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PROCESS OPTIMIZATION FOR AMYLOGLUCOSIDASE BY A MUTANT STRAIN OF *ASPERGILLUS NIGER* IN STIRRED FERMENTER

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Abstract

The present study was designed to optimize the process parameters for the production of amyloglucosidase bya mutant strain of *Aspergillus niger* in stirred fermenter by. For this purpose, various cultural conditions like rate of fermentation, process pH, rate of agitation and size of inoculum was investigated. The maximum production (25.15 U/mL/min) of amyloglucosidase was achieved at the agitation speed of 200rpm. The production of amyloglucosidase was found to be maximum (25.08 U/mL/min) at pH 5 of the medium. The optimum productivity (25.15 U/mL/min) of the enzyme was achieved with 4% inoculum after 48 h of incubation. The process temperature was optimized at 30^{o}C throughout the study.

Introduction

Amyloglucosidase has the characteristic property of hydrolyzing the starch into glucose (Kelly *et al*., 1983; Ford, 1999; Reilly, 1999). Glucose is used in various food industries (Polakovic & Bryjak, 2004). Various microorganisms are reported for the extracellular release of the amyloglucosidase (Pamboukian *et al.,* 1999). Most frequently used fermentation techniques for the biosynthesis of amyloglucosidase enzyme are solid state and submerged fermentation (Ramadas *et al.,* 1996, Metwally, 1998; Nandakumar *et al*., 1999; Imran *et al.,* 2012). However, submerged fermentation is preferably used at industrial scale, due to the agitation for proper air supply and easy availability of nutrients than the solid state fermentation (Prescott & Dunn, 1987). *Aspergillus* species possessed a superior ability to secrete amyloglucosidase (Gwiazdowska *et al.,* 2004). So, *Aspergillus niger* is preferred for the commercial production of amyloglucosidase (Pandey & Radhakrishan, 1993; Arassaratnam *et al*., 1997; Haq *et al*., 2002; Khalaj *et al*., 2001; Omemu *et al*., 2005; Spier *et al*., 2006; Costa *et al*., 2007). The production of amyloglucosidase is also dependent on incubation temperature and medium pH. The pH of the medium exerts a reflective effect on amyloglucosidase production. Any change in the hydrogen ion concentration greatly inhibited the growth of fungi as well as the enzyme production (Schmidell *et al*., 1988; Pavezzi *et al.,* 2008). Slightly acidic is suitable for the production of amyloglucosidase (Aguero *et al.,* 1997). Level of inoculum is reported to be an important factor in the biosynthesis of amyloglucosidase in stirred fermeter (Narang & Satyanarayana, 2001). Optimum supply of oxygen and nutrients are very essential for aerobic fermentation. Increased agitation could lead to the breakage of hyphae of a number of microorganisms (Beiny & Thomas, 1991). The main objective of present study was optimization of cultural conditions for the enzyme production by *A. niger* in biofermenter.

Materials and Methods

Microorganism: The microorganism used in the present study was obtained by various physical and chemical mutagenic treatments as already reported by Malik *et al*., (2011).

Fermentation medium: The fermentation medium used in the present study was optimized by Malik *et al*., (2011).

Vegetative inoculum: Twenty five mL of fermentation medium was added in 250 mL conical flask, cotton plugged and was then sterilized. One ml of conidial suspension prepared, as mentioned above, was poured to the flask with the help of a pipette under aseptic conditions. Vegetative inoculum was placed at 200rpm in an incubator shaker for 24h, keeping the temperature of 30^{o}C (Malik *et al*., 2011).

Fermentation technique: Scale up studies was carried out in a stirred glass fermenter of 7.5 liters capacity (Model: Bioflo-110 Fermenter/Bioreacter, USA) with working volume of 5 liters. The fermentation medium (4.5L) was sterilized in an autoclave at 121^{o}C for 20 min. at the pressure of 15 lb/in^{2}. After that vegetative inoculum at the rate of 4% was added to the sterilized fermentation medium. The temperature of fermenter was maintained at 30^{o}C throughout the incubation period. The agitation speed was fixed at 200 rpm with the aeration rate of 1.0 L -1min^{-1}. Sterilized silicone oil was added as antifoaming agent during fermentation (Pandey & Radhakrishan, 1993).

Enzyme assay: The assay of amyloglucasidase was carried out according to the method of Cadwell *et al*., (1968). One unit of activity is the amount of enzyme, which liberates one mg of glucose per hour from 5% soluble starch. The enzyme activity was then converted into U/mL/min. The transmittance was observed at 546 nm on spectophotometer. The transmittance was converted to mg of glucose from standard curve.

