UNIVERSITY of LIFE SCIENCES in Lublin
FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF BIOCHEMISTRY
20-033 Lublin, Akademicka 12
Phone +48 81 445 66 08
www.biochwet.up.lublin.pl

The influence of substrate concentration (Michaelis Constant) on the velocity of sucrose hydrolysis

The principle of method

Invertase (β-fructo-furanosidase) belongs to hydrolases that cleave glycosidic bond in sucrose and break it down into glucose and fructose. This process is called inversion and is related to the name of enzyme. Optimum pH for this enzyme is ranged between 4-7, but at pH = 10 it is completely inactive.

The activity of invertase can be measured based on the methods for the detection of reducing sugars because together with the progress of hydrolysis the amount of single sugars (glucose and fructose) in the incubation mixture increases.

Reducing sugars react with copper reagent and copper I is synthesized. In order to determine quantitatively the amount of copper I which was synthesized during the oxidation of sugars the reaction with phosphomolybdenum reagent should be performed. The intensity of obtained colour is proportional to the amount of reducing sugar in the sample.

Protocol:

Add 0,5cm^3 of Copper reagent, 0,7cm^3 of H_2O dest and 0,1 cm^3 0,05M NaOH to the set of 7 tubes marked from 1 to 7.

In addition to tube no 1 add 0,1 cm^3 of enzyme and 0,1 cm^3 H_2O dest – it is blank sample for colorimetric measurements.

Prepare set of 6 tubes marked from A to F and pipete sucrose of appropriate concentration:

A – 1 cm^3 **0,8M** sucrose
B – 1 cm^3 **0,6M** sucrose
C – 1 cm^3 **0,5M** sucrose
D – 1 cm^3 **0,4M** sucrose
E – 1 cm^3 **0,3M** sucrose
F – 1 cm^3 **0,1M** sucrose

Tubes A-F should be incubated in water bath for 5min in 37° C. After incubation add 1 cm^3 of enzyme (invertase) to each tube and immediately incubate for further 15 min in similar conditions. Note time "0" – time when substrate was mixed with enzyme and enzymatic reaction was initiated. It should be kept in mind that after adding the enzyme the concentration of substrate is half lower.

After 15 min. of incubation in water bath transfer appropriate amounts of solutions from set of tubes marked A-F to set of tubes marked 2-7:

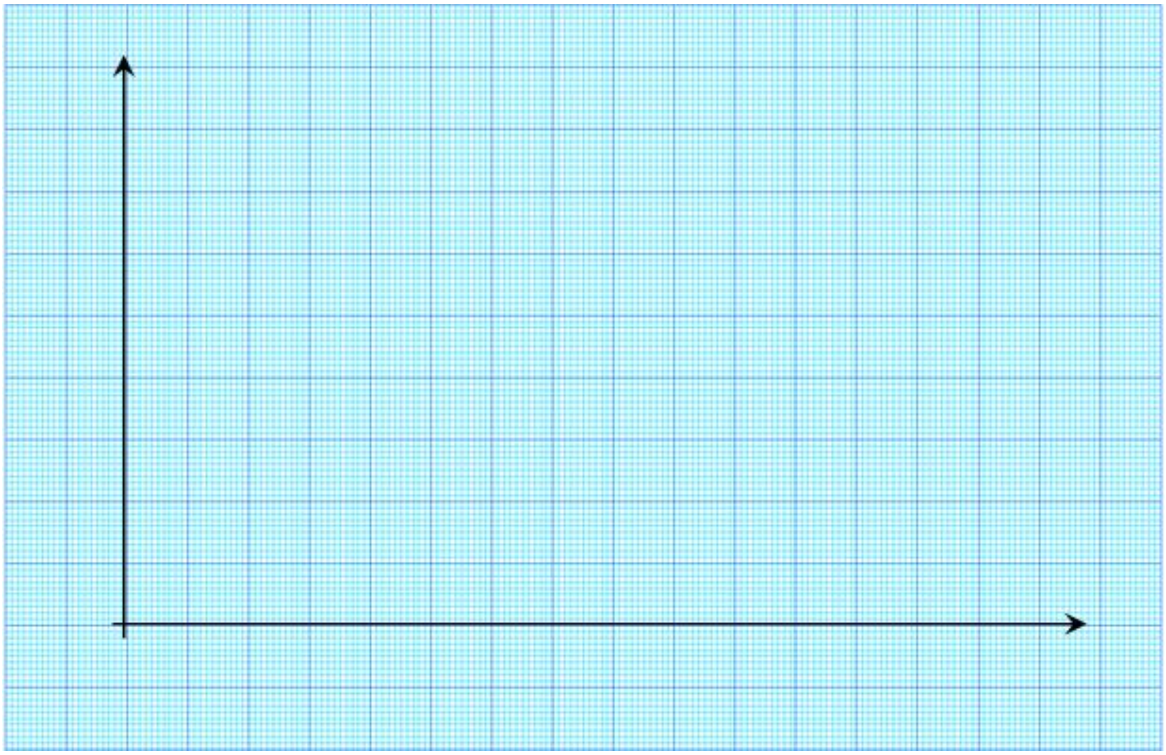
A – 0,2 cm^3 of incubation mixture to tube no **2**
B – 0,2 cm^3 of incubation mixture to tube no **3**
C – 0,2 cm^3 of incubation mixture to tube no **4**
D – 0,2 cm^3 of incubation mixture to tube no **5**
E – 0,2 cm^3 of incubation mixture to tube no **6**
F – 0,2 cm^3 of incubation mixture to tube no **7**

All tubes 1-7 heat in boiling water bath for 8 min. Chill carefully under the stream of tap water and add to each tube 1cm^3 of phosphomolybdenum reagent. Each tube should be diluted with distilled water approximately 10 times (ask the teacher). Measure absorbance at wave length of 610 nm against the solution from tube 1 (also similarly diluted). Give the results in the table.

No of tube	**2**	**3**	**4**	**5**	**6**	**7**
Final concentration of substrate in incubation mixture	0.4	0.3	0.25	0.2	0.15	0.05
Absorbance (A)						

The calculation of results

1. Draw the plot of dependencies between absorbance and the concentration of substrates after 15 min of incubation.
2. Calculate Michaelis Constant



Lublin, 28th July 2017.