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PRODUCTION OF a-AMYLASE THROUGH SOLID SUBSTRATE FERMENTATION OF BANANA STALKS BY *Bacillus subtilis*

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Study was carried out to optimize moisture level, fermentation time, pH of the medium, incubation temperature along with effect of peptone and surfactants on a-amylase production by B. *subtilis* in solid substrate fermentation (SSF) of banana stalks. Addition of peptone, sodium dodecyl sulphate (SOS) and tween-80 enhanced the production of a-amylase. The a-amylase activity was maximum (15.22 U/mL) at 24 hours of incubation, with banana stalks (70% moisture) as substrate in a medium, containing 0.3% peptone, 0.1% SOS and 0·1% tween-80 at pH 7 and 35°C.

Key Words: a-amylase, *Bacillus subti/is*, banana stalk, solid substrate fermentation, surfactants.

INTRODUCTION

a-amylase is secreted by a wide variety of microorganisms, including fungi, yeasts and bacteria. The end products of a-amylase action on starch are oligosaccharides with varying chain length with an a-configuration and a-limit dextrins which constitute branched oligosacchardies (Pandey *et a/.*, 2000). Originally, e-a myiases were added during dough preparation to generate fermentable compounds. Besides generating fermentable compounds, a-amylase also have an anti-staling effect in bread baking and improving the softness retention of baked goods (Oe-Stefanis and Turner, 1981). The increased guminess of a-amylase treated bread is associated with the production of branched maltodexrtrins *of* OP 20-100 (De Stefanis and Turner, 1981).

Besides their use in the saccharification or liquefaction of starch, these enzymes are also used for the preparation of stable, viscous starch solutions for the warp sizing of textile fibers, the clarification of haze in beer or fruit juices and for the pretreatment of animal feed to improve its digestibility. A growing area of application of a-amylase is in the field of laundry and dishwashing detergents (Spendler and Jorgensen, 1997). *Bacillus subti/is* a-amylase is particularly useful in industrial and agricultural processing applications due to its high degree of stability and activity upon a broad range of starches (Nicholson and Chambliss, 1987).

This paper reports the optimization of some fermentation parameters and effect of peptone (additional nitrogen source) and surfactants on SSF for a-amylase production by B. *subti/is* grown on banana stalks.

MATERIALS AND METHODS

All the samples were fermented in duplicate and all the measurements were in triplicate.

Substrate

Banana stalks obtained from fruit market of Faisalabad, were dried (70°C) and ground to powder form (40 mm mesh) and stored in polyethylene bags till use as substrate for a-amylase production.

Fermentative Organism and sporulation medium

Pure culture of bacteria *Bacillus subti/is* obtained from Nuclear Institute for Biotechnology and Genetic Engineering, (NIBGE), Faisalabad was raised (pH 7, 35°C) on nutrient agar slants. The sporulation medium contained (g/100mL): dextrose/glucose 2.00, yeast extract, 0.3; peptone, 0.5; agar, 2; NaCI, 3.5; Na2HP04.2H20, 1.1; $NaH_2P04.2H20$, 0.61; KCI, 0.3 and $MgS04.7H_20$, 0.3.

Inoculum preparation

For the preparation of inoculum, the spores were directly transferred from sporulation slants into the conical flask containing 100 mL presterilized 1% glucose solution. The spore suspension was poured into a sterilized conical flask and spore concentration adjusted at 10^7_10^8 spores/mL

Fermentation process

Conical flasks containing banana stalks (5g) moistened with mineral salt solution containing (g/100 ml) NaCI 3.5, Na2HP04.2H20 1.1, $NaH_2P04.2H20$ 0.61, KCI 0.3, $MgS0_4.7H_20$ 0.3 were autoclaved (at 121°C) and inoculated with 5 mL of homogenous spore suspension. The flasks were incubated at pH 7 and 35°C under still culture conditions for optimum fermentation period. To the fermented biomass samples 100 mL of 0.02M sodium phosphate buffer was added and placed in shaker (120 rpm) for half an hour. It was filtered and the filtrates were centrifuged at 10,000 rpm at _1O°C. The supernatants were subjected to enzyme assay.

