

## Production of Glucoamylase by *Fusarium moniliforme* under Solid-State Fermentation

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**Summary:** Production of glucoamylase employing *Fusarium moniliforme* under solid-state fermentation was optimized. Different substrates, like rice bran, green gram bran, black gram bran, wheat bran and maize bran, were investigated to select the best substrate. Accordingly, wheat bran showed the highest glucoamylase activity. The maximum glucoamylase activity under optimal conditions was  $96.59 \pm 3.56$  U/ g of wheat bran. The optimum conditions were: yeast extract as additive 1 % (w/w), incubation period of 96 h, incubation temperature  $30 \pm 2$  °C, inoculum level 4 ml, moisture content of the wheat bran 60 %, and amount of wheat bran was 15 g at pH 5.0.

### Introduction

Glucoamylase (1, 4- $\alpha$ -D glucan glucohydrolase, glucoamylase EC 3.2.1.3) is an exo-acting enzyme that catalyses the hydrolysis of  $\alpha$ -glucans, from the non-reducing ends by mainly cleaving  $\alpha$ -1, 4 – glycosidic linkages and a few  $\alpha$ -1,6 linkages, but at a very slow pace [1, 2, 3]. This enzyme hydrolyses starch to yield 100 % glucose, and it preferentially acts on polysaccharides with a high molecular weight [4]. The production of high glucose syrups from starch, is one of the most important applications of glucoamylases, and these are also used in the production of ethanol as well as in baking and brewing industries [3].

Traditionally, glucoamylases have been produced by submerged fermentation (SmF). In recent years, however, the solid-state fermentation (SSF) processes have been increasingly applied for the production of this enzyme [5]. SSF compared to SmF is more simple, requires low capital, has superior productivity, reduced energy requirement, uses less water and produces lower waste water, has easier control of bacterial contamination and requires low cost of downstream processing [6-8]. In the SSF process, the solid substrate not only supplies the nutrients to the culture, but also serves as an anchorage for the microbial cells. The moisture content of the medium changes during fermentation as a result of evaporation and metabolic activities, and thus, maintaining the optimum moisture level of the substrate is very important [9].

Agro-industrial residues are generally employed as substrates for the SSF processes and

enzyme production is not an exception to that [5]. The selection of an appropriate solid substrate plays an important role in the development of an efficient SSF technology. The cost and the availability of the solid substrate are the important considerations. In order to attain higher enzyme titres, a number of factors need to be optimized, including a suitable microorganism and processing parameters [10]. There are few reports on the production of glucoamylases under SSF processes [11-13, 5, 14]. The aim of the present study was to investigate the production of glucoamylase using different agro-industrial residues and optimise the factors that influence the production of glucoamylase by *Fusarium moniliforme* in SSF.

### Results and Discussion

In the present studies, five different substrates, namely black gram bran, green gram bran, wheat bran, rice bran and maize bran were used for the production of glucoamylase. Culture media containing 10 g of each substrate were subjected to fermentation for 96 hours at pH 5.0; temperature,  $30 \pm 2$  °C; moisture, 50 % and inoculum size, 5 ml. The results are shown in Fig. 1. All the solid substrates supported microbial growth and glucoamylase formation. However, wheat bran was proved to be superior to other substrates. A high enzyme activity ( $60.12 \pm 1.80$  U/ g) was obtained in a medium containing wheat bran as the substrate followed by rice bran ( $50.63 \pm 2.03$  U/ g) and black gram bran ( $20.98 \pm 1.5$  U/ g). It has also been reported previously that wheat bran was found to be the best

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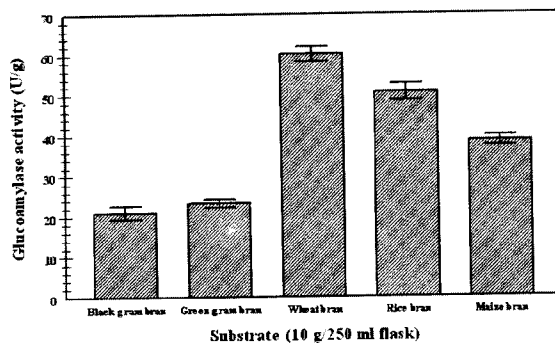


Fig. 1: Effect of substrate on the production of glucoamylase by *F. moniliforme*.

substrate for glucoamylase production [5, 15-16]. In subsequent experiments, therefore, wheat bran was used as a substrate for the production of glucoamylase.