Results and Discussion

The rate of agitation was investigated for amyloglucosidase production in stirred fermenter. Figs 1, 2, 3 and 4 showed the effect of agitation rate on the enzyme production. The fermentation was carried out at 150, 200, 250 and 300 rpm. The production of enzyme

was found to be maximum (25.15 U/ml/min), when the agitation rate was maintained at 200 rpm. When the agitation rate was decreased *i.e.*, 150 rpm the enzyme production was quite low. An increase in the rate of agitation above 200 rpm had an adverse effect on amyloglucosidase production as well on the pellet formation. At higher speed of agitation, the fungus form a diffused hyphal mass instead of pellets. It might be due to low supply of the oxygen because the pellets were broken to form a hyphal mass, which resulted in the decrease of growth of the fungus and ultimately the enzyme production. Therefore, 200 rpm was optimized for the enhanced production of amyloglucosidase in the fermenter. Present finding are in agreement with the earlier reports, that increased agitation could lead to the breakage of hyphae of a number of microorganisms (Beiny & Thomas, 1991). Secondly, at high speed, foaming appeared, which inhibited the production of biomass and hence amylase production. Similar results were also reported by Pamboukian *et al.* (1999).

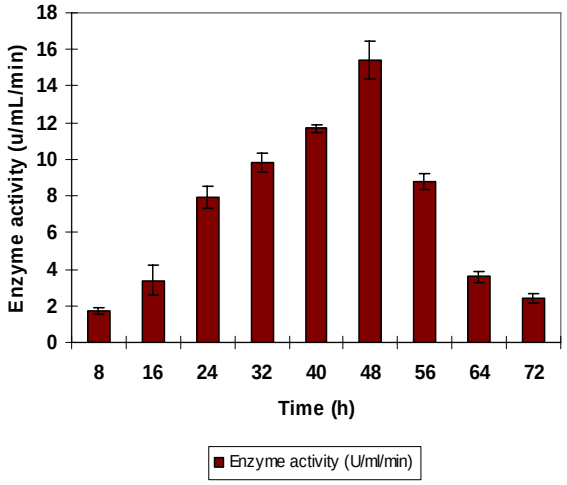

Fig. 1. Production of amyloglucosidase in fermentor by *A. niger* (mutant) at 150 rpm.

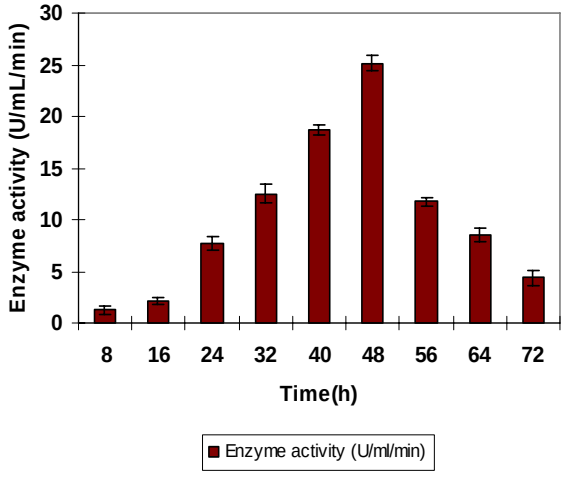

Fig. 2. Production of amyloglucosidase by *A.niger* (mutant) in fermenter at 200 rpm.

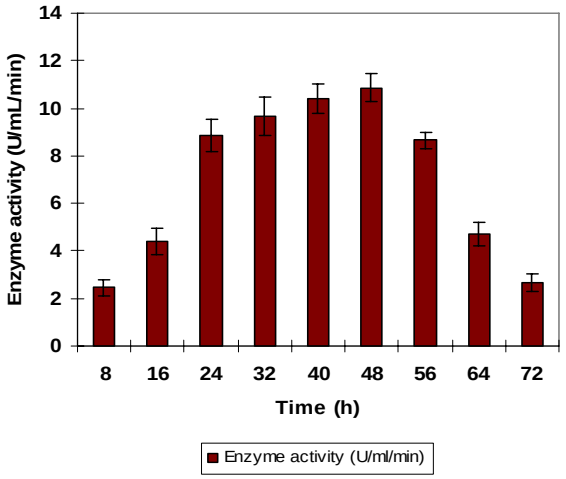

Fig. 3. Production of Amyloglucosidase in fermentor by *A. niger* (mutant) strain at 250 rpm.

