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Bio-processing of banana peel for alpha amylase production by *Aspergillus oryzae* employing solid state fermentation.

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Abstract

Alpha amylase (α amylase) is one of the main enzymes used in various sectors such as food, textile, paper and detergent industries. Microbial production of α amylase using solid state fermentation (SSF) system appears as a promising technology now. Several parameters such as incubation period, incubation temperature, initial pH of the media, substrate content etc. affect the production of α amylase in SSF system. The present study focuses on the production of α amylase from the fungus *Aspergillus oryzae* and also optimization of various fermentation parameters using banana peel as a substrate. Incubation period of 96 hours, incubation temperature 35°C, initial pH of the medium at 5.0 and 50 g of substrate were found to be optimum for the production of á amylase by *Aspergillus oryzae* through SSF process.

Keywords: á amylase, *Aspergillus oryzae,* solid state fermentation (SSF)

1. Introduction

Amylase is an enzyme that catalyses the hydrolysis of starch into sugars.Two major classes of amylases are there: α amylase and glucoamylase. The á-amylases are calcium metalloenzymes which catalyse the hydrolysis of internal 1,4- glycosidic linkages in starch to low molecular weight product , such as glucose, maltose and maltotriose units (Gupta *et al.*, 2003, Kandra *et al.*, 2003). Glucoamylase hydrolyses single glucose unit from the non reducing end of amylose and amylopectin in a step wise manner (Anto *et al.*, 2006, Pandey *et al.*, 2005). A amylases are widely distributed in nature and have been reported to be produced by plant, animal and microbial sources (Pandey*et al.,* 2005, Reddy *et al.,* 2003). However microbial amylase has predominant application in the industrial sector. Major advantages of using fungi for amylase production are the bulk production capacity and ease of manipulation. Many species of *Aspergillus* are used as a source of α amylase (Pandey *et al.,* 2005). Production of α amylase has been investigated through Submerged fermentation(SmF) and Solid state fermentation (SSF) (Norouzian *et al.,* 2006, Miranda *et al.,* 1999). Submerged fermentation is advanced and commercially more viable (Hashemi *et al.,* 2010). But in the recent years, the solid state fermentation has also emerged as a well developed technique (Bhatnagar *et al.,* 2010). Again being the various synthetic media used for alpha amylase production very expensive, agro industrial residues are generally considered as the best substrates for alpha amylase production through solid state fermentation (Ellaiah *et al.,* 2002). Baysal *et al.,* (2003) have reported the use of rice husk and wheat bran for the production of alpha amylase through SSF. Ramachandran *et al.,*(2004) have checked the potential of coconut oil cake as substrate for the production of alpha amylase using *Aspergillus oryzae.*

Alpha amylases have potential application in a wide number of industrial processes such as food fermentation, textile, paper, detergent, brewing and distillation industries etc. Fungal amylases could also

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be potentially useful in the pharmaceutical and fine chemical industries. However, the use of alpha amylases now has expanded in many other fields such as clinical, medicinal and analytical chemistry (Gupta *et al.,* 2003, Pandey *et al.,* 2000).

Hence the present study was undertaken to analyse the production of alpha amylase from the fungus *Aspergillus oryzae* through solid state fermentation using banana peel as a substrate. In this study, the influence of incubation time, incubation temperature, initial pH of the medium, substrate content on alpha amylase production by the fungus was also investigated.

2. Materials and methods

2.1 Substrate

Banana peel was used as a substrate for alpha amylase production through SSF. Banana peel was collected from household wastes.

2.2 Microorganism and culture maintenance

Aspergillus oryzae MTCC No. 3107, used in the present study was obtained from MTCC, Institute of Microbial Technology (IMTECH), Chandigarh, India. The culture was maintained on CYEA slants. The slants were grown at 30°C for 4 days and stored at 4°C for further use.

