

# PRODUCTION OF STARCH-DIGESTING GLUCOAMYLASE FROM *Aspergillus niger* ATCC 10864

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## ABSTRACT

Production of starch-digesting glucoamylase by *Aspergillus niger* ATCC 10864 grown in liquid media containing 5 types of starch, i.e. soluble starch, corn starch, cassava starch, sticky-rice starch and rice starch was examined. The highest amount of glucoamylase was obtained when rice liquid medium was used as carbon source. The optimal liquid culture conditions for glucoamylase production were as follows: 2 % (w/v) rice starch as carbon source mixed with 1 % (w/v) peptone (as nitrogen source) and the pH was adjusted to 7.0 followed by shaking in a 30.° C shaking incubator at the speed of 200 rpm. In addition, when the three types of minerals, i.e.  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  were added together in the above culture, the yield of glucoamylase was higher than those when each of the three minerals was added separately. Studies on cell growth and glucoamylase in rice liquid medium showed that much more glucoamylase was produced extracellularly during the stationary phase, the experimental phase reaching a maximal activity of about 101.45 U/ml.

**Keywords:** starch-digesting, glucoamylase, rice liquid medium, *Aspergillus niger*

## 1. INTRODUCTION

Starch-degrading enzymes of microbial origin have a number of industrial application [1]. The mould extracellular enzyme, glucoamylase ( $\alpha$ -1,4-glucan glucohydrolase (EC 3.2.1.3)) is one of the most important enzymes used in food processing as well as in the commercial production of D-glucose from starch and has received considerable attention because of its ability to hydrolyze starch almost completely into glucose [2]. Glucoamylase was an enzyme that hydrolyzed  $\alpha$ -1,4-glucosidic linkages in starch, thereby removing successive glucose units from the non-reducing ends of the chains. Subsequently it was demonstrated that the enzyme also hydrolyzes  $\alpha$ -1,6- and  $\alpha$ -1,3-glucosidic bonds, although at much slower rates. Consequently the enzyme and the many fungi producing it have received considerable attention. Of prime importance in this context are the fungi belonging to the *Aspergillus niger* group which have properties highly suited to the industrial scale saccharification of starch [1]. The aim of this present study was to determine the optimal condition for production of starch-digesting glucoamylase by *A. niger* ATCC 10864 in liquid culture, and cell growth and glucoamylase production in rice liquid medium by *A. niger* ATCC 10864.

## 2. MATERIALS AND METHODS

### Microorganism

*Aspergillus niger* ATCC 10864 was selected for this study as the producer of glucoamylase, and was maintained on PDA slants at 4° C and subcultured monthly.

### Media

The chemically defined medium [3] for the batch cultivation contained the following per litre:

20 g soluble starch, 7.3 g  $(\text{NH}_4)_2\text{SO}_4$ , 4.5 g  $\text{KH}_2\text{PO}_4$ , 1.0 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0 g NaCl, 0.1 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 7.2 mg  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.3 mg  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.3 mg  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ , 3.5 mg  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  and 6.9 mg  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .

### Shake-flask cultivation

For shake-flask cultivation, 70 ml of medium in 250 ml conical flask were sterilized for 15 min at 121° C. The flasks were each inoculated with 1.0 ml spore suspension in 0.1% (v/v) Tween 80

( $10^6$  spores/ml) and incubated in a rotary shaking incubator (200 rpm) at 30 ° C for 5 days. Samples were removed every 24 h. The culture broth was centrifuged for the estimation of enzyme activities.