*F. moniliforme* was cultured in SSF medium of wheat bran with an initial moisture content of 40, 50, 60, 70, 80 %; values being set before autoclaving. The optimum moisture content for best glucoamylase production ( $65.89 \pm 3.14$  U/g) was 60 % at 96 h. The lowest enzyme activity ( $40.79 \pm 1.23$  U/g) was with 80 % initial moisture content (Fig. 2). SSF were distinguished from submerged cultures by the fact that microbial growth and product formation occur at or near the surfaces of solid materials with low moisture contents [17]. The critical importance of moisture content, in solid-state fermentation and its influence on the biosynthesis and secretion of enzyme, can be attributed to the interference of moisture in the physical properties of the solid particles. High substrate moisture results in decreased wheat bran porosity, which in turn prevents oxygen penetration [18]. At the same time, low moisture level led to poor microbial growth and poor accessibility to nutrients.

The impact of inoculum size on glucoamylase production was determined by varying the levels of inoculum (3–7 ml) to 10 g of the wet wheat bran (60 % moisture). After 96 h, fermented biomass was analysed, and maximum enzyme activity ( $69.83 \pm 2.17$  U/g), was observed with 4 ml inoculum size (Fig. 3). A further increase in spore density caused a gradual decrease in glucoamylase synthesis. Inoculum density is an important consideration for SSF since higher inoculum levels, beside increasing the water content of the medium, are inhibitory in

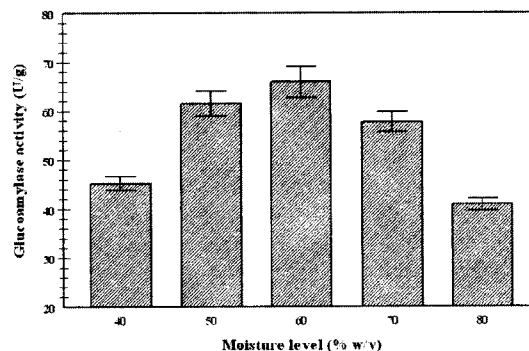


Fig. 2: Effect of initial moisture content on the production of glucoamylase by *F. moniliforme*.

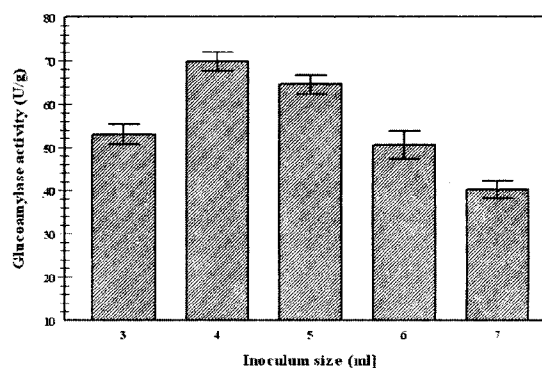


Fig. 3: Effect of inoculum size on the production of glucoamylase by *F. moniliforme*.

nature, and lower inoculum levels require more time for fermenting the substrates in SSF [19, 20].

The effect of initial pH of the medium on the synthesis of glucoamylase was determined by growing *F. moniliforme* on wheat bran at  $30 \pm 2$  °C for 96 h using different moistening media set at different pH values. The results shown in Fig. 4 indicate that maximum enzyme yield ( $70.52 \pm 2.3$  U/g) was obtained at pH 5.0 after 96h of incubation at  $30 \pm 2$  °C under optimum conditions. Optimal pH is very important for the growth of microorganism and its metabolic activities. As the metabolic activities of the microorganism are very sensitive to changes in pH, glucoamylase production by *F. moniliforme* is found to be affected if pH level is higher or lower compared to the optimum value. The obtained results showed a good coincidence with the reports from literature. *Aspergillus niger* NCIM-1245 produced maximum glucoamylase at pH 4.7 [11]. Moreover,

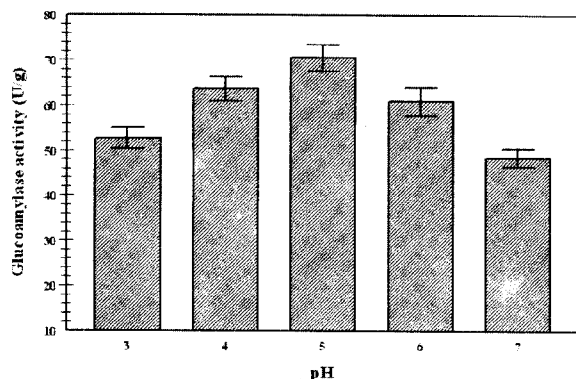


Fig. 4: Effect of pH on the production of glucoamylase by *F. moniliforme*.