2.3 Preparation of substrate

Banana peels were cleaned by washing in tap water and cut into small pieces followed by homogenization in blender. 15g of banana peel wastes were taken in 250ml conical flask and moistened with nearly 40ml salt solution consisting the following in g/l :0.8g NaCl,0.8g KCl, 0.1g$CaCl_2$, 2g Na_2HPO_4, 0.2g $MgSO_4$, 0.1g $FeSO_4$, 8g glucose,2g NH_4Cl, pH 6.2. The flasks were autoclaved at 121°C for 20 minutes. Then the flasks werecooled to room temperature.

2.4 Inoculum preparation

Fungal spores were transferred aseptically to 100ml conical flasks containing 50ml of autoclavedPDA medium(autoclaved at 121^{0}C for 15 minutes). The flasks were kept in incubator at 30ºC for 48 hours. The suspension was then used as inoculums.

2.5 Production of alpha amylase under SSF

To each substrate containing flask, 30%(w/v) of inoculum was added. The flasks were then incubated in a rotary incubator at 30°C. Enzyme production was checked after every 24 hours for 5 days.

2.6 Extraction of crude enzyme

After incubation, fermented waste samples of each flask were mixed with 0.1M Sodium phosphate bufferat the ratio of 1:10 (w/v)), pH 6.9. The flasks were shaken at 150rpm for 60minutes. The material was filtered through muslin cloth. The filtrate was collected and centrifuged at 5000rpm for 10 minutes at room temperature. The supernatant was collected and used as crude enzyme extract for determining amylase activity (Prasanna*et al.,* 2005).

2.7 Measurement of enzyme activity

The activity of alpha amylase was assayed by incubating 0.5 ml of enzyme with 0.5 ml of starch (0.5 % w/v) prepared in 0.1 M phosphate buffer, pH 7. After incubation at 60°C for 10 minutes the reaction was stopped by adding 1ml of 0.5M acetic acid. The reducing sugar released were assayed colorimetrically by addition of 1 ml of 3,5 Dinitrosalicyclic acid reagent (Miller, 1959). One unit of á amylase activity is defined as the amount of enzymes that releases 1 µ mol of reducing sugar as glucose or maltose per minute under assay condition and expressed as U/g of dry substrate (Sodhi *et al.,* 2005).

2.8 Optimization of process parameters

Various fermentation parameters were optimized for maximum production of á amylase by *Aspergillus oryzae* through SSF. The various parameters studied were fermentation period (24-120 hours), incubation temperature (25°C - 50°C), initial pH of the media (4-8) and substrate content (20g-60g).

2.8.1 Difference in incubation periods

Inoculated Conical flasks were incubated for varying time periods (24-120hours) at pH 7. The enzyme activity was calculated and results were plotted in graphs.

2.8.2 Effect of incubation temperature

The effect of temperature on enzyme production was investigated. The inoculated flasks were incubated at 25°C, 30°C, 35°C, 40°C, 45°C, and 50^{0}C AT pH 7 for 48 hours. The enzyme activity was observed and plotted in graphs.

2.8.3 pH of medium

The initial pH of the substrate was adjusted at 4,5,6,7, 8 and 9 in separate conical flasks. The variation in pH was carried out by adding acid or base buffer as per requirement. The SSF flasks were then

incubated for 48 hours hours at 30^{0}C. The enzyme activity was observed for different pH and plotted in graphs as shown.

2.8.4 Substrate amount

Conical flasks containing different substrate levels (20-60g) were inoculated and incubated at 30^{o}C for 48 hours at pH 7. The enzyme activity was observed for different substrate amounts.

3. Results

3.1 Effect of incubation time on alpha amylase production

Table1shows the variation in á amylase activity with incubation time. Incubation time of 96 hours was found to be optimum for the enzyme production (5.4±0.3 U/g). Further increase in incubation time decreased the enzyme secretion. The trend (Fig. 1) indicates that alpha amylase production increases with the rise in incubation time, but after 96 hours the enzyme secretion decreased gradually.

