International Journal of Microbiological Research 2 (3): 204-207, 2011
ISSN 2079-2093

Production of Glucoamylase by *Aspergillus oryzae* Under Solid State Fermentation Using Agro Industrial Products

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Abstract: The use of agro industrial products (wheat bran and sugar cane bagasse) present a great potential as substrate and support the low production costs for glucoamylase production under solid-state fermentation by *Aspergillus oryzae*. The effect of various factors on amylase production was examined. Cultivation was carried out at temperatures 30, 40, 50, 60 and 70°C for 120 hrs. Study of influence of pH initial in SSF was conducted with pH 3.0, 4.0, 5.0, 6.0 and 7.0. The results showed that wheat bran and sugar cane bagasse powder at the ratio of 1:1 was the best for optimum production of glucoamylase. The maximum yield was achieved with optimized process parameters such as incubation period (120 hours), pH 5.0 and fermentation temperature (60°C). In kinetic characterization of enzymes the Michaelis-Menten relationship is well expressed that shows the real potential of this organism for production of glucoamylase.

Key words: Glucoamylase · *Aspergillus oryzae* · Solid state fermentation · Agro residues

INTRODUCTION

Amylases are among the most important enzymes and are of great significance in present day biotechnology, having approximately 25% of the enzyme market [1]. New amylases could be potentially useful in the pharmaceutical and fine-chemical industries if enzymes with suitable properties could be identified. With the advent of new frontiers in biotechnology, the spectrum of amylase applications has expanded into many other fields, such as clinical, medicinal and analytical chemistry [2].

The amylase family has two major classes, namely amylase (EC 3.2.1.1) and glucoamylase (EC 3.2.1.3). α- amylase can hydrolyze starch into maltose, glucose and maltotriose by cleaving the 1, 4-α-D-glucosidic linkages between adjacent glucose units in the linear amylose chain [3, 4]. Glucoamylase (GA) hydrolyzes single glucose units from the non-reducing ends of amylose and amylopectin in a stepwise manner and produce glucose as the sole end-product from starch and related polymers. Unlike α-amylase, most glucoamylases are also able to hydrolyze the 1, 6-α-linkages at the branching points of amylopectin, at a lower rate than 1, 4-linkages [3]. It is an important group of enzymes in starch processing. They are second to the proteases in worldwide distribution and sales among industrial enzymes. GA has manifold applications in industry. This enzyme is used in dextrose production, in the baking industry, in the brewing of low-calorie beer and in whole grain hydrolysis for the alcohol industry [5]. Glucoamylase is present almost exclusively in filamentous fungi and far less in bacteria and yeasts.

Because of their short growth period, the enzymes from microbial sources generally meet industrial demands. Growth conditions and nutrients promote high yields of microbial amylases [5]. However, carbon sources such as dextrin, fructose, glucose, lactose, maltose and starch are very expensive for commercial production of these enzymes. Being an agrarian country, various agricultural byproducts like wheat bran, rice husk, sugarcane bagasee, cotton seed meal, sunflower meal, oat bran are abundantly available for the conversion to products of economic importance. Bioconversion of these agricultural byproducts to enzymes like glucoamylase offers an alternative for their utilization [6].

Traditionally, glucoamylases have been produced by submerged fermentation (SmF). However, in recent years the solid-state fermentation (SSF) process is receiving a renewed surge of interest, primarily because of low-cost

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and increased productivity and prospects of using a wide range of agro industrial residues as substrates [6, 7]. This process has been applied increasingly for the production of glucoamylase. Agro-industrial residues are generally considered best substrates for the SSF processes and enzyme production by SSF is not an exception to that [8].

Various byproducts like cereal bran and flours, potato residue and other starchy waste materials have been utilized as fermentation substrate for glucoamylase production by filamentous fungi [9, 10, 11]. There has been an extensive study for glucoamylase production by *Aspergillus niger* on wheat bran in SmF and SSF [12]. Recently, *Aspergillus oryzae* has also been explored on various agro-residues as solid substrates for production of glucoamylase under SSF [13]. The present study was concerned with the optimization of cultural conditions for improved production of glucoamylase from the mixture of wheat bran and sugar cane bagasse powder.

MATERIALS AND METHODS

Fungal Isolates: *Aspergillus oryzae* was isolated from soil samples and characterized. It was maintained on Czapak Dox agar (CZA) slants at 30°C for 5 days.

Substrates: Commercially available wheat bran and sugarcane bagasse were obtained from the local market. These substrates were dried in an oven at 70°C to a constant mass, ground in a mill to 10 mm particle size and used as inducer substrates for GA production in SS in equal ratio [13].

Inoculum Preparation: Inoculum was prepared by transferring 5day-old slant culture into 250-mL Erlenmeyer flask containing 20 g. of substrate mixture with 50% moisture and incubated for 5 days at 30°C. After incubation, fermented mixture was mixed with 0.1% Tween-80. After 20 min, the mixture was filtered off through sterile glass wool to get spores. Spore count was determined by serial dilution and spread plating method [13].

