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Bio-Processing of Banana Peel for α-Amylase Production by *Bacillus subtilis*

SHAISTA KOKAB, M. ASGHAR, K. REHMAN, M.J. ASAD AND O. ADEDYO†
Department of Chemistry, University of Agriculture, Faisalabad–38040, Pakistan
†Department of Chemical Engineering, University of Waterloo, Ontario, Canada

ABSTRACT

Alpha amylase was produced by *Bacillus subtilis* utilizing banana peel in a solid state fermentation (SSF). The effect of varying incubation period, substrate level, pH of the medium, incubation temperature, peptone (nitrogen source) and micronutrients on the production of α-amylase was investigated. The maximum activity of α-amylase (9.06 IU/mL/min) was recorded after 24 hours of SSF at pH 7 and 35°C temperature of the optimum banana peel medium containing, 50 g fresh chopped banana peel (substrate), 0.2% peptone, 0.02% $MgSO_4.7H_2O$, 0.04% $CaCl_2.2H_2O$ and 0.1% KH_2PO_4. The enzyme produced by *Bacillus subtilis* can be used in industrial processes after characterization.

Key Words: Banana peel; Fermentation; α–amylase; *Bacillus subtilis*

INTRODUCTION

Alpha amylase is a hydrolytic enzyme and in recent years, interest in its microbial production has increased dramatically due to its wide spread use in food, textile, baking and detergent industries (Asghar *et al*., 2000). Besides its use in the saccharification or liquefaction of starch, the enzyme is also used for the warp sizing of textile fibres, the clarification of haze formed in beer or fruit juices, and for the pretreatment of animal feed to improve the digestibility (Hanes & Stedt, 1988). A growing new area of application of α-amylase is in the fields of laundry and dish washing detergents (Van der Maarel *et al*., 2002). Asia is the largest producer of banana. It has been estimated that about 20,000 tones of banana peel are discarded annually from their processing plants, posing serious environmental problems. A study of sugars in banana peels showed that the peel contained 14.6% glucose and 56% sucrose (Goewert & Nicholas, 1980).

This paper reports the optimization of fermentation parameters for α-amylase production by *Bacillus subtilis* through solid state fermentation (SSF) of banana peel for possible commercialization of the process.

MATERIALS AND METHODS

Microorganism. *B. subtilis* obtained from NIBGE, Faisalabad and maintained on nutrient agar slants, at pH 7 and 35°C temperature was used in the present investigation.

Preparation of substrate. Banana peel used as substrate was obtained from fruit market of Faisalabad and chopped into small pieces of uniform size (40 mm mesh).

Inoculum preparation. The spores of *B. subtilis* were transferred aseptically to a 500 mL conical flask containing 100 mL of pre-sterilized inoculum medium containing g/100 mL: glucose, 2; yeast extract, 0.3; peptone, 0.5; NaCl, 1.5; $Na_2HPO_4.2H_2O$, 1.1; $NaH_2PO_4.2H_2O$, 0.61; KCl, 0.3; and $MgSO_4.7H_2O$, 0.01 in laminar air flow. The flask was then kept on shaker (120 rpm) at 37°C for 24 h. The homogenous spore suspension (10^6-10^7 spores/mL) was used as inoculum.

Solid state fermentation. All the treatments were run in duplicate. The pH pf the fresh chopped banana peel (86.27% moisture) was adjusted to 7 with MHCl/MNaOH and sterilized in autoclave (SANYO) for 15 min at 121°C. After cooling, inoculum (5 mL) was added to each flask in the laminar air flow (DALTON) with the help of sterilized pipette. The flasks were then incubated at 35°C for 24 h (unless otherwise mentioned) without shaking in incubator (Gallenkamp). The SSF media flasks were gently shaken after every 12 h for uniform mixing of the substrate and microorganism.

Optimization of process parameters. The growth medium of banana peel was fermented with *B. subtilis* for optimization of different parameters for α-amylase production. The experiments were carried out systematically in such a way that the parameter optimized in one experiment was maintained at its optimum level in the subsequent experiments.

Parameters

Incubation period. Growth media containing 50 g of banana peel were incubated for varying time periods (12-60 h) at pH 7 and 35°C.