Table 1: Effect of incubation period on alpha-amylase production

Banana peel substrate	
Time (hours)	Enzyme activity (U/g)
24	2.3± 0.10
48	3.4± 0.25
72	4.2± 0.15
96	5.4± 0.30
120	3.9±0.20
144	1.5± 0.20

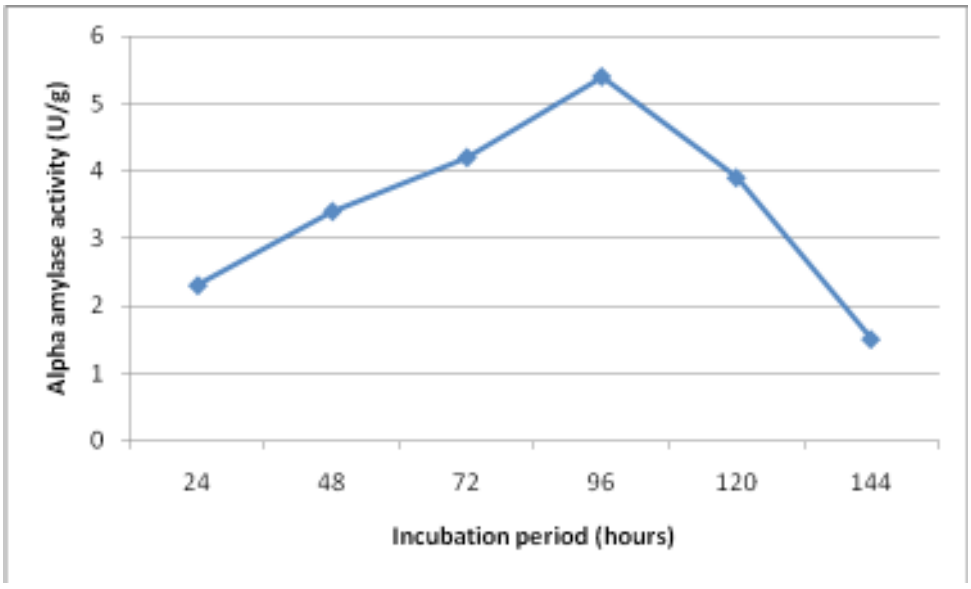

Fig. 1: Effect of incubation period on alpha-amylase production

3.2 Effect of incubation temperature on alpha amylase production

Temperature plays a vital role in growth of microorganism as well as their enzyme production activity. Table 2 shows the variation in á amylase activity at different incubation temperature (25^{o}C-50^{o}C). Maximum enzyme production (6.45±0.23 U/g) was obtained at 35^{o}C. In all the other flasks maintained at various incubation temperatures, the enzyme activity was found less as compared to that of at 35^{o}C (Fig. 2).

Table 2: Effect of incubation period on alpha-amylase production

Banana peel substrate	
Incubation temperature (°C)	Enzyme activity (U/g)
25	4.2± 0.14
30	4.6± 0.25
35	6.45± 0.18
40	5.2± 0.20
45	3.1±0.10
50	1.2± 0.23

