STUDIA UBB. CHEMIA, LV, 3, 2010

STARCH HYDROLYSIS WITH COMMERCIAL ENZYME PREPARATES

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ABSTRACT. There has been a growing interest in the use of starch containing wastewaters in bioprocessing technologies with various commercial available amylolytic enzyme preparates. In this paper for the enzymatic hydrolysis of residual starch in waste waters is described.

Keywords: *Amylase, starch hydrolysis, bioprocessing*

INTRODUCTION

Starch is the most abundant storage polysaccharide in plants. Hence, its application is very important in the food industries such as in the production of oligosaccharides and glucose by starch hydrolysis. Acid splitting is the traditional method for the production of glucose syrup. However, the procedures are not suitable for industrial mass production since the products are rather complicated and require high purification costs. And since the cost of using starch-hydrolyzed enzymes is lower and its procedures are much simpler, it has become the main method in starch hydrolysis [1,2]. A number of enzymes are used in starch hydrolysis. Glucoamylase is one of the key enzymes used for starch processing which has extensive uses in the manufacture of crystalline glucose or glucose syrup, either as soluble or immobilized enzymes. The enzyme hydrolyzes _-1,4- and -1,6-glycosidic linkages of starch to produce glucose [3].

Microbial communities can be found in the most diverse conditions, including extremes of temperature, pressure, salinity and pH. These microorganisms, called extremophiles, produce biocatalysts that are functional under extreme conditions.The biocatalysts obtained from these microorganisms could be applicable in similarly diverse conditions [4].

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Thermophilic extremophiles have attracted most attention. In particular extremophilic proteases, lipases and polymer-degrading enzymes, such as cellulases, chitinases and amylases have found their way into industrial applications.

The reasons to exploit enzymes that are stable and active at elevated temperatures are obvious. At elevated temperatures the solubility of many reaction components, in particular polymeric substrates, is significantly improved. Moreover, the risk of contamination, leading to undesired complications, is reduced at higher temperatures. Enzymes from microorganisms that can survive under extreme pH could be particularly useful for applications under highly acidic or highly alkaline reaction conditions, for example in the production of detergents and starch hydrolysis. Several enzymes used for starch-hydrolysis (e.g. amylases, pullulanases, glucoamylases and glucosidases that are active at low pH) have been isolated [5].

In this work, with the aim to develop an efficient process for the enzymatic hydrolysis of residual starch in wastewaters, the use of several commercial available amylolytic preparates was tested. The optimal conditions were determinated in each case.

RESULTS AND DISCUSSION

The enzymatic activity of commercial available amylolytic preparates was first determinated in a prescreening test by measuring the concentration of starch solution before and after 10 min. enzymatic treatment, using the collorimetric method (see experimental part).

One international unit (IU) of amylolytic preparate is defined as that amount of enzyme which hydrolyse 1 μmol of starch per min under the specified conditions.

Table 1. Enzymatic activity of commercial available preparates at pH 7 and 25 °C

Enzyme preparate	Absorbance at 580 nm		Enzymatic activity (IU)
	0 min	10 min	
Fungamyl 800L	0,965	0,604	4,5
DextrozymeDX1.5X	0,416	0,385	0,9
Finizym W	2,867	1,885	4,1
Dextrozyme GA	0,392	0,333	1,8
Shearzyme	2,984	2,737	1,0
Termamyl	2,673	2,643	0,1
Liquizyme	1,072	0,976	1,1

The most efficient catalyst for enzymatic hydrolysis of starch proved to be Fungamyl 800L (4.5 IU) and Finizym W (4.1 IU).

1. The influence of enzyme-substrate ration

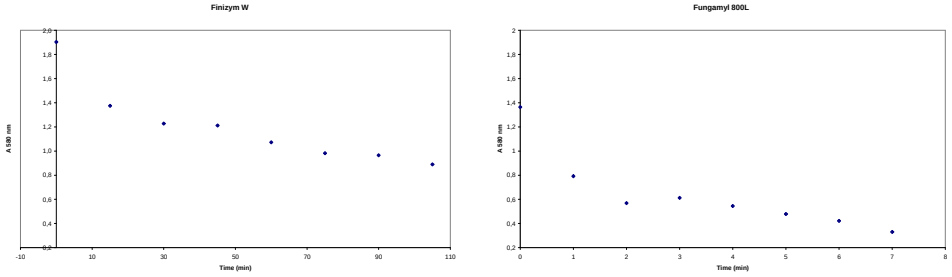
Using 3 mL of starch solution and the two best enzyme praparates, the evolution of enzymatic hydrolysis was monitored by measuring the starch concentration after 10 min. as described, using the calibration curve.

Table 2. The influence of enzyme-substrate ratio at pH 7 and 25 °C

Entry	Enzyme preparate	Volume enzyme preparate (μL)	Enzymatic activity (IU)
1	Finizym W	100	0,1
2		200	***7,9***
3		300	4,9
4		400	3,0
5	Fungamyl 800L	1	1,7
6		2	3,0
7		3	7,3
8		4	***10,4***
9		5	8,4
10		6	9,1
11		7	9.8
12		8	9,9
13		9	9,7

As observed in Table 2, Fungamyl 800L is more active as Finizym W. The best results were obtained when 200 μL of Finizym W (Table 2, entry 2) or 4 μL of Fungamyl 800L (Table 2, entry 8) were used for hydrolysis 3 ml 4% starch solution at pH 7 and 25 °C.

