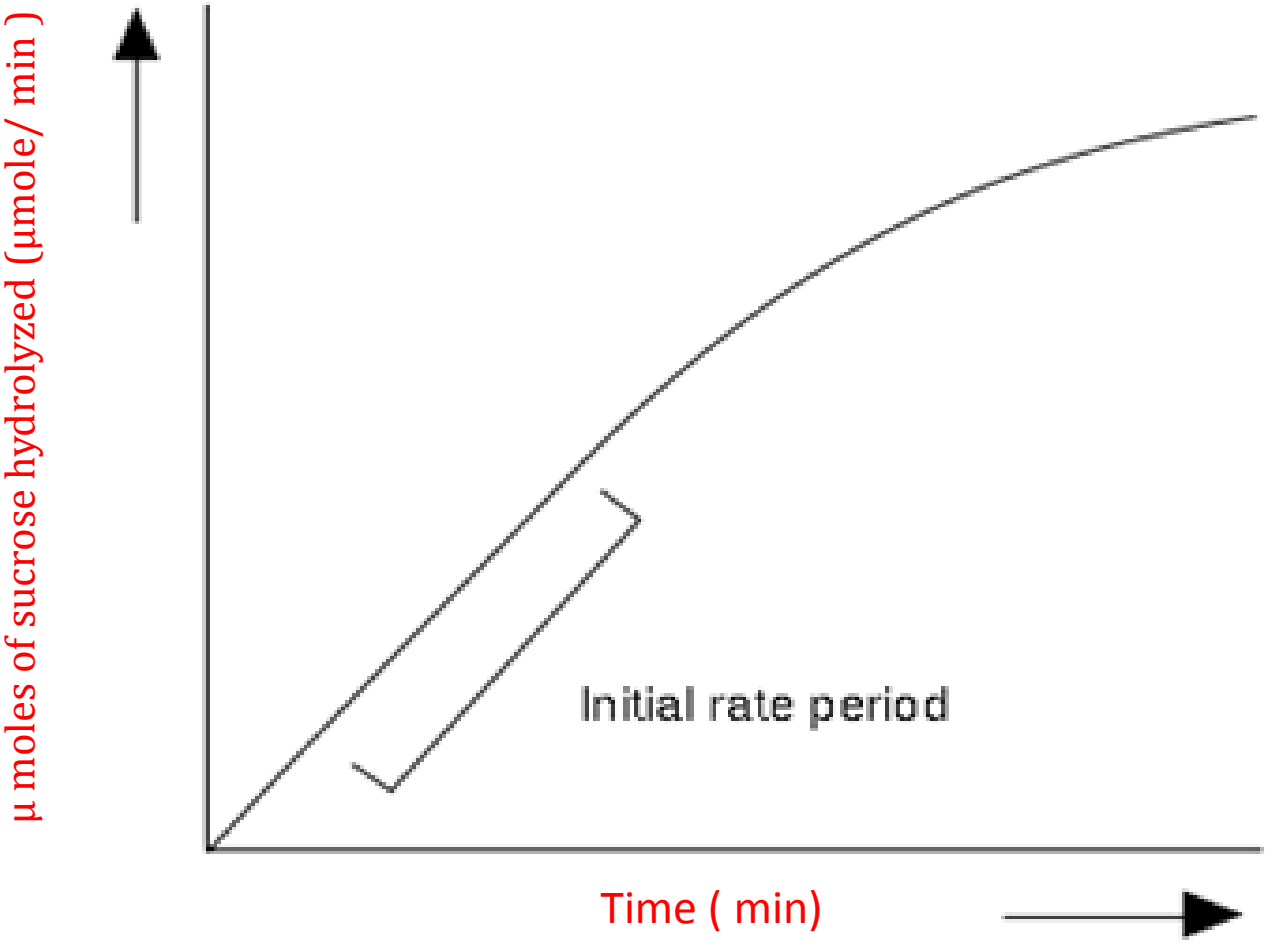
Part II: Effect of Time Incubation On The Rate Of An Enzymatic Reaction .

Table (5)

Tube	Absorbance 540nm	Micromoles of sucrose hydrolyzed
A		
B		
C		
D		
E		
F		

Part II: Effect of Time Incubation On The Rate Of An Enzymatic Reaction .

Results:



Discussion

In part I:

You well plot the standard curve and comment on its shape

In part II:

You well find the concentration of micro M of reducing sugar from the standard curve from their absorbance you obtained

Thank You