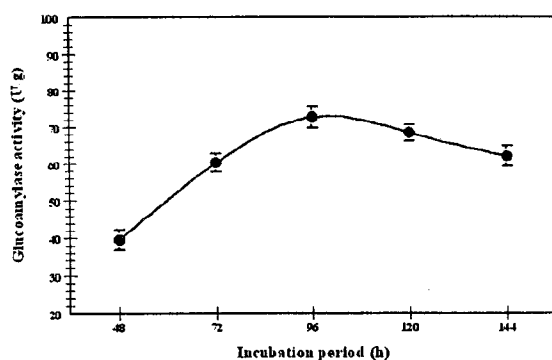


Fig. 5: Effect of incubation period on the production of glucoamylase by *F. moniliforme*.

Eljaiah *et al.*, [5] optimised the conditions for production of glucoamylase employing *Aspergillus* sp. A<sub>3</sub> under SSF. The maximum enzyme activity was observed at pH 5.0.

SSF was carried out for 48, 72, 96, 120 and 144 hours with 10 g of wheat bran as substrate at pH 5.0 and  $30 \pm 2^\circ\text{C}$  temperature. The strain used produced high titres of enzyme ( $72.69 \pm 2.89$  U/g) at 96 h incubation (Fig. 5). The biosynthesis of glucoamylase decreased after 96 hours incubation period, thereby being an important parameter that has to be controlled for optimum enzyme formation. It varies from organism to organism due to variation in the lag and log phases of growth among different microbial strains [5]. Ramadas *et al.*, [21] also reported 96 h as the optimum period for glucoamylase synthesis by *Aspergillus niger* in SSF.

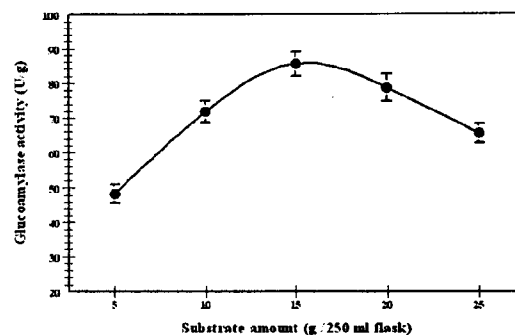


Fig. 6: Effect of substrate amount on the production of glucoamylase by *F. moniliforme*.

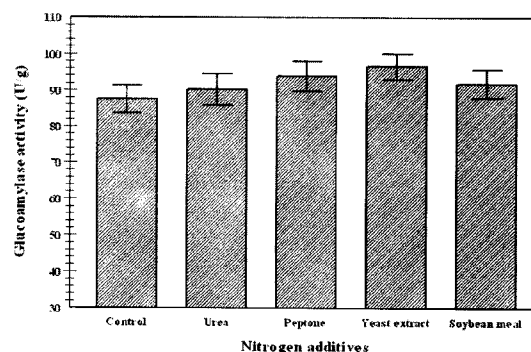


Fig. 7: Effect of nitrogen additives on the production of glucoamylase by *F. moniliforme*.

The level of substrates is vital in SSF especially in tray processes. The level of substrate also influences the porosity and aeration of the substrate in flasks. Different amounts (5, 10, 15, 20 and 25 g) of wheat bran were tried to study their effect on glucoamylase production in 250 ml conical flasks. Maximum enzyme activity ( $85.48 \pm 3.52$  U/g) was observed in the flasks containing 15 g of wheat bran (Fig. 6). Lower and/or higher substrate levels gave comparatively lower enzyme yields.

Various complex organic nitrogen sources were added separately to the solid substrate medium at a concentration of 1% (w/w) to assess their effects on glucoamylase production. Addition of different nitrogen sources resulted in enhanced production of glucoamylase. Maximum enzyme yield ( $96.59 \pm 3.56$  U/g) was observed with yeast extract followed by peptone ( $93.78 \pm 4.12$  U/g) and soybean meal ( $91.89 \pm 3.56$  U/g) respectively (Fig. 7).