Substrate level. Conical flasks containing different substrate levels (20-60 g) were inoculated (5 mL) and incubated for 24 h (optimum) at pH 7 and 35°C.

pH of the medium. pH of 50 g (optimum) chopped banana peel was adjusted at different levels viz., 4, 5, 6, 7 and 8 before inoculation and incubation for 24 h.

Incubation temperature. SSF media of banana peel (50 g) were inoculated (5 mL) and incubated at pH 7 under different conditions of temperature viz., 25, 30, 35, 40 and

45°C for 24 h.

Additional nitrogen source. The SSF medium was supplemented with varying concentration of peptone (0.1, 0.2, 0.3, 0.4 and 0.5%) to investigate its effect on growth of *B. subtilis* and enzyme production under preoptimized conditions.

Inorganic nutrients. Effect of varying concentrations of $MgSO_4.7H_2O$, $CaCl_2.2H_2O$ and KH_2PO_4 on α-amylase production by *B. subtilis* in optimum banana peel medium was also studied by conducting three experiments one after the optimization of other nutrient.

Enzyme extraction. α-amylase was extracted from SSF medium by a simple contact method (Ramesh & Lonsane, 1989). After specified incubation period (in each case), 100 mL sodium phosphate buffer of pH 6.9 was added into each experimental flask. The flaks were shaken (150 rpm) for half an hour and the material was filtered through Whatman filter paper No. 1. The filtrate was centrifuged at 1000 rpm for 10 min at –10°C. The supernatant was carefully collected and used as crude enzyme extract.

Enzyme assay. α-amylase activity was determined by the spectrophotometric method described by Bernfeld (1955) in an assay mixture containing, enzyme extract, starch as substrate and DNS as coupling reagent. One unit of α-amylase activity was defined as the number of µ moles of maltose liberated by 1 mL of enzyme solution per minute.

RESULTS AND DISCUSSION

Different fermentation parameters were optimized for α-amylase production by conducting a series of experiments and the results are discussed as under:

Substrate characteristics. In an initial experiment dried (60°C) banana peel ground to powder form, fresh mashed peel and fresh chopped peel were used as substrates to study the growth of *B. subtilis* and α-amylase production. In each case, moisture was maintained at 80% level. It was noted that the organism did not show any commendable growth in the dried powder due to ceramalization of sugars in the peel as it turned black on oven drying. The mashed peel also did not support the microbial growth and it sporulated on the substrate surface due to formation of a sticky paste posing aeration and mixing problems. The microorganism showed good growth on chopped banana peel and was selected to be used as substrate in SSF for α-amylase production.

Fermentation period. Duplicate flasks containing 50 g of fresh chopped banana peel were autoclaved, inoculated (5 mL) and incubated at 35°C for 12, 24, 36, 48, 60 and 72 h. The maximum activity of α-amylase (6.97 IU/mL/min) was noted in enzyme extracts harvested after 24 h of SSF at pH 7 and 35°C temperature (Table I). Results are in line with those of Krishna and Chandrasekaran (1996) who optimized solid state flask culture for production of α-amylase by *B. subtilis* using banana stalks as a substrate. The SSF was found to produce maximum α-amylase when incubated for 24 h at pH 7 and 35°C. Tonkova *et al*. (1993) produced α-amylase by *B. licheniformis* 44 MB 82-G, using glucose as carbon source and optimum enzyme activity of culture medium was recorded after 96 h.

Substrate level. Fermentation media containing 20, 30, 40, 50 and 60 g banana peel were sterilized, inoculated and incubated for 24 h at pH 7 and 35°C. It was observed that 50 g banana peel in the fermentation medium yielded maximum (7.14 IU/mL/min) α-amylase activity (Table I) after 24 h. A further increase in substrate did not increase the enzyme yield significantly because 5 mL inoculum was added to each flask and increase in substrate level only could not effect the growth of organism. Krishna and Chandrasekaran (1996) reported maximum α-amylase production by *B. subtilis* utilizing 10 g banana stalk as substrate moistened with mineral salts solution to 70% moisture content. The difference in substrate level may be due to the reason that they used dried (70°C) powder of banana stalks and we utilized fresh chopped banana peel (86.27% moisture).

