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A STUDY OF THE OPTIMAL CONDITIONS FOR STARCH HYDROLYSIS THROUGH THERMOSTABLE α - AMYLASE

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ABSTRACT

The present work determines the optimal conditions for starch hydrolysis by thermostable α -amylase (EC 3.2.1.1) produced by Bac.subtilis strain XK-86. The hydrolysis reaction has the greatest rate at pH = 7.0, starch substrate concentration 250 g.l^{-1}, enzyme concentration – 12 enzyme units per ml suspension and 90^{o} C temperature. We show that the enzyme is inhibited by high concentrations of the substrate (above 250 g.l^{-1}), as well as by glucose. We establish that the enzyme under consideration gives a high reaction rate of the hydrolysis and high concentration of the reducing sugars in a relatively short period of time – 4 hours and 15 min.

Keywords: starch hydrolysis, thermostable α - amylase.

INTRODUCTION

Starch hydrolysis is a widely used process in various industries. The production of low molecular mass products from starch substrate underlies the sugar, brewing, spirits, textile, some food processing and other industries. At present starch hydrolysis is carried out in two basic ways – acidic and enzymatic. The older and more traditional method is acidic hydrolysis which requires highly acidic medium (pH = 1 – 2) obtained through mineral acids; high temperatures (150 – 230^{o} C) and high pressure [1 - 4]. As a result of the thermal processing, acidic hydrolysis produces unnecessary byproducts which contaminate the end-product - hydrolysate. The enzymatic hydrolysis of starch is carried out under milder conditions: lower temperatures (up to 100^{o} C), normal pressure, pH of the medium around 6 – 8 [2, 5, 6]. At the same time enzymatic hydrolysis is characterized by high reaction rate, high stability of the enzyme towards the denaturizing action of solvents, detergents, proteolythic enzymes, and a decrease in the viscosity of the reaction medium at higher temperatures, etc. Most often, enzymatic hydrolysis is carried out with the enzyme α-amylase (EC 3.2.1.1) from different sources and less often β- amylase is employed. The bacterial α-amylase enzymes attack the polysaccharide molecules in the inner part of the chain. They act on the amylose of starch so that they destroy the spiral of the polysaccharide chain and thus the characteristic blue color with iodine disappears. The viscosity of the starch solutions is quickly lowered. In the beginning dextrines are obtained, and if the enzyme acts continuously, maltose accumulates, in which one of the molecules of glucose has a free glucoside group and hence reducing properties.

The basic parameters which affect the hydrolysis process – temperature, pH of the medium, concentration of the substrate and concentration of the enzyme –

usually vary depending on the source of the enzyme. Most often the hydrolysis with thermally resistant -amylase is carried out at temperature 90 – 100° C [2, 6], concentration of the substrate in the suspensions varying from 20 % to 35 % [2, 5, 6, 7], pH between 6 and 8, and enzyme concentration 0.03 – 1 % [2, 6, 7].

The purpose of the present paper is to determine the optimal conditions for enzymatic hydrolysis of starch through the use of thermally resistant bacterial -amylase, produced from strain of Bacillus Subtilis XK – 86 in the plant Biovet, Peshtera, Bulgaria.

EXPERIMENTAL

We employ thermostable -amylase, produced from strain of Bacillus Subtilis XK – 86 with level of activity 1000 Ug^{-1}. The enzyme level of activity is expressed in amiolytic units that give the amount of enzyme which hydrolyses 1 g of starch to 30 % level of hydrolysis at 30° C, pH = 6.0 in 10 min. "Purum"-grade starch, produced by Fluca is used as a substrate. D-glucose, p.a. grade, produced by Fluca is used as an inhibitor.

Phosphate buffer containing 1/15 mol l^{-1} solutions of KH_2PO_4 and Na_2HPO_4, pH = 7.0 is used as medium for carrying out the enzyme hydrolysis of starch.

The experiments are performed in a glass cell with 150 ml volume and constant stirring of the suspension. The suspensions of the substrate are prepared with phosphate buffer solution to which the starch is added gradually while constantly stirring. The obtained suspension is heated to the desired temperature and after its attainment, the enzyme is progressively added.