## Experimental

All experiments and enzyme activity measurements were run in triplicate and the data presented are mean values  $\pm$  S.E. The  $\pm$  S.E values have been shown as error bars in Figs.

### Microorganism

The fungus *Fusarium moniliforme* was obtained from National Fungal Culture Collection of Pakistan (NFCCP), Department of Plant Pathology, University of Agriculture, Faisalabad. It was maintained on potato dextrose agar (PDA) slants at 4 °C and sub-cultured fortnightly.

### Inoculum Development

Inocula were prepared by transferring spores from 5-6 days old slant culture, into 500 ml Erlenmeyer flask containing 150 ml of sterile Vogel's medium [22]. The composition of inoculum medium was (g/ l): glucose, 20.0; trisodium citrate, 2.5;  $\text{KH}_2\text{PO}_4$ , 5.0;  $\text{NH}_4\text{NO}_3$ , 2.0;  $(\text{NH}_4)_2\text{SO}_4$ , 4.0;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2; peptone, 2.0; and microelement solution, 10 ml. The microelements solution contained (g/ L):  $\text{CuSO}_4$ , 0.08;  $\text{H}_2\text{MoO}_4$ , 0.05;  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.07;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.043; and  $\text{Fe}_2(\text{SO}_4)_3$ , 0.05. pH of the medium was adjusted to 5.0 using 1M HCl/ 1M NaOH. The flasks were incubated on a rotary shaker at 150 rpm at 30 °C for 48 h to get homogenous spore suspension ( $1 \times 10^7$ - $1 \times 10^8$  spores  $\text{ml}^{-1}$ ). The spore suspension was used as inoculum in the growth media to optimise the culture conditions for maximum production of glucoamylase.

### Production of Glucoamylase Under SSF

*F. moniliforme* was grown under SSF and process conditions were optimised for the enhanced production of glucoamylase. The growth was carried out considering different parameters like suitable substrate, initial moisture levels, initial pH, inoculum size, incubation period, amount of substrate and various nitrogen additives, affecting the production of glucoamylase. The conditions were optimised by adopting search technique varying parameters one at a time as described by Pandey and Radhakrishnan [11]. The static experiments were conducted in 250 ml Erlenmeyer flasks containing 5 g of substrate moistened with mineral salt solution g/ l; trisodium citrate, 2.5;  $\text{KH}_2\text{PO}_4$ , 5.0;  $\text{NH}_4\text{NO}_3$ , 2.0;  $(\text{NH}_4)_2\text{SO}_4$ , 4.0;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2) to 60 % moisture content.

Flasks were plugged with cotton and sterilized by autoclaving for 15 minutes at 121 °C. After sterilisation, the flasks were cooled and inoculated with 3 ml of inoculum ( $1 \times 10^7$ – $1 \times 10^8$  spores  $\text{ml}^{-1}$ ) and incubated at  $30 \pm 1$  °C for 96 h under various experimental conditions. The parameters once optimised was fixed for subsequent studies.

### Isolation of Glucoamylase

At the end of fermentation, the fermented biomass was treated with 50 ml of distilled water and agitated thoroughly on orbital shaker (150 rpm) for 30 minutes. The whole contents were filtered through muslin cloth. The residue was again treated with another 50 ml of distilled water, in the same way and filtered. The filtrates were pooled together, centrifuged at 10,000 rpm for 10 minutes at 4 °C and the clear supernatant was used as the enzyme source.

### Determination of Enzyme Activity

Glucoamylase activity was determined by using 1 % soluble starch solution in 50 mM 2-(N-morpholino)-ethanesulphonate monohydrate (MES monohydrate) buffer (pH 5.5) at 40 °C for 40 min. The released glucose was measured using a glucose oxidase (GOD) method as described by Iqbal *et al* [23] and Bhatti *et al.*, [24]. One unit (IU) of enzyme activity was defined as micromoles of glucose released per minute by the total amount of enzyme, extracted from 1 gram of the dry substrate under the above assay conditions.

### Conclusion

Based on the above findings, it could be concluded that wheat bran, enriched with minerals and nitrogen additives, could be used for the enhanced production of glucoamylase by *F. moniliforme* in SSF technology.

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