### **Analytical methods**

Microbial growth in submerged fermentation was estimated by measuring biomass dry weight. The culture liquid was centrifuged at 5,000 rpm for 15 min, at 4 °C. Glucoamylase activity was determined by the method of Ramadas, *et al.* [4]. The assay mixture consisted of 0.5 ml 1% soluble starch as substrate from Sigma, 0.4 ml 0.02 M sodium acetate (pH 4.0) and 0.1 ml of appropriately diluted enzyme. After incubation for 10 min at 60 °C, the reaction was stopped by adding 1.0 ml 1% DNS reagent (contained the following per litre: 10 g 3,5 - dinitrosalicylic acid, 2g phenol, 0.5g sodium sulfite, 200 g potassium sodium tartrate ( $\text{COOK}(\text{CHOH})_2\text{COONa}\cdot 4\text{H}_2\text{O}$ ), 500 ml 2 % NaOH) and heated for 5 min in boiling water. After cooling, the total volume was made up to 6 ml with distilled water and the  $A_{540}$  measured using glucose as standard. One unit of glucoamylase activity was defined as the amount of enzyme that formed 1  $\mu\text{mol}$  glucose in 1 min.

### **Determination of protein**

Protein was measured by the method of Lowry [5] using Folin-ciocaitu's phenol reagent from Sigma, with bovine serum albumin as a standard.

### **Determination of reducing sugar**

Reducing sugar was measured by the method of DNS (Miller, 1959) using 3-5 dinitrosalicylic acid from Sigma, with glucose as a standard.

### **Determination of residual starch**

Residual starch was determined by the optical method based on starch-iodine reaction [7], with soluble starch as a standard.

## **3. RESULTS AND DISCUSSION**

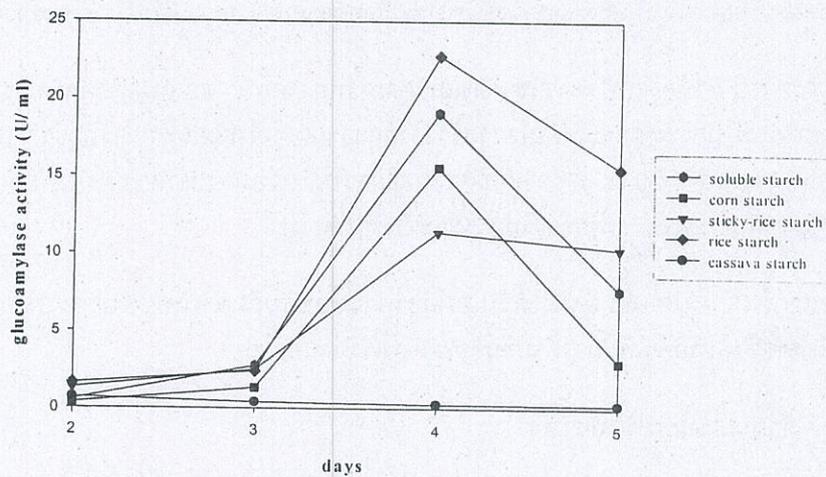
### **3.1 Production of glucoamylase by *A. niger* ATCC 10864**

When the organism was grown on the 5 different starch types under otherwise identical conditions enzyme production on 4 of the substrates reached a maximum after 4 days and therefore it declined. Of these, rice starch gave the highest maximum and final yields. Cassava starch medium showed no significant enzyme production.

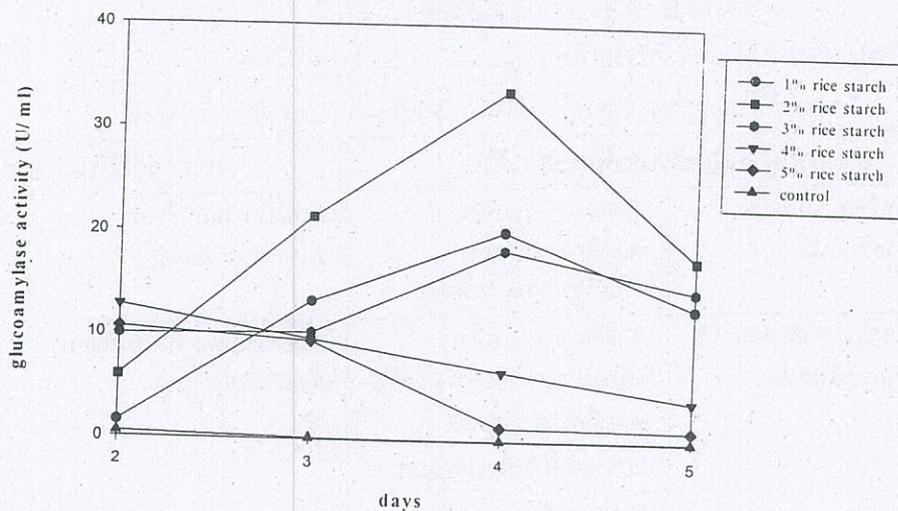
### 3.2 Optimization of glucoamylase production by *A. niger* ATCC 10864

