

One Step Industrial Enzymatic Technology of Starch Hydrolysis to Glucose

Kvesitadze G.^{1,2,*}, Khvedelidze R.², Urushadze T.², Kutateladze L.², Zakariashvili N.², Jobava M.², Sadunishvili T.^{1,2}

¹ Georgian National Academy of Sciences, 52 Rustaveli Ave., 0108, Tbilisi, Georgia

² Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Georgia. 240 David Aghmashenebeli Alley, 0159 Tbilisi, Georgia

*corresponding author: e-mail: kvesitadze@hotmail.com

Abstract

Starch degrading enzymes like amylase have received great attention because of their technological significance and economic benefits. As a result of screening of Durmishidze Institute of Biochemistry and Biotechnology collection of mycelial fungi, accounting 2500 individual strains, 39 strains producing amylases have been revealed. Three promising enzyme producer strains of genus *Aspergillus* have been selected and technical preparations of their amylases obtained. The hydrolysis process of starch of different concentrations with the technical preparation of *Aspergillus niger* p8-3 at 68°C was studied. 94- 96 % yield of glucose was reached at incubation of 30% and 40% starch with technical preparations of fungal enzymes during 8 hours.

Keywords: microscopic fungi, amylases, hydrolysis, glucose, technology

1. Introduction

Application of amylases (α - and glucoamylases) is an obligatory technological operation in the production of glucose-fructose syrup from starch containing substrates (wheat, corn, potato, maize, buckwheat peas, etc.) have been prevalent for many decades and a number of microbial sources exist for the efficient production of this enzyme (Brahmachari et al., 2017). Various manufacturers use different approaches to starch hydrolysis using amylases but the principles are the same – gelatinization, liquefaction and saccharification of starch. In modern starch-based industries process of obtaining fermentable sugar glucose from starch containing raw materials by means of amylases is running in two steps, involving two enzymes of different origin: bacterial α -amylase and fungal glucoamylase (Uthumporn et al., 2010). These two enzymes cannot be used simultaneously due to their different heat stability, with a challenge of thermostability of fungal amylases (Haki and Rakshit, 2003). Consequently, the selection of stable, operable at pasteurization and higher temperatures amylase preparation with both α - and glucoamylase activities will allow conducting a process of starch hydrolysis in one step, using only one enzyme preparation.

2. Materials and Methods

The screening was performed by deep cultivation in a liquid medium of following composition, %: starch-6,0; NaNO₃-0,91; KH₂PO₄-0.1; MgSO₄·7H₂O-0,05, KCl-0.05, FeSO₄ -0.0002; malt sprouts –1.5 per 50 ml in 50-ml Erlenmeyer conic flasks, on temperature-controlled rotary shaker (180-200 rpm) at 30°C for 72 hours. Determination of α -amylase activity was conducted according to staining value of blue starch-iodine complex. Glucoamylase activity was determined using a glucooxidase reagent (Bergmeyer H.V. and Bernt, 1974). To obtain enzymatic preparations, filtrate of culture liquid was cooled and glass filtered. Ethyl alcohol was added (3.5:1) to chilled enzyme solution under the conditions of gradual stirring. Pellet was removed by centrifugation (4000 rpm, 10 min) and lyophilized. Optimum conditions of starch enzymatic hydrolysis was established according to yield of produced glucose at different substrate enzyme ratio, pH, temperature and duration of hydrolysis

3. Results and Discussion

Durmishidze Institute of Biochemistry and Biotechnology (DIBB) owns a unique collection of mycelial fungi. As a result of screening, several strains producing stable forms of amylases with different degree of α - and glucoamylase activities were revealed. Conduction of the fermentation processes at pasteurization temperature (65°C) is of great importance as it minimizes pollution of the reaction medium. Temperature optima of amylases technical preparations from selected three microscopic fungi strains were established to be within the range 67-80°C. Different strains of *Aspergillus niger* expressed different α -amylase and glucoamylase activities at different temperatures. *A. niger* B-6: high α -amylase activity at 67°C and glucoamylase activity at 62°C; *A. niger* 6-12: high α -amylase activity at 72°C, and glucoamylase activity at 65°C; *A. niger* p8-3: high α -amylase activity at 82°C and high glucoamylase activity at 65°C (Fig.1).