In these cases, the concentration of starch was monitored for 2 hours (Figure 1) measuring the absorbance as described earlier.

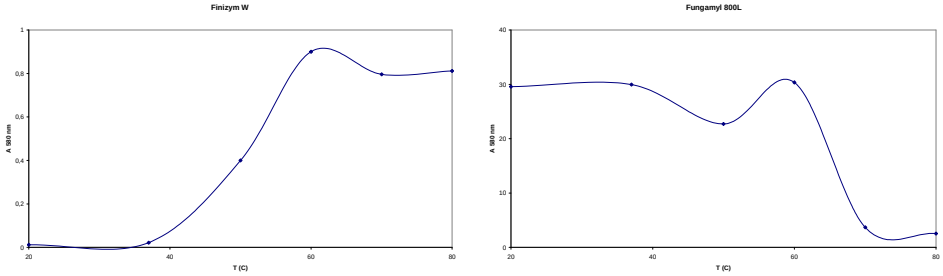


200 μL Finizym W 4 μL Fungamyl 800L

Figure 1. Decreasing of starch concentration in time

2. The influence of temperature on the enzymatic hydrolysis of starch

The temperature dependence of the hydrolytic activity of tested enzyme preparates is shown in Figure 2. The optimum reaction temperature was at 60 °C for both cases.

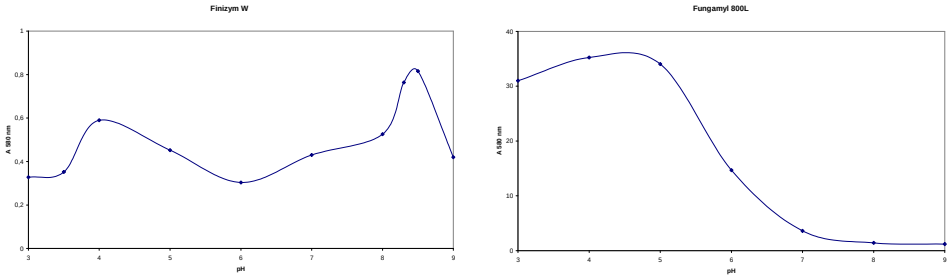


200 µL Finizym W 4 µL Fungamyl 800L

Figure 2. The inffluence of temperature on the enzymatic hydrolysis of starch

3. The influence of pH on the enzymatic hydrolysis of starch

The effect of pH on the enzymatic activity of tested enzyme preparates was studied by varying the pH of the reaction medium from 3 to 9 at an interval of 1 units and the pH profile is shown in Figure 3.



200 µL Finizym W 4 µL Fungamyl 800L

Figure 3. The inffluence of pH on the enzymatic hydrolysis of starch

CONCLUSIONS

Some commercial available amylolytic enzyme preparates were tested for the hydrolysis of residual starch in waste waters from the food industry. For the two best preparates, the optimum conditions (substrate-enzyme ratio, temperature and pH) were experimental determinated.

Table 3. The optimum conditions for enzymatic hydrolysis of starch

Enzyme preparate	Substrate*-preparate ratio (V/V)	Temperature (°C)	pH
Finizym W	15	60	8.5
Fngamyl 800L	750	60	4

* 4% starch solution

EXPERIMENTAL SECTION

Materials and methods

The next enzyme preparates, commercial available by Novozym, Denmark, were used: Fungamyl 800 L (a fungic α-amylase from *Aspergillus oryzae*), Dextrozyme GA and Dextrozyme DX 1.5X (α-amylases), Finizym W (a fungic β-D-glucanase with residual phospholipase activity), Thermamyl (an α-amylase from *Bacillus licheniformis*), Shearzyme 500 L (an endo-1,4-xilanase from *Aspergillus oryzae*) and Liquizyme Supra.

Stoc solutions

- 4% starch solution in tested buffer
- 6% acetic acid
- 0.0005 N iodine solution

Methods

The amounts of unhydrolyzed starch was determined spectrophotometrically in presence of iodine, measuring the absorbance of the blue inclusion complexe at 580 nm [6].

The hydrolysis experiments were performed at controlled temperature and pH using starch solutions (4%, 10 mL) in the corresponding buffer. After adding the enzyme, samples (3 mL) were taken at 0 and 10 min and 6% acetic acid solution (1 mL) was added for stopping the hydrolysis. In each sample, iodine solution (0.0005N, 1 mL) was added and the absorbance of formed blue complex was measured at 580 nm.

ACKNOWLEDGEMENTS

The financial support from the Romanian Ministry of Education and Research (PNCD II Project Nr. 51001/2007, Valorificarea soluțiilor reziduale de amidon în scopul modernizării producției alimentare REZAMIDO) is gratefully acknowledged.

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