Enzyme Production in Solid-state Fermentation: The static experiments were conducted in 250-mL Erlenmeyer flasks containing 20 g of substrate moistened with water. Flasks were plugged with cotton and sterilized by autoclaving for 15 min at 121°C. After sterilization, the flasks were cooled and inoculated with 10 % (by mass per volume) inoculum having 10^6-10^7 spores/ml. The flasks were incubated at (30±1) °C for 5 days [13].

Isolation of Glucoamylase: At the end of fermentation, the whole fermented mixture was treated with 100 ml of distilled water and agitated thoroughly on orbital shaker at 100 rpm for 2 hrs. The crude enzyme was filtered through muslin cloth. The residue was again treated with another 100 ml of distilled water in the same way and filtered. The filtrates were pooled and centrifuged at 10,000 rpm for 10 min at 4°C to remove the suspended particles and supernatant was used as enzyme source for assaying glucoamylase [13].

Glucoamylase Assay: GA activity was determined by taking an appropriate amount of the enzyme followed by the addition of 1 % soluble starch solution in 50 mM citrate buffer (pH=5.5) at 50°C for 20 min. The released glucose was measured with 3, 5-dinitrosalicyclic acid (DNSA) reagent [14] using glucose as a standard. Glucoamylase activity unit (U) was expressed as the amount of enzyme releasing one µ mole of glucose equivalent per minute per ml.

Optimization of the Production Process: The SSF of the selected substrates (wheat bran and sugarcane bagaase) was optimized by varying process conditions like pH (3-7), temperature (30-70°C) and substrate concentration resulting in the studies of kinetic parameters.

RESULTS AND DISCUSSION

Glucoamylase Production in Solid State Fermentation: In the solid state fermentation, the production of glucoamylase was reached maximum of 330 µg/ ml/ min at 5 days of incubation period. Further increase in incubation period did not show any significant increase in enzyme production rather it was decreased. Thus, optimum time of the enzyme synthesis was to be 5 days after inoculation.

Effect of pH: In the present study, the glucoamylase production by *A. oryaze* was found maximum at pH 5 (Fig. 1). Further increase in the pH resulted in decrease of the activity of glucoamylase.

Effect of Temperature: The maximum production of glucoamlyase was obtained at 60°C. Increase in incubation temperature decreased the production of the enzyme (Fig. 2). It might be due to that at high temperature, the growth of fungus was inhibited and hence, enzyme formation was also inhibited.

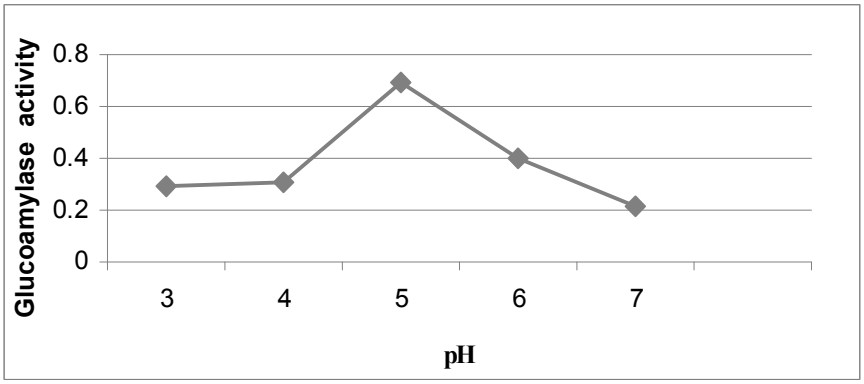

Fig. 1: Effect of pH on GA production by *A. oryaze* under optimum conditions: fermentation time 120 hrs, temperature (60)°C, inoculum size 10 % (by mass per volume), substrate 20g

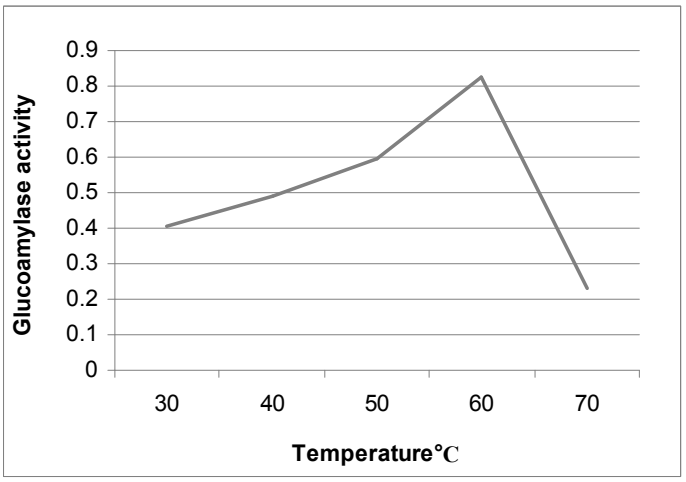

Fig. 2: Effect of Temperature on GA production by *A. oryaze*-under optimum conditions: fermentation time 120 hrs, pH 5, inoculum size 10 % (by mass per volume), substrate 20g