Optimization of SSF parameters

In the first experiment the growth medium of banana stalk (5g) was fermented with 40, 50, 60, 70 and 80% moisture level for optimization of substrate: water ratio. In the second experiment the growth medium of banana stalk (70%) was fermented for 12, 24, 36, 48 and 72 hrs for optimization of fermentation period with *Bacillus subti/is.* In the third experiment growth media (70% moisture) adjusted at different pH values were fermented for 24 hours. Growth media of banana stalks were adjusted at pH 4 and incubated at varying temperatures for 24 hours, in the fourth experiment. Varying concentrations of peptone and surfactants (SOS and Tween-80) were used in three independent experiments in such a way that a parameter optimized in an experiment was maintained at it's optimum level in subsequent investigation.

Enzyme Assay

The activity of enzyme in culture filtrates was determined by the assay method of Bernfeld (1955) using starch as substrate and dinitro salicylic acid (ONS) as coupling reagent. The absorbances of the coloured complex were noted at 540 nm against reagent blank. One unit of enzyme activity was defined as the amount of enzyme to release 1urnole of maltose/min.

RESULTS AND DISCUSSION

Moisture level

Growth media of banana stalk (5g) containing different moisture levels (40, 50, 60, 70 and 80%) were fermented with *Bacillus subti/is* for 24 hours. The results showed that a-amylase activity increased steadily upto 14.38 U/mL with 70% moisture and decreased thereafter to 13.66 U/mL with 80% moisture. The results are in accordance with Krishna and Chandrasekran (1996) who reported 70% moisture level as optimum for a-amylase production by *Bacillus subtilis* in SSF of banana stalk.

Fermentation Period

It was noted that initially the enzyme production increased by increasing fermentation period upto 24 hours but decreased thereafter. The growth medium harvested after 24 hours showed maximum (14.66 U/mL) a-amylase production. These results are in line with Lealem and Gashe (1994) who obtained maximum enzyme activity after 72 hours when *Bacillus sp.* A 001 was grown in starch salt medium. Tunkova *et al.* (1993) noted maximum a-amylase activity after 96 hours incubation of *Bacillus /icheniformis* 44 MB 82-6 with glucose as carbon source. The difference in incubation time may be attributed to different carbon sources and microbial strains employed.

Effect of pH

Maximum a-amylase production (14.67 U/mL) was recorded in the banana stalk medium (70% moisture) adjusted at pH 7 and incubated for 24 hours under SSF conditions (Fig. 1). An increase in enzyme production was observed by an initial increase in pH from 5 to 7. A further increase in pH (from 7 to 9) caused a gradual decrease in enzyme yield. These results are in agreement with those of Keating and Kelly (1996) who got maximum a-amylase activity by *Bacillus sp.* at pH 5. Terui (1973) got optimal a-amylase activity in starch medium fermented with *Bacillus sp.* at pH 6.8. The variation may be due to the difference in substrate characteristics and pH optima of different *Bacillus* strains.

Effect of Temperature

Banana stalks in SSF media were inoculated and incubated at different temperatures viz. 25, 30, 35, 40 and 45°C and harvested after 24 hours. a-amylase production was maximum (14.68 UlmL) in the medium fermented at 35°C. An increase in incubation temperature from 35°C to 45°C caused a decrease in enzyme yield (Fig. 2). These results are in accordance with Mamo and Gessesse (1999) who produced maximum a-amylase by *Bacillus sp.* at 55°C. Lin *et al.* (1998) also reported maximum growth and a-amylase production by *Bacillus sp.* at 55°C.

Effect of Peptone

Five different levels of peptone i.e. 0.1, 0.2, 0.3, 0.4 and 0.5% were used as additional nitrogen source in banana stalk medium (70% moisture) at pH 7. Results showed that a-amylase production by *Bacillus subtilis* was enhanced by the addition of peptone and peaked (14.81 U/mL) with 0.3% level (Fig. 3). Further increase in peptone through 0.4 %upto 0.5% led to a steady decrease in yield of a-amylase. Our results are comparable to those of Krishna and Chandrasekran (1996) who observed maximum a-amylase activity with 0.5% peptone by *Bacillus sp.* Terui (1973) produced maximum a-amylase by *Bacillus sp.* using starch as carbon source and 0.5% peptone as additional nitrogen source.

