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Starch Enzyme Hydrolysis – Experimental and Kinetics

Ina Karadashka[1], Stilijan Tchaoushev[2]

[1] Central Scientific and Research Laboratory, University of Chemical Technology and Metallurgy, Bulgaria, ina_karadashka@yahoo.com

[2] Department of Chemical Engineering, University of Chemical Technology and Metallurgy, Bulgaria

Abstract In order to study the condition of starch hydrolysis with thermostable α-amylase the influence of the substrate concentration, temperature and pH were investigated. The kinetics parameters – K_m and V_{max} were determined using Michaelis – Menten equation and the other its forms – Lineweaver - Burk, Hanes - Woolf and Eadie - Hofstee. It was found that reaction rate has maximum value at pH 5.8 and temperature of 75 ºC. The values of K_m are between 60.42 – 65.72μg.ml^{-1}, and V_{max} has values between 10.89 and 11.35 μg.ml^{-1}.min^{-1}.

Keywords: starch hydrolysis, α-amylase.

1. Introduction

The great application of starch hydrolysis products in the food, pharmaceutical, textile, leather and paper industries increased interest in the study and optimization of the hydrolysis process.

Strong catalytic properties of enzymes at mild conditions and their higher selectivity make the enzyme hydrolysis more preferred method for starch hydrolysis in comparison with acid hydrolysis which requires the use of mineral acids at high temperatures and pressures [3, 4, 9]. The different structure of the two polysaccharides, amylose and amylopectin, which build the starch determine their different chemical properties [7]. Amylose has a long linear structure made of glucose residues connected with α-1.4 connections. Glycosyl residues in the structure of amylopectin form branched chains driven by the presence of α-1,4 and α-1,6 glycosidic linkages.

α-amylase (EC 3,2,1,1) is an endoenzyme from the group of amiolitic enzymes. It catalyzes the hydrolysis of α-1,4 internal glycosidic linkages in polysaccharides, in a disorderly manner, resulting in a mixture of glucose and maltose . The conditions under which the enzyme reaction takes place - pH of the medium, temperature, pressure, concentration of enzyme and substrate determine its effectiveness. The study of the kinetics of the enzyme reaction is limited to the determination of its rate under the given experimental conditions and detection of the kinetic parameters K_m and V_{max} [11].

The purpose of this work is to determine the optimum temperature and pH of the medium for carrying out the hydrolysis of soluble starch with a thermostable α-amylase in order to calculate the values of the kinetic parameters K_m and V_{max} using the equations of Michaelis - Menten [6], Lineweaver - Burk [5], Hanes - Woolf [1], Eadie - Hofstee [2], and comparison of the values obtained.

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2. Materials and methods

2.1. Materials

A soluble starch (POCh, cat. number 789820424) was used as a substrate. Working substrate solutions at concentrations from 0.50 $g.l^{-1}$ to 3.00 $g.l^{-1}$ were prepared in phosphate buffer (pH 5.8) containing 0.02 $mol.l^{-1}$ potassium dihydrogen phosphate and 0.02 $mol.l^{-1}$ disodium hydrogen phosphate three hydrate. It was used thermostable α-amylase from *B. licheniformis*. The working enzyme solution was prepared by appropriate dilution with phosphate buffer. Iodine and potassium iodide were used to prepare an iodine solution. For this purpose 0.50 g iodine and 5.00 g potassium iodide were diluted in volumetric flask of 100 ml. The working iodine solution was diluted 100 times. As a stopping reagent was used 0.1 $mol.l^{-1}$ hydrochloric acid.

2.2. Experimental procedure

The iodine assay method [10] with some modifications was used. Into a test tube was added 0.20 ml of substrate solution (with concentration from 0.50 $g.l^{-1}$ to 3.00 $g.l^{-1}$) and kept for two minutes at 75 ºC in the thermostable water bath (MLW W3). To these solutions was added 0.20 ml of the working enzyme solution and incubated exactly for five minutes. The reaction was stopped by adding of 0.20 ml hydrochloric acid (0.1 $mol.l^{-1}$). After mixing 4.00 ml iodine solution was added to the reaction mixture. The test tube was cooled into a cold water for five minutes. Absorbance was measured at wavelength 590 nm using spectrophotometer (Cary 100 Varian) against a blank containing 4.00 ml iodine solution, 0.40 ml phosphate buffer and 0.20 ml hydrochloric acid (0.1 $mol.l^{-1}$) and the path length 1 cm. Absorbance values were converted to starch concentration (a concentration of consumed starch during the hydrolysis for the minute) by using of starch calibration curve.