#### 3.2.1 Effects of different concentrations of carbon source

The effect of adding different concentrations of carbon sources on the production of glucoamylase was studied in liquid medium. The addition of rice starch (each at 1, 2, 3, 4 and 5 % w/v) and control (without carbon source) was compared. From the results (Fig. 2) it is apparent that biosynthesis of the enzyme was maximally induced in the presence of 2 % (w/v) rice starch ( $P < 0.01$ ).



**Figure 1.** Production of starch-digesting glucoamylase by *A. niger* ATCC 10864 with different carbon sources over 5 days.



**Figure 2.** The effect of various concentrations of carbon source for production of starch-digesting glucoamylase of *A. niger* ATCC 10864.

### 3.2.2 Effect of nitrogen source on glucoamylase synthesis by *A. niger* ATCC 10864

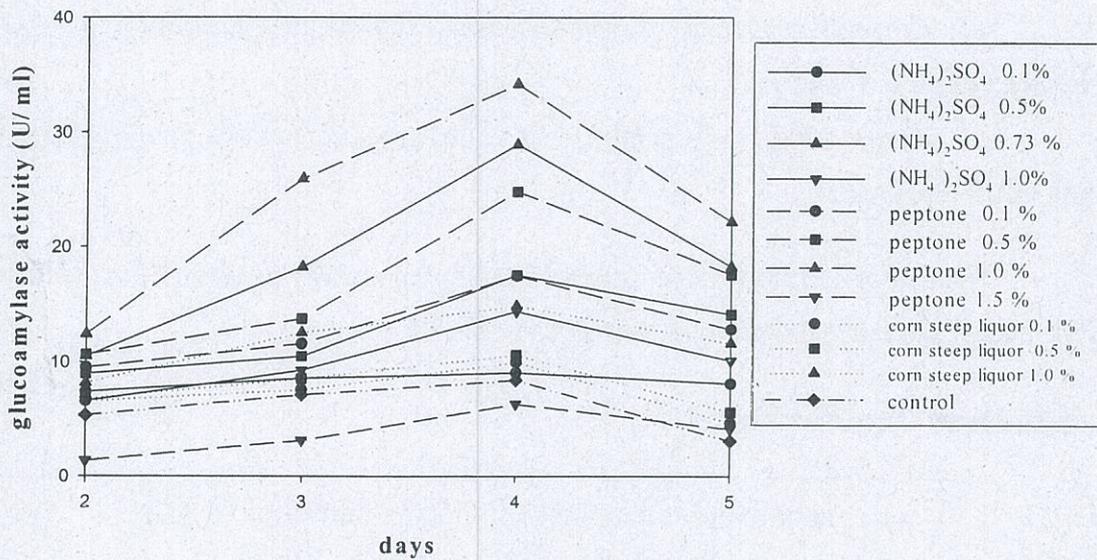
The effect of nitrogen source supplementation on glucoamylase activity of *A. niger* ATCC 10864 is shown in Figure 3. Supplementation with 0.1, 0.5, 0.73 and 1.0 % (w/v)  $(\text{NH}_4)_2\text{SO}_4$  0.1, 0.5, 1.0 and 1.5 % (w/v) peptone 0.1, 0.5 and 1.0 % (w/v) corn steep liquor and control (without nitrogen source) were compared. When the medium was supplemented with 1.0 % (w/v) peptone, *A. niger* ATCC 10864 produced a higher glucoamylase activity than 1% (w/v)  $(\text{NH}_4)_2\text{SO}_4$  and 1% (w/v) corn steep liquor ( $P < 0.01$ ).