Effect of SOS

To study the effect of SOS (a surfactant) different concentrations of SOS (0.05, 0.1, 0.15, 0.2 and 0.25%) were added to the fermentation medium along with pre-optimized moisture and peptone (0.3%). Maximum a-amylase activity (15.00 U/mL) was obtained with 0.1% SOS. A further increase in its concentration resulted in lower a-amylase yield (Fig. 4). Our results are comparable with those of Goes and Sheppard (1999) who produced a-amylase by *Bacillus sp.* and observed maximum activity with 0.5% SOS in potato peel medium.

Fig. 1 Effect of pH on a-amylase production by *B. subtilis* in SSF of banana stalk

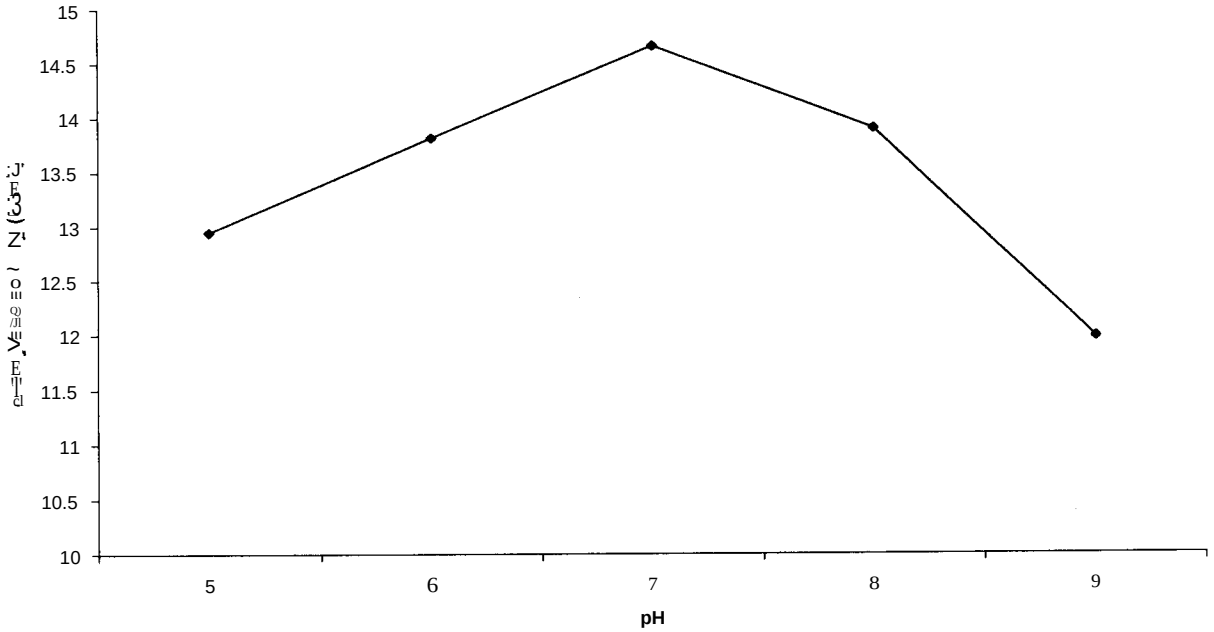

Fig. 2 Effect of temperature on a-amylase production by *B. subtilis* in SSF of banana stalk