Table I. Effect of varying fermentation period and substrate level* on α-amylase production by *Bacillus substilis*

Fermentation period (hours)	α-amylase activity (IU/mL/min)	Substrate level (g)	α-amylase activity (IU/mL/min)
12	5.60	20	3.27
24	6.97	30	4.53
36	5.90	40	5.49
48	4.35	50	7.14
60	3.97	60	7.20

* Substrate level was varied and SSF was carried out for 24 h at pH 7 and 35°C

pH of medium. Results showing the effect of pH on α-amylase production by *B. subtilis* in SSF of banana peel are presented in Fig. 1. The maximum activity of α-amylase (7.19 IU/mL/min) was observed in the fermentation medium adjusted at pH 7. At pH 4, activity was low due to more acid but as the pH increased to 7 the enzyme induction increased. At pH 8, comparatively lower enzyme production was observed. Different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth (Lehninger, 1982). Our results are in line with Terui (1973) who reported 6.8 as optimum pH for the production of α-amylase by *B. subtilis*.

Incubation temperature. *Bacillus subtilis* showed maximum production of α-amylase (7.31 IU/mL/min) in the banana peel medium (50 g) incubated at 35°C for 24 h at pH 7. A decrease or increase in incubation temperature caused a decrease in enzyme production by *B. substilis* (Fig. 2). The optimum temperature observed for the production of α-amylase from Banana stalk using *B. subtilis* was also 35°C

Fig. 1. Effect of varying pH of the medium on α-amylase production

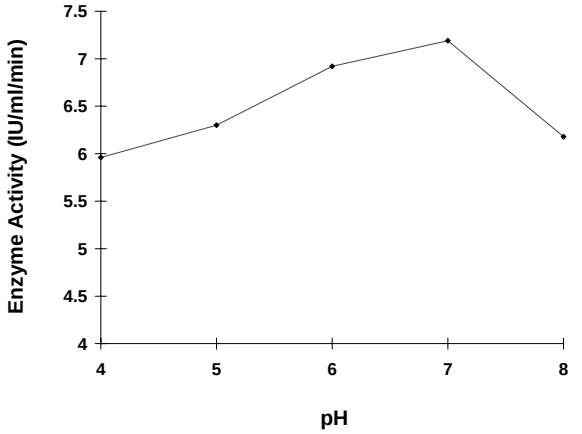


Fig.2. Effect of varying incubation temperatures on α-amylase production by *Bacillus subtilis*

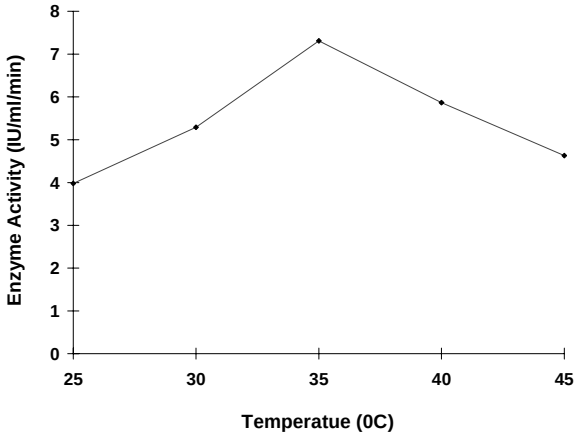


Fig. 3. Effect of varying concentrations of peptone on α-amylase production by *Bacillus subtilis*

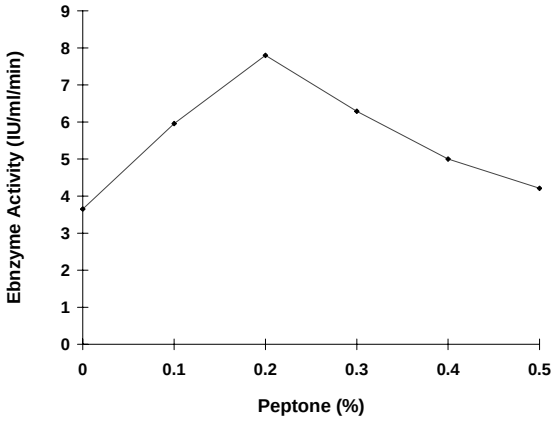
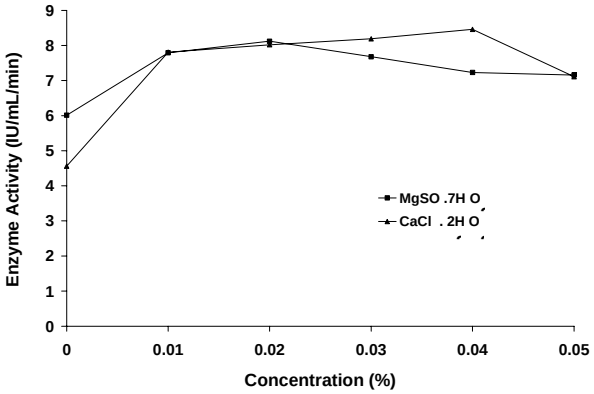