### 3.2.3 Effect of initial pH on the production of glucoamylase by *A. niger* ATCC 10864

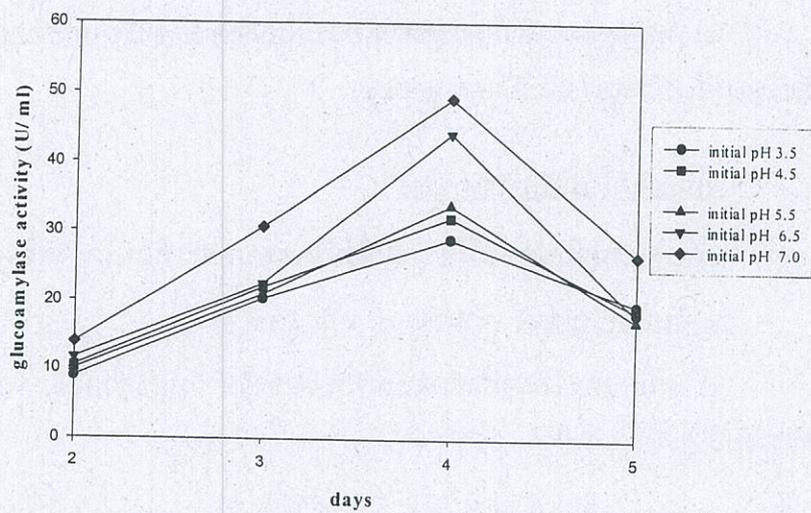
The effect of initial pH on the growth and biosynthesis of the enzyme was determined in the culture medium at different initial pH (3.5, 4.5, 5.5, 6.5 and 7.0). It was observed that the maximal growth of the organism was obtained at 7.0 ( $P < 0.01$ ) (Figure 4).

### 3.2.4 Effect of incubation temperature on the production of glucoamylase by *A. niger* ATCC 10864

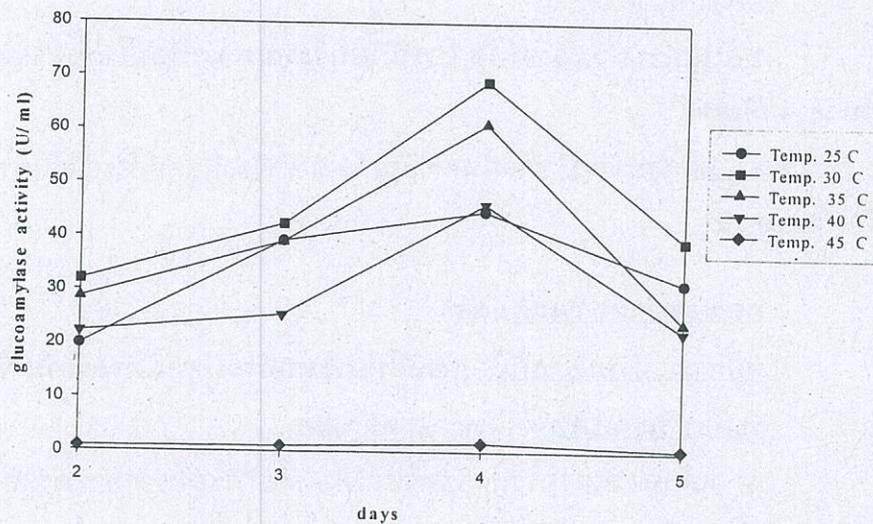
Fermentative production of glucoamylase in the culture broth containing 2% rice starch was carried out at various temperatures (25, 30, 35, 40 and 45 °C) for 5 days. It was found that growth of the organism as well as biosynthesis of the enzyme was maximal at 30 °C ( $P < 0.01$ ), where the enzyme production was very low at 25 °C and at or above 40 °C (Figure 5).



**Figure 3.** Effect of different nitrogen source on glucoamylase synthesis by *A. niger* ATCC 10864 when cultured for 5 days.