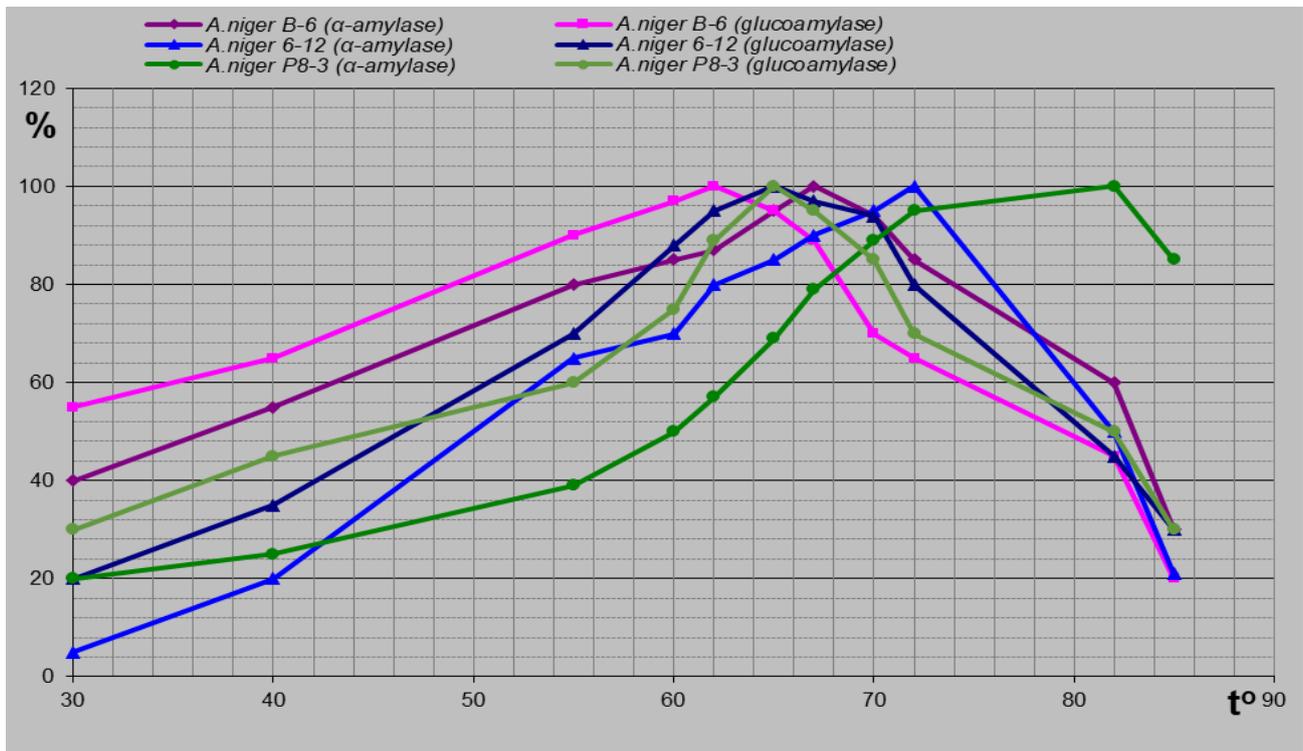


Fig 1. Temperature optima of amylolytic activities of *Aspergillus niger* strains

Crude preparations of most active amylases were obtained from culture liquids by precipitation with ethyl alcohol. Exhaustive hydrolysis process of starch solutions of different concentrations (1, 3, 5, 10, 30 and 40 %) with the enzyme preparation of *Aspergillus niger* p8-3 at 68°C was studied. In case of starch low concentrations (1-10%) exhaustive hydrolysis of the substrate needs 10–60 minutes; in case of high concentrations, hydrolysis takes longer time. 96% yield of fermentable glucose was reached by hydrolyses of 30% and 40% starch with amylase enzyme preparation of *A. niger* p8-3 during 8 hours. Based on these data we have elaborated original technology of starch enzymatic hydrolysis. Initially, for gelatinization of insoluble in cold water starch, it is gradually heated to 80°C (thermal effect) and first portion of enzyme preparation is added. At this temperature, optimum for α-amylase of our enzyme preparation partial hydrolysis of starch to dextrins and oligosaccharides during 15-20 minutes takes place. As a result, starch is liquefied with concomitant loss in viscosity. Further, system is cooled to 65°C and new portion of the enzyme preparation is added. Glucoamylase at optimum for its action temperature catalyses saccharification, resulting in the production of glucose (96%) and disaccharide maltose (3-4%).

4. Conclusions

Aspergillus niger p8-3 strain have been selected from microscopic fungi collection of DIBB, producing heat stable amylases with complete set of enzymes. A one step starch hydrolysis technology has been elaborated based on enzyme technical preparation containing heat stable α-amylase with optimum temperature of action at 80°C and glucoamylases with optimum temperature of action at 65°C capable to starch efficient hydrolysis to glucose with 94-96% yield. The offered technology is: ecologically friendly as compared to chemical hydrolysis; cost-effective as is based on application of one fungal enzyme preparation differently from alternative technologies based on the use of two enzyme preparations: bacterial α-amylase and fungal glucoamylase.

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