At various intervals, samples of 1.00 ml volume are taken from the obtained hydrolysate and placed in a volumetruk flask of 100 ml. Immediately 0.5 ml 2 mol/l^{-1} HCl is added to the sample to stop the hydrolysis. The reducing sugars obtained during the starch hydrolysis are determined by the complexometric method we have developed earlier [8]. According to this method, 25 ml distilled water and 25 ml of both Fehling I and II solutions are added to the sample hydrolysate. The sample is heated with a boiling water bath for 5 min. The flask is cooled down and filled up to the mark with distilled water, after which the sample is homogenized. The red residue of Cu_2O thus obtained is filtered through a paper filter for small crystalline residues. A sample with 25 ml volume is taken from the filtrate and transferred to an Erlenmeyer flask of 300 ml volume. 8 ml acetate buffer solution (2 mol.l^{-1}, pH = 5 - 5.1) and 0.10 – 0.15 g pyridilazoresorcin (PAR : KNO_3 1:100) are added to the sample in the flask. The excess Cu^{2+} cations are titrated with a standard solution of ethylendiaminetetraacetic acid (EDTA), until the color of the solution changes from red to yellow – green. The amount of the reducing sugars like glucose or inverted sugar contained in the sample is calculated from the quantity of the Cu^{2+} from the Fehling solution which have undergone a reaction with the sugar, by using the Bertrand tables [4].

RESULTS AND DISCUSSION

To study the optimal conditions for the hydrolysis of starch with the a – amylase enzyme XK – 60 under consideration, we have varied the following factors: concentration of the substrate, concentration of the enzyme, temperature of the hydrolysis, pH and the influence of inhibitors.

In order to determine the optimal concentration of the substrate we have done experiments in the range from 100 to 300 g.l^{-1} starch. From the results presented in Table 1 it is evident that for substrate concentrations 250 and 300 g.l^{-1}, the level of hydrolysis is almost the same (30.1 % and 30.3 % respectively). We supposed that this was most likely due to the inhibiting action of the reducing sugars obtained during hydrolysis at substrate concentrations higher than 250 g.l^{-1}. To prove this hypothesis, we investigated the influence of the concentration of the glucose added to the substrate on the rate of the enzymatic reaction. As can be seen from Fig. 1, the addition of glucose in the beginning of the hydrolysis process significantly reduces the reaction rate of the process. The reduction rate is proportional to the amount of added glucose.

These data proved our assumption for the inhibiting action of the reducing sugars obtained during the hydrolysis process on the employed enzyme at higher substrate concentrations (greater than 250 g.l^{-1}).

It is well known that the maximum rate of the enzymatic reaction is proportional to the enzyme concentration. This is why determining the optimal

Table 1. Studying the influence of the substrate and enzyme concentrations on the level of enzymatic starch hydrolysis (30 min after the beginning of hydrolysis).

Influence of the substrate concentration		Influence of the enzyme concentration*	
Concentration of the substrate g.l^{-1}	% hydrolysis of starch	Enzyme concentration: units per ml suspension	% hydrolysis of starch
100	18.2	6	8.2
150	22.5	8	12.4
200	27.1	10	25.2
250	30.1	12	30.1
300	30.3	14	30.2

*All experiments are performed under the following conditions: 250 g.l^{-1} substrate concentration, hydrolysis temperature 90^{o} C; pH = 7.

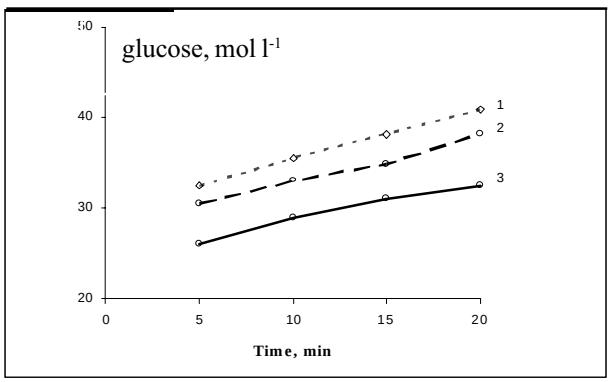

Fig. 1. Influence of the addition of glucose: reducing sugars plotted versus time. Glucose: curve 1 – none added; curve 2 – 0.05 mol l^{-1}; curve 3 – 0.1 mol l^{-1} Substrate concentration 130 g.l^{-1}; 90^{o} C; pH = 7; enzyme concentration 12 units per ml suspension.