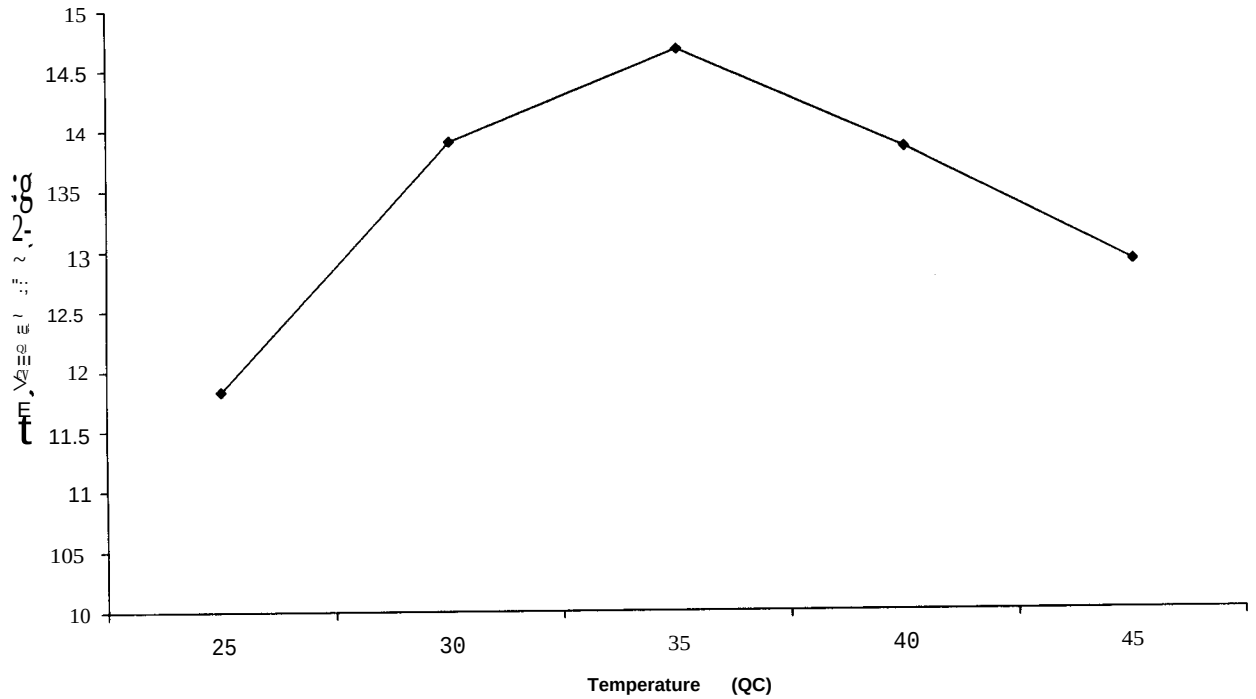

Fig. 3 Effect of varying concentrations of peptone on a-amylase **production by B. *subtilis***

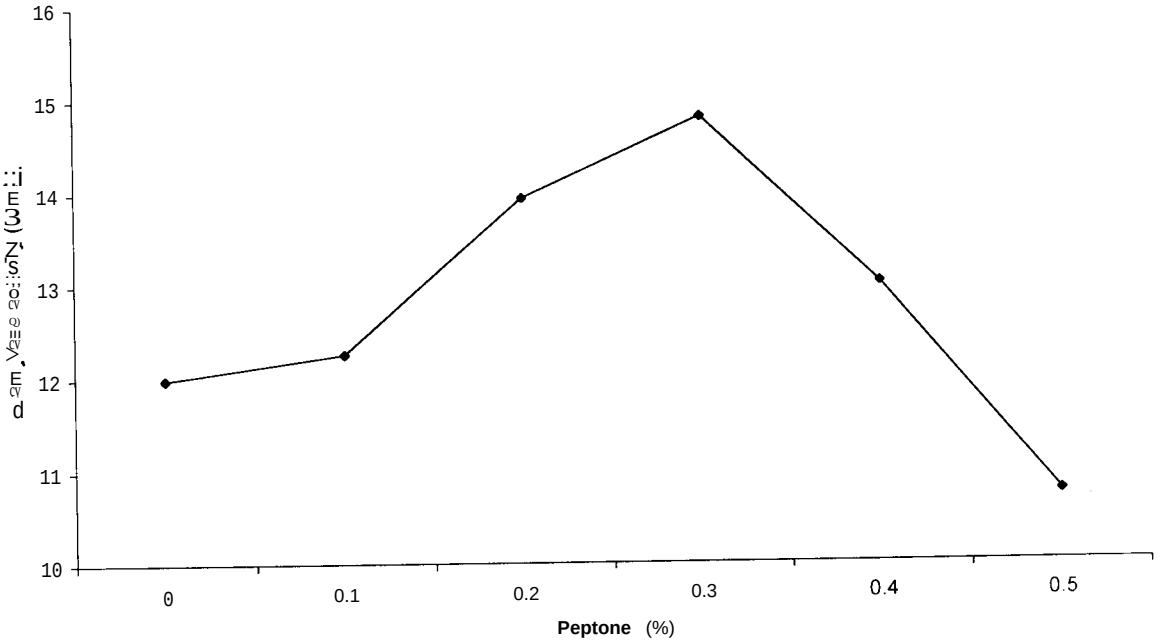

Fig. 4 Effect of varying concentrations of surfactants on a-amylase production by *Bacillus subtilis*