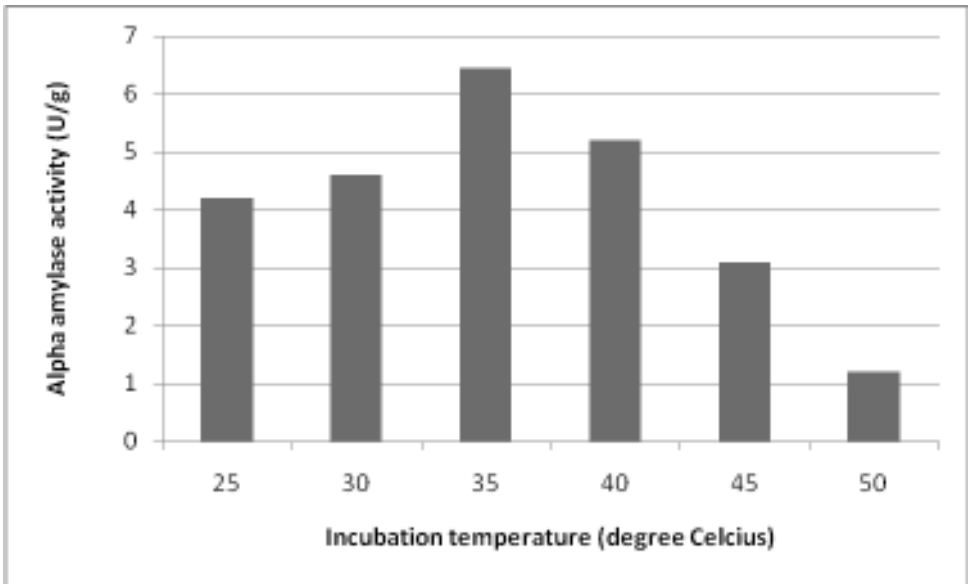

Fig.2: Effect of incubation temperature on alpha amylase production

3.3 Effect of initial pH of the media on alpha amylase production

In the present study, the influence of pH on alpha amylase activity had been studied by varying pH ranging from pH 4 to 9. The effect of initial pH in the production of alpha amylase is shown in Table 3. Maximum enzyme activity was observed at pH 5.0 (5.7±0.31U/g). The use of alkaline buffer for enzyme reaction resulted in a sharp decline in the enzyme activity (Fig. 3).

Table 3: Effect of initial pH of the medium on alpha-amylase production

Banana peel substrate	
Initial pH	Enzyme activity (U/g)
4	4.3± 0.22
5	5.7± 0.21
6	4.7± 0.15
7	3.85± 0.18
8	3.2±0.25
9	1.5± 0.30

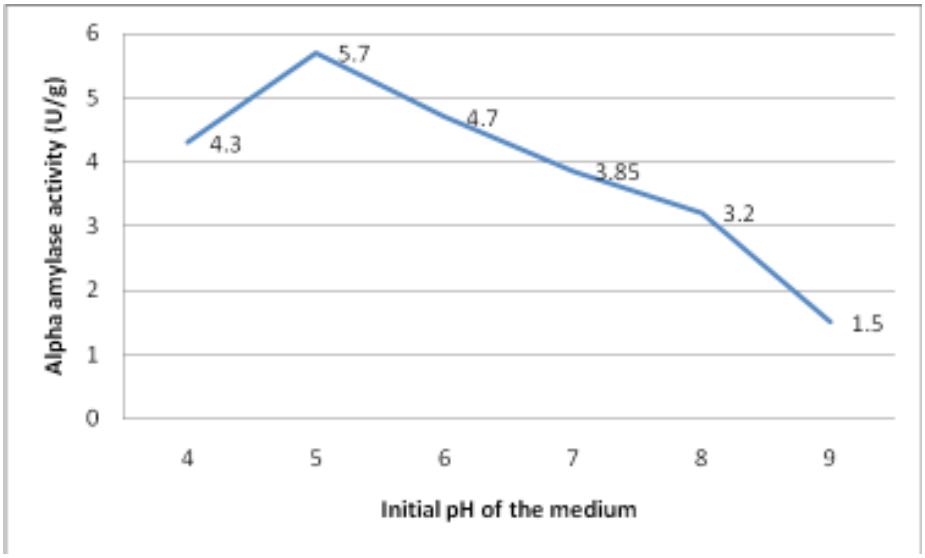

Fig. 3: Effect of initial pH of the medium on alpha-amylase production

3.4 Effect of substrate concentration on alpha amylase production

The amount of the substrate used in the SSF is an important factor for the production of alpha amylase. Table 4 shows the amylase activity at different substrate concentrations. Maximum enzyme activity was found at 50g of substrate (6.55±0.1 U/g). Further increase in the substrate content resulted in no significant increase in the enzyme activity (Fig. 4).