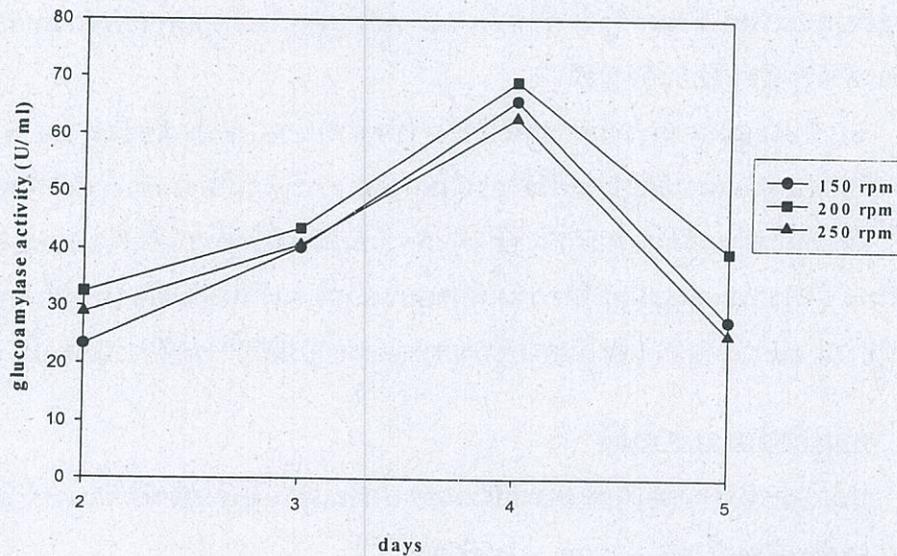
**Figure 4.** Effect of different initial pH in liquid medium on production of glucoamylase by *A. niger* ATCC 10864 when cultured for 5 days.



**Figure 5.** Effect of different growth temperature on glucoamylase activity by *A. niger* ATCC 10864 when culture for 5 days.

### 3.2.5 Effect of shaking speed on production of glucoamylase by *A. niger* ATCC 10864

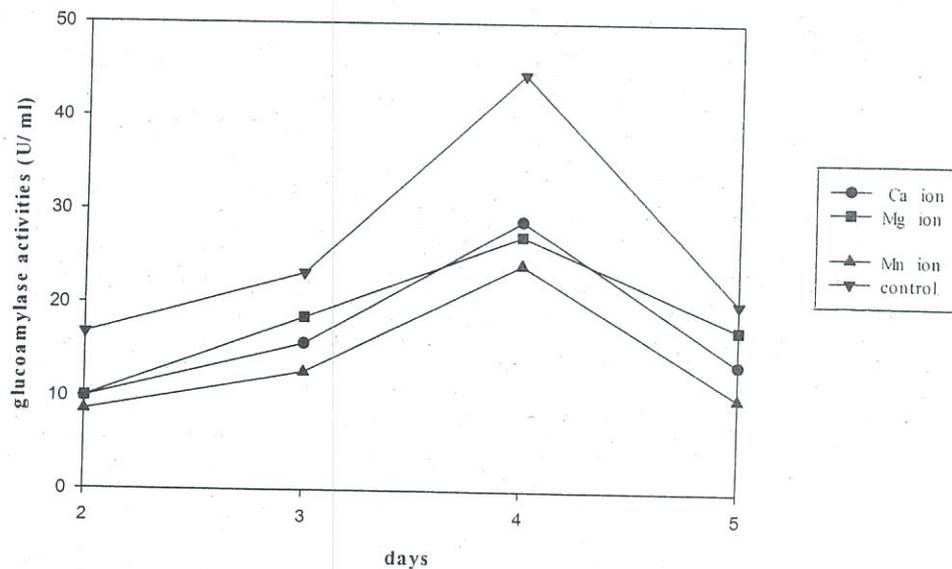
Three different shaking speeds (150, 200 and 250 rpm) of shake flask cultured were compared. It was observed that the glucoamylase activity was highest at 200 rpm ( $P < 0.01$ ) (Figure 6).