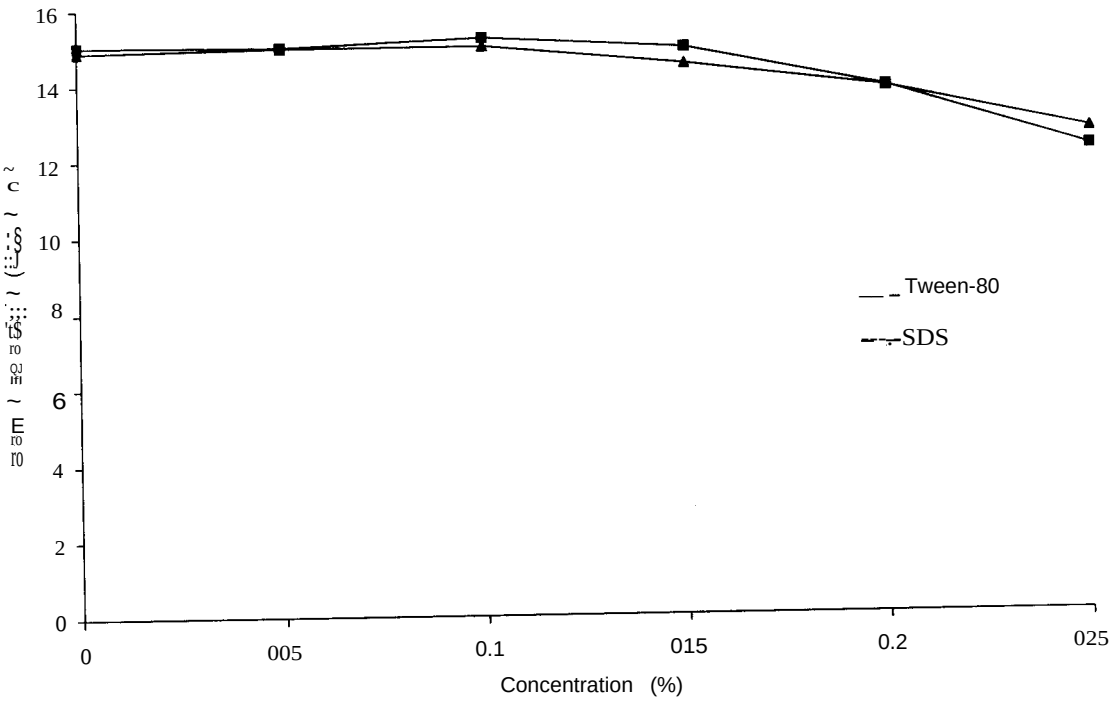

Table 1. Activity of a-amylase produced by *B. subilis* in SSF of banana stalk with varying moisture levels.

Moisture (%)	a-amylase activity (U/mL)
40	10.64
50	12.83
60	14.00
70	14.38
80	13.66

Table 2. Activity of a-amylase produced by *B. subilis* at varying incubation periods.

Fermentation period (hours)	a-amylase activity (U/mL)
12.00	11.92
18.00	13.27
24.00	14.66
36.00	13.99
48.00	11.87

Effect of Tween-80

Results indicated that production of a-amylase by *Bacillus subtilis* increased with the addition of tween-80 (another surfactant) into the optimum growth medium. a-amylase activity was found to be maximum with 0.1% tween-80 in the preoptimized banana stalk medium (Fig. 4). The activity decreased with a further increase in concentration upto 0.25%.

The surfactants have the potential to increase enzyme yields in SSF by increasing penetration of water into the solid substrate matrix and increasing surface area for microbial growth. Results of our project accord with those of Goes and Sheppard, (1999) who noted maximum -amylase activity in potato peel medium fermented by *Bacillus sp* with 0.1% tween-80.

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