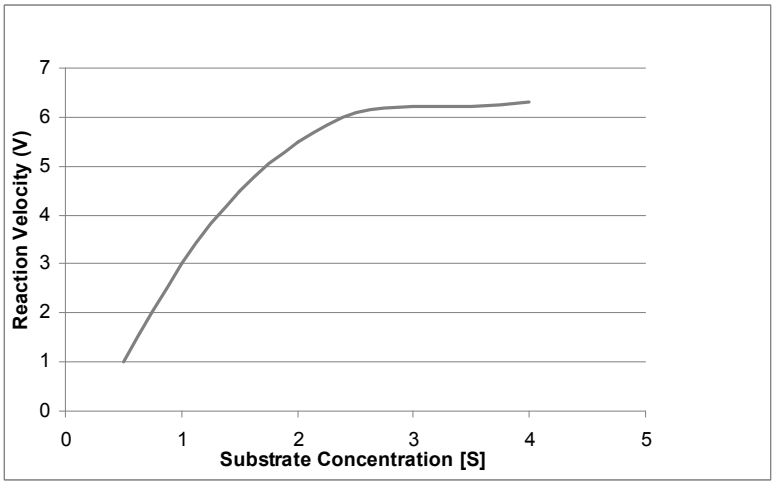

Fig. 3: Michaelis-Menten kinetics showing hyperbolic plot of glucoamylase activity

Enzymatic Kinetic Parameters: The kinetic parameters of the enzymatic activity using starch as a reaction limited substrate could be expressed using the simple Michaelis-Menten kinetics (Fig. 3) expressed by equation. The resulted plot has a slope equal K_m/V_{max} (K_m = substrate saturation constant and V_{max}= maximum velocity) an intercept equal $1/V_{max}$. This result showed that enzyme used in this study had a higher catalytic activity for starch. From the kinetic studies, it can be concluded that its industrial potential is high.

The kinetic parameters of glucoamylase activity give remarkable value of industrial applicability. The obtained results indicate that *Aspergillus oryzae* has potential for the production of glucoamylase of relatively improved stability at different conditions.

ACKNOWLEDGEMENT

The authors acknowledge Amity University for supporting this research on solid state fermentation.

REFERENCES

1. Rao, M.B., A.M. Tanksale, M.S. Gathe and V.V. Deshpande, 1998. Molecular and biotechnological aspects of microbial proteases. Microbiology and Molecular Biology Review, 62: 597-635.
2. Pandey, A., P. Nigam, C.R. Soccol, V.T. Soccol, D. Singh and R. Mohan, 2000. Advances in microbial amylases. Biotechnology Applied Biochemistry, 31: 135-152.
3. Fogarty, W.M., 1983. Microbial Amylases. In: Microbial Enzyme and Biotechnology, Applied Science Publishers, London, UK pp: 1-90.
4. Antranikian, G., 1992. Microbial Degradation of Starch. In: Microbial Degradation of Natural Products, G. Winkel, (Ed.), VCH Publishers, Weinheim, Germany, pp: 27-51.
5. James, J.A and B.H. Lee, 1997. Glucoamylases: Microbial sources, industrial applications and molecular biology-A review. J. Food Biochemistry, 21: 1-52.
6. Babu, K.R. and T. Satyanarayana, 1995. Amylase production by thermophilic *Bacillus coagulance* in solid-state fermentation. Process Biochemistry, 30: 305-309.
7. Park, Y.S., S.W. Kang, J.S. Lee, S.I. Hong and S.W. Kim, 2002. Xylanase production in solid-state fermentation by *Aspergillus niger* mutant using statistical experimental design. Applied Microbiology Biotechnol., 58: 761-776.
8. Ellaiah, P., K. Adinarayana, Y. Bhavani, P. Padmaja and B. Srinivasula, 2002. Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species. Process Biochemistry, 38: 615-620.
9. Biesebeke, R., E.N. Record, N. Van Biezen, M. Heerikhuisen, A. Franken, P.J. Punt and C.A. Van Den Hondel, 2005. Branching mutants of *Aspergillus oryzae* with improved amylase and protease production on solid substrates. Applied Microbiology and Biotechnol., 69: 44-50.
10. Joshi, V.K., A. Pandey and D.K. Sandhu, 1999. Waste treatments in fermentation technology. In Biotechnology: food fermentation. V.K. Joshi and A. Pandey, (Edn.). Vol. 2, Trivendrum, India: Educational Publication and Distribution, pp: 1291-1338.
11. Arasaratnam, V., K. Mylvaganam and K. Balasubramaniam, 2001. Improvement of glucoamylase production by *Aspergillus niger* in solid-state fermentation with paddy husk as support. J. Food Science and Technol., 38: 334-338.
12. Kaur, P., H.S. Grewal and G.S. Kocher, 2003. Production of α-amylase by *Aspergillus niger* using wheat bran in submerged and solid state fermentations. Indian J. Microbiol., 43: 143-145.
13. Zambare, V., 2010. Solid State Fermentation of *Aspergillus oryzae* for Glucoamylase production on agro residues. International J. Life Scie., 4: 16-25.
14. Miller, G.L., 1959. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. Analytical Chemistry, 31: 426-429.