2.3. Effect of substrate concentration on the initial reaction rate

In order to study the effect of substrate concentration on the reaction rate the experimental procedure was performed at different initial concentration – from 0.50 $g.l^{-1}$ to 3.00 $g.l^{-1}$, temperature 75 ºC and pH 5.8 followed the experimental procedure. The kinetic constants - K_m and V_{max} were determined using Michaelis - Menten equation and the other its forms – equations of Lineweaver - Burk, Hanes - Woolf and Eadie - Hofstee. It was used a linear fit regression analysis for the last three equations in order to compare the obtained results.

2.4. Effect of temperature on the reaction rate

The temperature effect on the reaction rate was studied at five different temperatures, respectively 60, 70, 75, 80 and 90 ºC for five minutes at pH 5.8, working substrate solution 2.00 $g.l^{-1}$ and the same dilution of the enzyme solution.
It was found that the reaction rate is highest at pH 5.8 and temperature 75 ºC. The results are represented in Table 1.

Table 1. Influence of temperature on the reaction rate

Temperature, ºC	v_0, $\mu g.ml^{-1}.min^{-1}$	pH	v_0, $\mu g.ml^{-1}.min^{-1}$
60	15.11	5.5	2.99
70	16.34	5.8	3.98
75	16.52	5.9	3.82
80	16.08	6.0	3.50
90	14.31	6.5	2.95

2.5. Effect of pH value on the reaction rate

The reaction rate was determinate at different pH values in the range from 5.5 to 6.5 at room temperature, concentration of substrate solutions 2.00 $g.l^{-1}$ and one and the same dilution of the enzyme solution.

3. Results and discussions

Kinetic parameters

Michaelis - Menten model

The kinetic parameters in this model (K_m and V_{max}) were determined by fitting the Michaelis - Menten equation [6, 11] to the experimental data obtained according to eq. (1):

$$v_0 = \frac{V_{max}.C_S}{K_m + C_S} \quad (1)$$

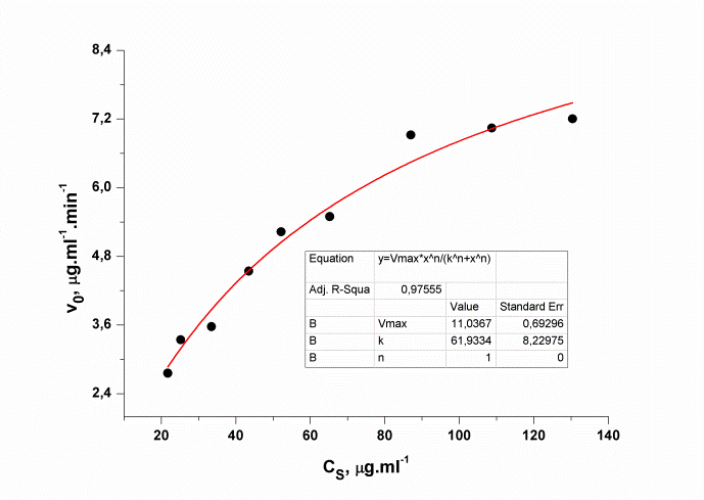

Figure 1. Michaelis-Menten plot of the experimental data

Lineweaver- Burk model

The experimental data were fit to the following equation:

$$\frac{1}{v_0} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} . \frac{1}{C_S} \quad (2)$$

The plot [5, 11] is represented as $\frac{1}{v_0} = f\left(\frac{1}{C_S}\right)$

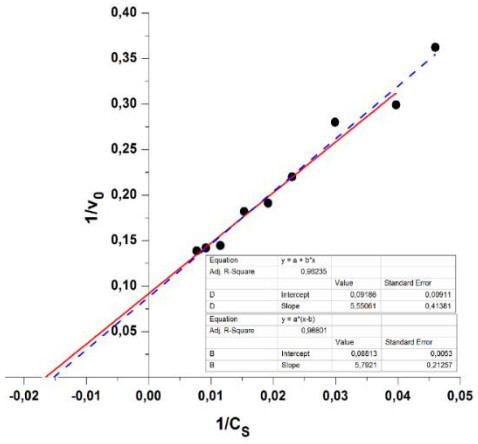

Figure 2. Lineweaver- Burk graph of the experimental data

Hanes – Woolf model

The data was fit according to the equation:

$$\frac{C_S}{v_0} = \frac{1}{V_{max}}.C_S + \frac{K_m}{V_{max}} \quad (3)$$

Hanes - Woolf plot [1, 11] is represented as $\frac{C_S}{v_0} = f(C_S)$,

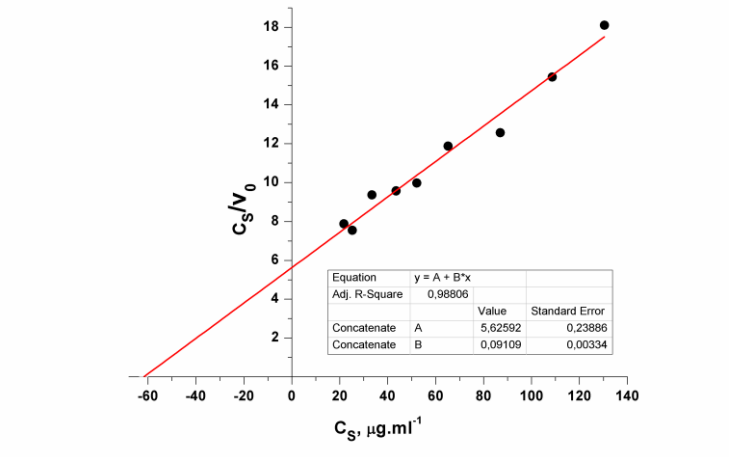

Figure 3. Hanes – Woolf plot of the experimental data

Eadie – Hofstee model

$$v_0 = V_{max} - K_m.\frac{v_0}{C_S} \quad (4)$$

Eadie – Hofstee plot [2, 11] is represented as $v_0 = f\left(\frac{v_0}{C_S}\right)$.

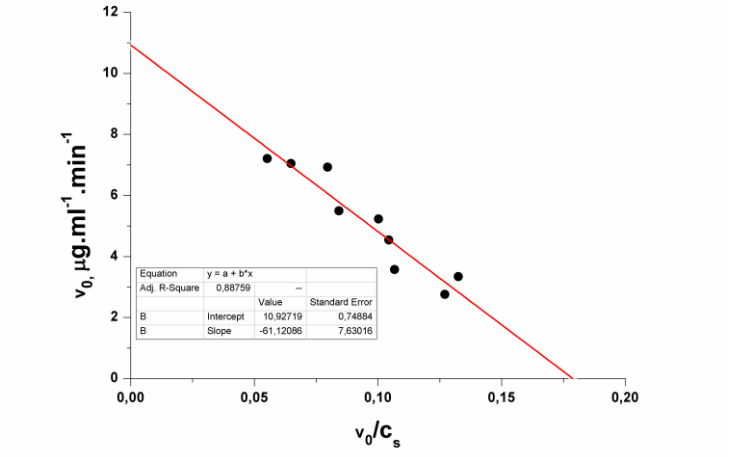

Figure 4. Eadie – Hofstee plot of the experimental data

The values of K_m and V_{max} obtained through different methods are represented in Table 2. The results obtained by Lineweaver - Burk method (full data range)/ were higher than those obtained by the other three methods. Previous studies have shown that at low substrate concentrations may be observed deviations, which affects the value of the slope. It is therefore recommended this model to be applied at high substrate concentrations.

The evaluation of V_{max} with high precision by Lineweaver-Burk method requires much higher concentrations (the values of 1/S are closer to the origin). Inaccuracy of measurements in lower concentration range strongly influence on the slope of K_m/V_{max} line and hence on the value of V_{max}.

The second approach provides better distribution of most of the values of V and in this way is possible more accurate determination of the slope of $1/V_{max}$, but in most cases the cut is small, which prevents precise determination of K_m by this method. The third method has the disadvantage that the variable v is a component of both the function and the argument. As a good approach may be advisable first to determine V_{max} by Lineweaver - Burk method, which gives a more accurate value for the cut or method Eddie - Hofstee which determines the slope of the line. Then from the graph V = f(S) is possible to find S , i.e. this substrate concentration at which $V = V_{max}/2$ [8].

Table 2. Values of K_m and V_{max}

Equation	K_m, μg.ml^{-1}	V_{max}, μg.ml^{-1}.min^{-1}
Michaelis – Menten	61.93	11.04
Lineweaver- Burk (blue dash line)	65.72	11.35
Lineweaver- Burk (red line)	60.42	10.89
Hanes - Woolf	61.76	10.98
Eadie – Hofstee	61.12	10.93

Conclusion

It was carried out enzymatic hydrolysis of soluble starch with a thermostable α-amylase. It has been found that the optimum conditions for the reaction are pH of the medium 5.8 and temperature 75 ºC. The kinetics parameters of the enzyme reaction derived from the equations of Michaelis - Menten, Lineweaver - Burk, Hanes - Woolf and Eadie - Hofstee are comparable in value.

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