Table 4: Effect of substrate concentration on alpha-amylase production

Banana peel substrate	
Substrate content (g)	Enzyme activity (U/g)
20	3.0± 0.40
30	3.5± 0.14
40	4.5± 0.22
50	6.55± 0.15
60	4.65±0.26
70	1.5± 0.30

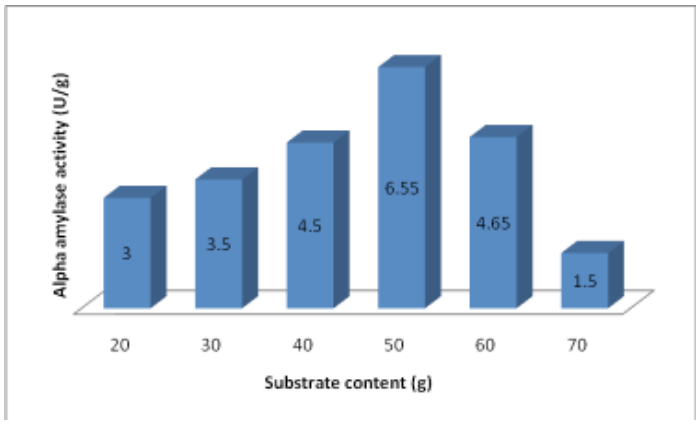

Fig. 4: Effect of substrate concentration on alpha-amylase production

4. Discussion

The present study emphasised on the optimization of the α amylase production by *Aspergillus oryzae* using banana peel as substrate through solid state fermentation.

It was found that with the increase in incubation period, the production of alpha amylase increases. But after 96 hours of growth, the capacity of the fungus to produce the enzyme declines, being the optimal incubation time of 96 hours. This may be due to lack of nutrients, accumulation of toxic substances or proteolysis of alpha amylase as explained by several workers (Chamber *et al.,* 1999, Shafique *et al.,* 2009). Abu *et al.,* (2005) also reported maximum amylase production by *Asprgillus* sp. after an incubation period of 96 hours.

In the present investigation, it was found that 35°C was optimum incubation temperature for alpha amylase production by *Aspergillus oryzae*. It has been reported that fungal growth and enzyme production is maximum at temperature ranging from 30°C to 35°C (Shafique *et al.,* 2009, Dakhmouche *et al.,* 2006).

The pH of the growth medium is one of the physico chemical parameter responsible for morphological changes in the organism and in enzyme secretion. Maximum alpha amylase activity was observed at pH 5.0. Further increase in the initial pH of the medium resulted in the decrease in the enzyme activity. This may be due to the fact that most of the fungi require slightly acidic pH for their optimum growth (Liu *et al.,* 2008, Sun *et al.,* 2009). It is also reported that Alpha amylase production by microbial strain strongly depends on the extracellular pH as it influences many enzymatic reaction as well as transport of various components across the cell membrane (Ellaiah*et al.,* 2002). In contrast to the present findings, Alva *et al.,* (2007) achieved the maximum alpha amylase production at pH 5.8 by *Aspergillus* sp.

The present study also concludes that the amount of substrate is very much crucial for the production of alpha amylase by *Aspergillus oryzae* through SSF. The maximum enzyme production was seen at 50g of substrate. In other substrate concentrations, the enzyme production was comparatively low. This may be due to availability of limited inoculum for biosynthesis (Abe *et al.,* 2005).

5. Conclusion

The results obtained from the present study indicated *Aspergillus oryzae* MTCC No. 3107 as a potential strain for α amylase production using SSF process with banana peel as the substrate. The maximum α amylase activity was achieved at incubation period of 96 hours, incubation temperature 35^{0}C, initial pH of the medium at 5.0 and 50 g of substrate content. Banana peel waste also proved to be a good substrate for alpha amylase production. The findings of the present investigation will provide valuable information for the bulk production of the enzyme in future.

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