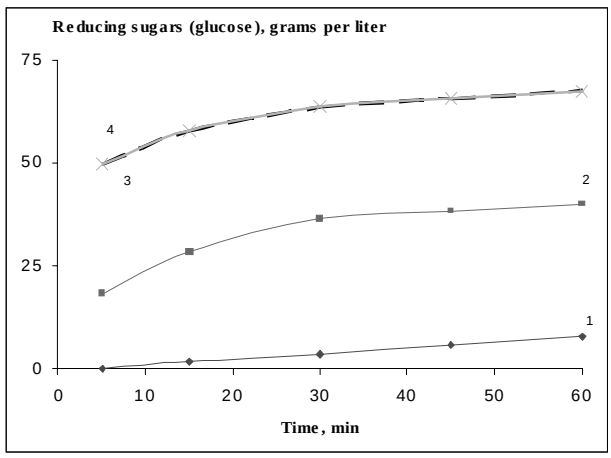

Fig. 2. Comparison of the concentrations of the obtained reducing sugars at different temperatures of hydrolysis; Substrate concentration 250 g.l^{-1}, pH = 7, enzyme concentration 12 units per ml suspension. Curve 1 – 30^{o} C; curve 2 - 60^{o} C, curve 3 - 90^{o} C, curve 4 - 100^{o} C.

concentration of the enzyme is very significant for the study of this process. We examined this influence in the concentration interval 6 – 14 enzyme units per ml suspension. Through experiments we established that for the enzyme employed, the optimal concentration was 12 enzyme units per ml suspension, since the use of higher concentrations did not lead to an increase in the rate of the enzymatic reaction (Table 1).

The results obtained from studying the influence of temperature on the hydrolysis rate are presented in Fig. 2. In order to determine the optimal temperature, we plotted the amount of reducing sugars obtained during hydrolysis over a period of 60min at four different temperatures – 30^{o} C, 60^{o} C, 90^{o} C and 100^{o} C. Temperatures higher than 100^{o} C were not examined due to the intensive boiling and formation of foam of the suspension at atmospheric pressure. As the rate of the enzy-

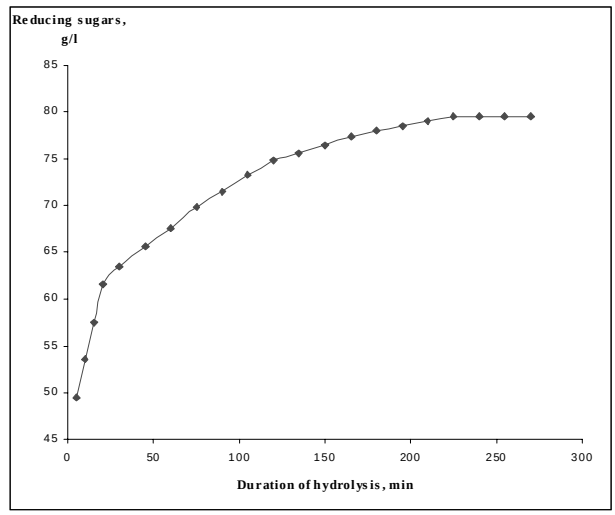

Fig. 3. Concentration of the reducing sugars as a function of the duration of hydrolysis Substrate concentration 250 g.l^{-1}, pH = 7, enzyme concentration 12 units per ml suspension, temperature 90^{o} C.

matic reaction is almost identical at 90° C and 100° C, we chose 90° C as the optimal temperature for carrying out of the hydrolysis. At this temperature, foam almost does not form if the suspension is continuously stirred.

The influence of pH at the rate of the enzymatic hydrolysis was studied in the range 6 – 8, maintained through phosphate buffers, containing 1/15 mol l^{-1} solutions of KH_2PO_4 and Na_2HPO_4. The maximum rate of the enzymatic reaction is observed at pH = 7 for the already determined optimal conditions for substrate and enzyme concentrations and temperature of hydrolysis 90°C.

Fig. 3 presents the hydrolysis curve – the concentration of the reducing sugars obtained as a function of time. The maximum amount of reducing sugars and hence the maximum level of hydrolysis at the predetermined optimal parameters is observed after 4 hours and 15 minutes, which shows that the enzymatic reaction is carried out with high rate. For hydrolysis with another thermally stable -amilase, produced from Bac. Licheniformis MB – 80 the duration is above 7 hours [2].

CONCLUSIONS

• The hydrolysis of starch with thermally stable -amilase, produced from strain of Bacillus Subtilis XK – 86, is carried out with maximum rate at pH=7, concentration of the substrate 250 g l^{-1}, concentration of the enzyme 12 units per ml suspension and temperature 90°C.

• The substrate exerts strong inhibiting influence on the enzyme activity at concentrations greater than 250 g.l^{-1}.

• We have established that with the studied enzyme we obtain a high rate of hydrolysis and thus high concentration of reducing sugars in the framework of a relatively short duration– 4 hours and 15 min.

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