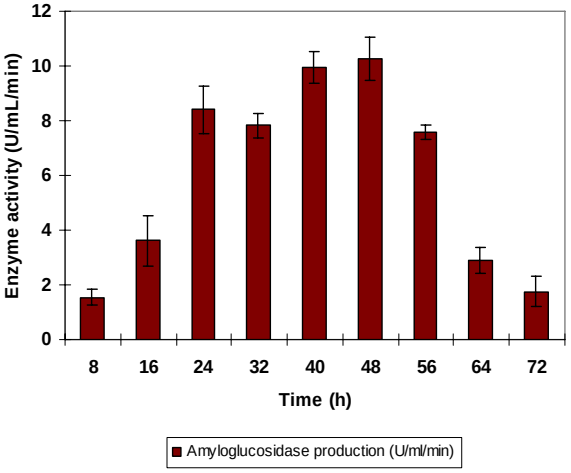

Fig. 4. Production of amyloglucosidase in fermenter by *A. niger* (mutant) at 300 rpm.

The effect of level of inoculum on the biosynthesis of amyloglucosidase by mutant strain of *A. niger* in stirred fermenter was also investigated (Figs. 5, 6 & 7). The 16 h old vegetative inoculum was used for inoculation. The inoculum was used at the level of 2%, 4% and 6% (v/v). At each level of inoculum, the process was monitored for 72 h. The biosynthesis of the enzyme was increased, with increase in the size of inoculum from 2% to 4%. At the level of 4% inoculum maximum amount of amyloglucosidase was produced (25.28 U/ml/min) after 48 h. When the level of inoculum was further increased, the production of amyloglucosidase decreased, although the maximum value of the enzyme was produced earlier after 40 h, but it was very low. Hence, 4% inoculum of *A. niger* produced maximum amount of the enzyme. Narang & Satyanarayana (2001) reported 5% inoculum as the optimum level of inoculum for maximum amylase production in stirred fermenter. Therefore, present results are comparatively more significant than others, as lesser amount of inoculum was sufficient to achieve maximum enzyme yield.

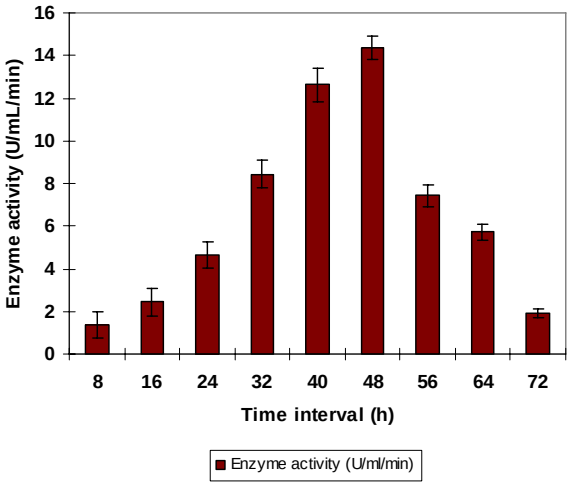

Fig. 5. Effect of inoculum size on Amyloglucosidase production in Fermenter by *A. niger* (mutant) with 2% inoculum.

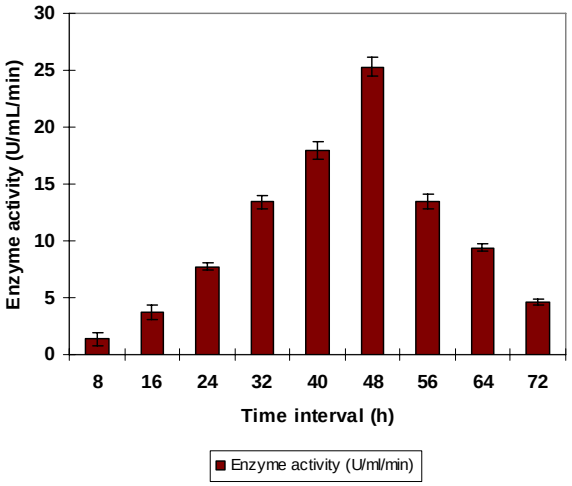

Fig. 6. Effect of inoculum size on Amyloglucosidase production in Fermenter by *A. niger* (mutant) with 4%inoculum.