**Figure 6.** Effect of different shaking speed on production of glucoamylase by *A. niger* ATCC 10864.

### 3.2.6 Effect of metal ions on production of glucoamylase by *A. niger* ATCC 10864

The effect of 3 divalent cations  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Mg}^{2+}$  was studied. A control containing all three metal ions was run for comparison (Figure 7). The result showed that 100  $\mu\text{g/ml}$  of  $\text{Ca}^{2+}$ , 3.5  $\mu\text{g/ml}$  of  $\text{Mn}^{2+}$  and 1,000  $\mu\text{g/ml}$  of  $\text{Mg}^{2+}$  stimulated the production of the enzyme ( $P < 0.01$ ).



**Figure 7.** Influence of metal ions on growth and production of glucoamylase by *A. niger* ATCC 10864 when cultured for 5 days.

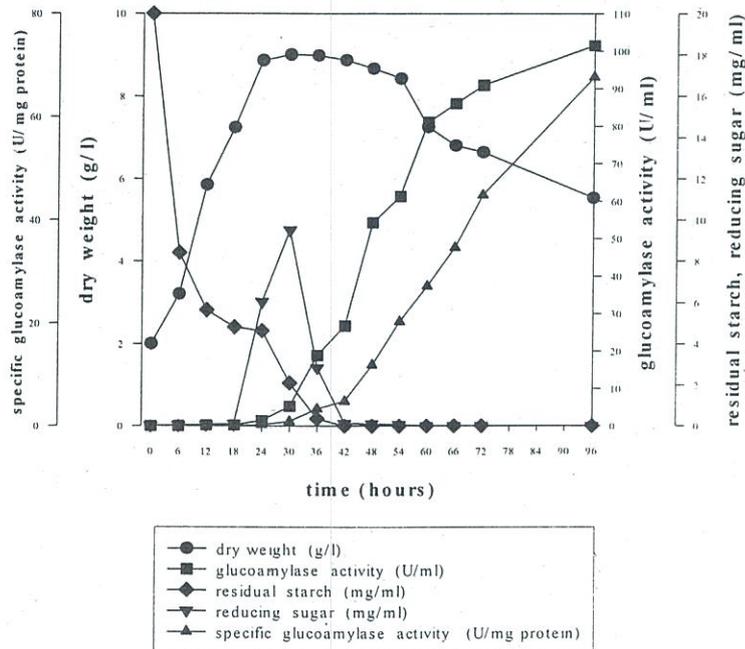
### 3.3 Growth and glucoamylase production by *A. niger* ATCC 10864 under optimal conditions

Culture media at optimized condition for glucoamylase production from *A. niger* ATCC 10864 were used. These were 2 % rice starch, 1 % peptone, initial pH 7.0, at 30 °C and 200 rpm shaker speed. Cell growth and glucoamylase production during a 96 h fermentation are shown in Figure 8. This strain had a fairly long log phase, almost 24 h. This was followed by rapid cell growth for about 24 h. Meanwhile, glucoamylase was secreted into the fermentation broth in limited amounts. Much more glucoamylase was secreted extracellularly during the stationary phase, reaching a maximal activity of about 101.45 U/ml.

#### 4. CONCLUSION

*A. niger* ATCC 10864 may be used successfully for the production of starch-digesting glucoamylase.

*A. niger* ATCC 10864 produced the highest amount of glucoamylase when rice medium was used as carbon source. The optimal liquid culture condition for obtaining maximum glucoamylase were as follows: 2 % (w/v) rice starch, (carbon source) was mixed with 1% (w/v) peptone (as nitrogen source) initial pH 7.0 followed by shaking in a 30 °C shaking incubator at the speed of 200 rpm. In addition, when the three types of minerals, i.e., CaCl<sub>2</sub>·2H<sub>2</sub>O, MgSO<sub>4</sub>·7H<sub>2</sub>O and MnCl<sub>2</sub>·4H<sub>2</sub>O were added together in the above culture, the glucoamylase was produced more than when each of the three minerals was added separately.



**Figure 8.** Time course of glucoamylase production, residual starch, reducing sugar and dry weight of cell during growth of *A. niger* ATCC 10864 on 2 % rice starch, 1 % peptone, at 30 °C and initial at pH 7.0.

## 5. ACKNOWLEDGEMENTS

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