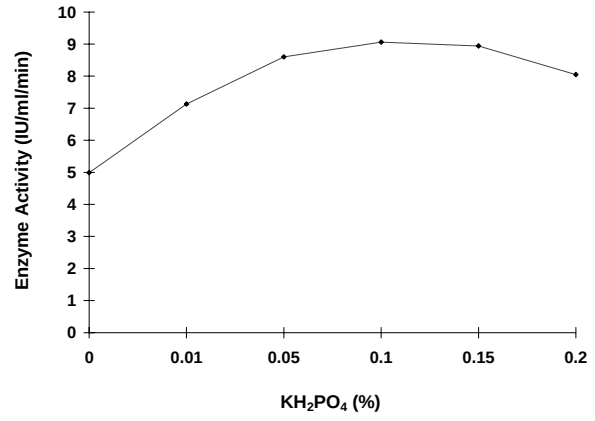
Fig. 4. Effect of varying concentrations of $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ on α-amylase production by *Bacillus subtilis*



as reported by Krishna and Chandrasekaran (1996).

Peptone. The source and concentration of nitrogen in the growth has an impact on microbial growth and enzyme production. The results showed maximum α-amylase yield (7.80 IU/mL/min) in the medium supplemented with 0.2% (w/w) peptone as nitrogen source (Fig. 3). The growth media containing less and more than 0.2% peptone exhibited lower enzyme activities. Results are comparable to those of Terui (1973) who observed the maximum enzyme yield at 0.5% (W/W) peptone concentration when *B. subtilis* was grown on starch medium.

Fig. 5. Effect of varying concentrations of KH_2PO_4 on α-amylase production by *Bacillus subtilis*



$MgSO_4.7H_2O$. The addition of $MgSO_4.7H_2O$ into the banana peel medium enhanced α-amylase production by *B. subtilis* and 0.02% $MgSO_4.7H_2O$ resulted in maximum α-amylase activity (8.12 IU/mL/min). The growth media containing less and more than 0.02% $MgSO_4.7H_2O$ showed lower activities of enzyme (Fig. 4). Bajpai *et al*. (1992) reported that 0.1% $MgSO_4.7H_2O$ gave maximum α-amylase production by *B. subtilis* from cheese whey. Upton and Forgarty (1977) produced maximum α-amylase from *Aspergillus oryze* using 8% starch with 0.1% $MgSO_4.7H_2O$.

$CaCl_2.2H_2O$. $CaCl_2.2H_2O$ was used to enhance microbial growth and α-amylase production. The enzyme activity increased with the addition of $CaCl_2.2H_2O$ and peaked (8.26 IU/mL/min) with 0.04% $CaCl_2.2H_2O$ which decreased,

thereafter (Fig. 4). Our results are in line with those of Asghar *et al*. (2002) who produced α-amylase by *Arachniotus sp*. in waste bread medium and noted maximum enzyme activity with 0.05% $CaCl_2.2H_2O$.

KH_2PO_4. Results shown in Fig. 5 revealed maximum α-amylase activity (9.06 IU/mL/min) with 0.1% KH_2PO_4 in the optimum SSF of banana peel medium. Alpha-amylase production was enhanced by addition of KH_2PO_4 upto 0.1% and decreased by further addition of this salt. Results are in line with those of Xiangi (1984) who obtained maximum α-amylase by immobilized *Aspergillus niger* when the medium contained 0.05% KH_2PO_4. Bajpai *et al*. (1992) observed that 0.1% KH_2PO_4 in cheese whey medium gives maximum α-amylase production by *B. subtilis*.

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