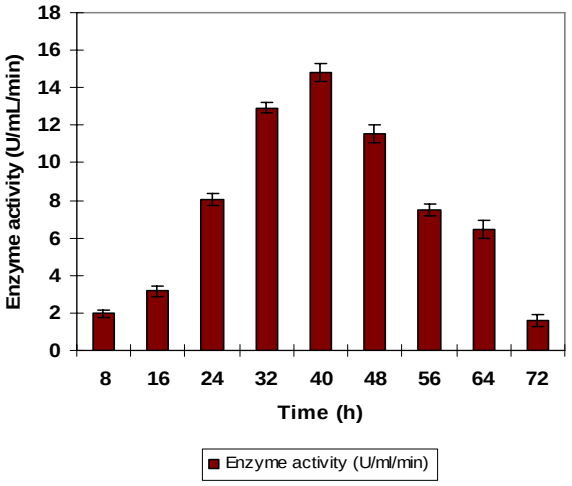

Fig. 7. Production of amyloglucosidase in fermenter by *A. niger* (mutant) with 6% inoculum.

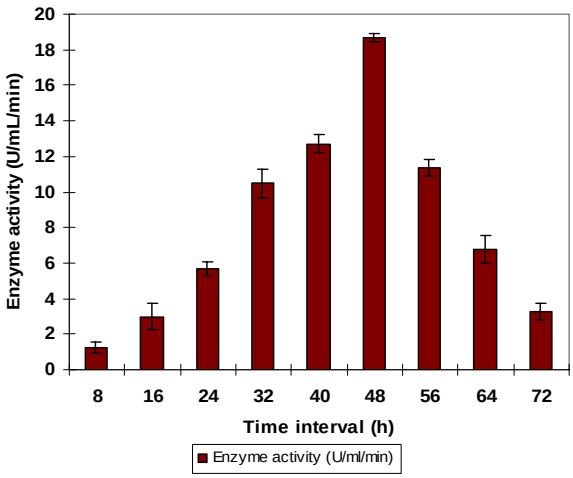

Fig. 8. Effect of pH on amyloglucosidase production by *A. niger* (mutant) at pH 4.

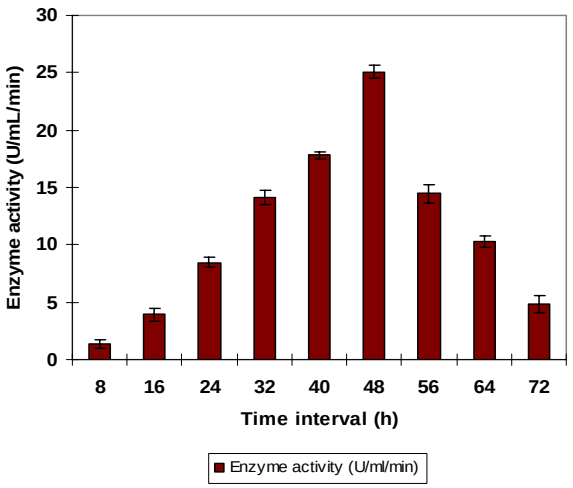

Fig. 9. Effect of pH on amyloglucosidase production by *A. niger* (mutant) at pH 5.

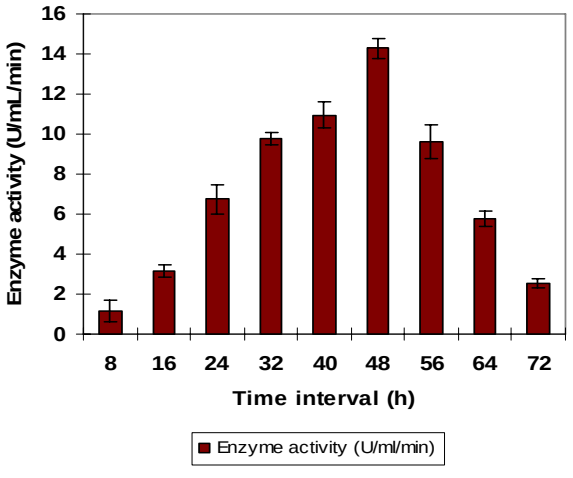

Fig. 10. Effect of amyloglucosidase production by *A. niger* (mutant) at pH 6.

The effect of process pH on the production of amyloglucosidase by *A. niger* mutant strain in the bio-fermenter (Figs. 8, 9 & 10) was studied. The pH of the fermentation medium was adjusted at 4.0, 5.0 and 6.0. The production of amyloglucosidase was found to be maximum (25.08 U/ml/min) when the pH of the medium was 5.0. An increase or decrease in pH reduced the enzyme production. So, pH 5 of the fermentation medium was selected for maximum production of amyloglucosidase by *A. niger* strain in the fermenter. The results of the present study depicts that the maximum enzyme biosynthesis was obtained at pH 5.0 which are in agreement with the previous reports (Ellaiah *et al.*, 2002; Aguero *et al.*, 1997; Mishra & Debnath, 2002).

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