

A STUDY OF PRODUCTION OF α - AMYLASE BY BACILLUS SUBTILIZE IN SOLID STATE FERMENTATION

¹ Ramamurthy, V., ² Anil Kumar, H.V. and ^{3*} Vadivazhagi.M.K

¹ Assistant Professor, P.G. and Research Department of Biochemistry, Marudupandiyar College,
Thanjavur – 613 403, Tamil Nadu, India

² Associate Professor, Department of Environmental and Laboratory of Applied Biological Science,
D.V.S. College of Arts and Science, Shimoga, Karnataka, India

³ Assistant Professor, Department of Biochemistry Sri Akilandeswari Womens College,
Vandavasi - 604 408, Tamil Nadu, India.

1. INTRODUCTION:

Enzymes are protein molecules with a molecular weight ranging from 10,000 to million, which initiate & control nearly every biochemical process in the body. Many micro-organisms produce extracellular enzymes. There are chiefly hydrolases & are involved primarily in degradation of macromolecules to units capable of being taken into the living cell. Amylase is an enzyme that involves break down starch into glucose molecules. They are 2 types α - amylase & β - amylase. Amylase find potential application in a number of industrial processes such as in the food, fermentation, textiles & paper industries. Bacillus is a genus of rod-shaped, gram positive bacteria. Bacillus includes both free-living & pathogenic species. Bacillus subtilis efficiently produces three types of enzyme α - amylase β -amylase & protein.

2. MATERIALS & METHOD

Micro Organism used:

Bacillus subtilis

Culture maintenance

Solid state fermentation:

Fermentation was carried out in 250 ml Erlenmeyer flasks containing 4g of sugar cane waste 0.25%, $MgCl_2$ 0.25%, KCL, ribose 0.25%, 100ml deionizer water which was autoclaved for 20 minutes at 121°C, a cell suspension of 1% -Bacillus subtilis was used as inoculum. Incubation was carried out for 15 days at 37°C.

- The parameters selected for this study were effect of P^H , effect of Temperature; effect of inoculum concentration, effect of incubation time & effect of substrate concentration.

Enzyme Extraction:

- Enzyme was extracted from the fermentation medium.

- 10ml of fermented medium was taken & subjected to centrifugation at >3000 rpm for 10mts.
- The supernatant was used for enzyme assay.

3. RESULTS:

In the present study α - amylase production in solid state fermentation was carried out. Cultural conditions such as effect of P^H , effect of temperature, inoculums Concentration, effect of substrate concentration. Monitored by using molasses substrate effect of different substrates such as molasses, rice bran, peel of black grapes & Pomegranate.

Effect of incubation period α - amylase production:

The α - amylase production was carried out in 250 ml Erlenmeyer flasks containing off 4 g of molasses. 0.05%- urea, 25%- $MgCl_2$, ribose 0.25%, 25ml deionizer water. The α - amylase production was monitored at effect of incubation periods were 12,24,36,48,60 & 72 hrs interval (Table -1) the maximum α - amylase 49% u/g) at 48 hrs interval, after which it decrease slightly (47.3 v/g) at 60 hrs interval. A prolonged incubation period beyond this period did not help to further increase in the enzyme yield.

Effect of P^H on α - amylase production:

The production of α - amylase by bacillus subtilis of different P^H level. The maximum α -amylase (47 U/G) was obtained when the P^H of the medium at. (Table-2) The maintained of favorable P^H in very essential for the success full production of α - amylase.

Effect of incubation Temperature:

The production of α - amylase by bacillus subtilis at different temperature (20^oc-40^oc) The maximum α -amylase (56 v/g) was obtain when the temperature of the medium was maintained at 30^oc. (Table-3)

Effect of inoculums volume:

The production of α - amylase by Bacillus subtilis of different inoculums volume was carried out. The maximum α -amylase (54.1 u/g) was obtained when the medium containing 7.5 ml of inoculums. Then slightly decreased in 10 ml inoculum containing medium. (Table – 4)

Effect of Substrate concentration:

The production of α - amylase by bacillus subtilis at different substrate concentration was carried out. The maximum α -amylase (51.2v/g) was obtained when the medium containing 10g substrate concentration. (Table – 4)

Effective of different substrate on α - amylase production:

A comparative study on the utilization of Carbon sources from the different substrates. A α - amylase production was high in peel of grapes as substrate about 61.16 The high production of α - amylase was

obtained in molasses 501 u/g but income of rice bran& peel of pomegranate production less 88 u/g & 27.77 u/g) respectively.

S.No	Time (hrs)	a-amylase (U/G)
1	12	11.7
2	24	19.8
3	36	42.9
4	48	49.1
5	60	47.3
6	72	39.5

Table 1: Effect of incubation time on a-amylase production:

S.No	pH	a-amylase (U/G)
1	3.5	29
2	4	37.2
3	4.5	43.2
4	5	47
5	5.5	39
6	6	36.5

Table 2: Effect of pH on a-amylase production

S.No	pH	a-amylase (U/G)
1	20	12.7
2	25	40.9
3	30	56
4	35	37.3
5	40	29.2

Table 3: Effect of temperature on a-amylase production

S.No	Inoculum volume	a-amylase (U/G)
1	1	13.1
2	2.5	29.3
3	5	46
4	7.5	54.1
5	10	49.3

Table 4: Effect of inoculum volume on a-amylase production

S.No	Substrate Con 1.	a-amylase (U/G)
1	1	12.8
2	2.5	37.5
3	5	39.1
4	7.5	43.4
5	10	51.2

Table 5: Effect of substrate concentration on a-amylase production

S.No	Substrate	a-amylase (U/G)
1	Molasses	50
2	Grapes	61.11
3	Rice bran	38.88
4	Pomegranate	27.77

Tabl.6. Effect of different substrate on a-amylase production

CONCLUSION:

This study in solid state fermentation contain bacillus subtiles. Which can be used for production of α - amylase by using molasses as substrate amylase is the most important industrial enzyme & are of great significance in present day biotechnology. The effect of temperature pH incubation period, inoculum volume, substrate concentration were also studied by this study of α - amylase production by bacillus

subtitles was found to be maximum 48hrs. incubation period the yield of α - amylase was 49.1 v/n for the pH 5 the maximum yield was 47 u/g at temperature 30°C the maximum yield was 56 u/g for the inoculum value of 7.5 ml yield amylase was 54.1 u/g the sub substrate concentration of 10 ml was found to be the optimum which gave a yield of 51.2 m/g fermentation.

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