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Production And Purification Of Amylase From Bacillus cereus

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Abstract: Alpha amylase is an important digestive enzyme used in medical studies and has several industrial and commercial applications. The present work evaluates the production of amylase using *Bacillus cereus* and purification by Aqueous Two Phase (ATP) Extraction system. The effect of different nutrient sources was investigated primarily and maximum amylase production was observed with beef extract medium. Further, the enzyme was purified by ATP composed of 40% (w/w) PEG 8000 and 30% (w/w) sodium citrate and also process parameters was optimized. Acceptable extraction was obtained in top phase with enzyme activity of 376.4 U at 40^0 C at pH 6.0 with optimum reaction time of 20 minutes.
Keywords: Beef extract, *Bacillus cereus*, Amylase, ATP, Kinetic parameters.

Introduction:

Amylase is the digestive enzyme. The key function of this enzyme is to break down starches in food so that they can be used by the body. Although amylases are derived from animal and plant sources, the *Bacillus cereus* (1, 2) as a source of this enzyme uses beef extract medium and results in good yield. The development techniques for the separation and purification of enzyme has many positive effects in the advancement of medical and biotechnological industry (3,4,5). Purification is troublesome because of system complexity and need to retain biological activity. ATP is widely used as the extraction and purification tool which makes the task simple and easy (6,7,8,9). In practice PEG/citrate, PEG/sulfate and PEG/phosphate are beneficial for large scale purification. The major advantage of this method is protein denaturation is impossible due to higher water content(10). The target enzyme move towards either of the phase and the presence of biomolecules to the other. The objective of this work is to purify amylase produced by bacillus cereus using ATP system and also optimization of process parameters and stability of the enzymes was studied (11,12).

Materials And Methods:

Chemicals: PEG 8000, sodium citrate, sodium phosphate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate utilized is purchased from HiMedia Laboratory, Mumbai. *Bacillus cereus* is obtained from MTCC, Chandigarh.

Culture:

Cell is grown in three different medium like glucose, wheat bran and beef extract. The growth curves for the above medium was observed and beef extract medium was optimized to obtain higher yield of amylase. Latter the cells are harvested by centrifugation and the extracellular enzymes are used for further analysis.

Optimisation Of Process Parameters:

Effect of Temperature:

The effect of temperature on amylase enzyme was carried out at different temperatures (40 ^{0}C, 50 ^{0}C, 60 ^{0}C, 70 ^{0}C, and 80 ^{0}C). The activation energy of the enzyme was subsequently determined.

Effect of pH:

The effect of pH on the amylase enzyme was carried out by varying the pH values (4.0, 5.0, 6.0, 7.0 and 8.0)

Effect of Heat:

The effect of heat on amylase enzymes was carried out at 70 ^{0}C for different reaction times (5, 10 , 15,20 and 25 minutes). The half life for the enzyme was calculated.

Effect of Reaction time:

The effect of reaction time was carried out for different time intervals and was optimized for the time at which the maximum activity was observed.

Phase Partitioning Experiments:

Effect of PEG and salt concentrations:

The effects of PEG/salt on the amylase enzyme was investigated.

Table 1.Compositions of PEG and Salts

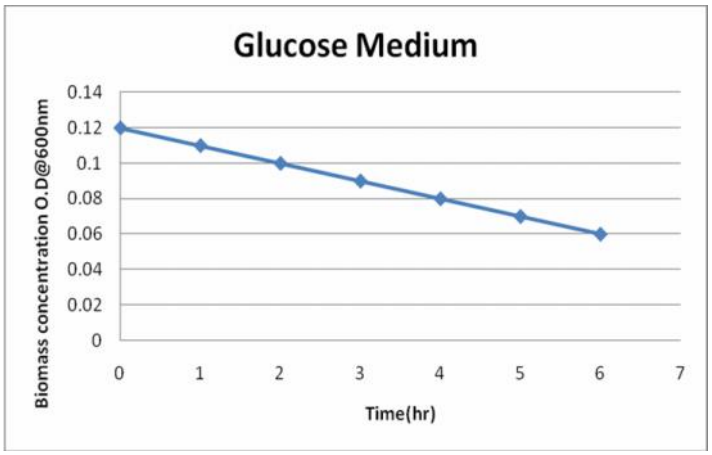
S.NO	PEG %(w/w)	Sodiumcitrat %(w/w)	Sodiumphosphate %(w/w)	K_2HPO_4 %(w/w)	KH_2PO_4 %(w/w)
1	50	25			
2	50		25		
3	50	30			
4	50		30		
5	40		25		
6	40	25			
7	40		30		
8	40	30			
9	50			25	
10	50				25

Results And Discussions:

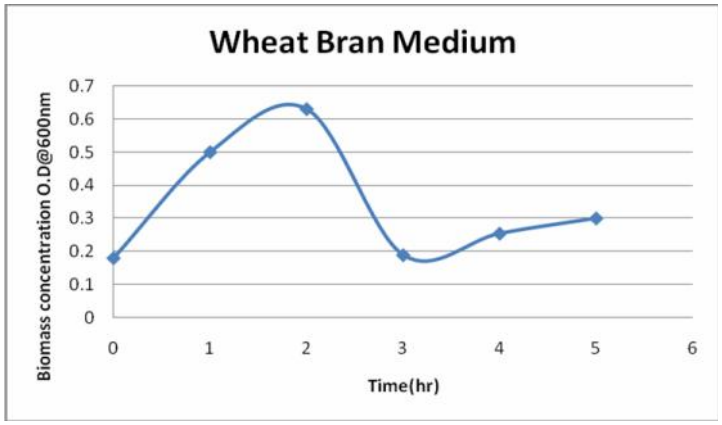
Effect of medium on amylase production:

The growth curves with different sources were obtained and it is shown in following figures

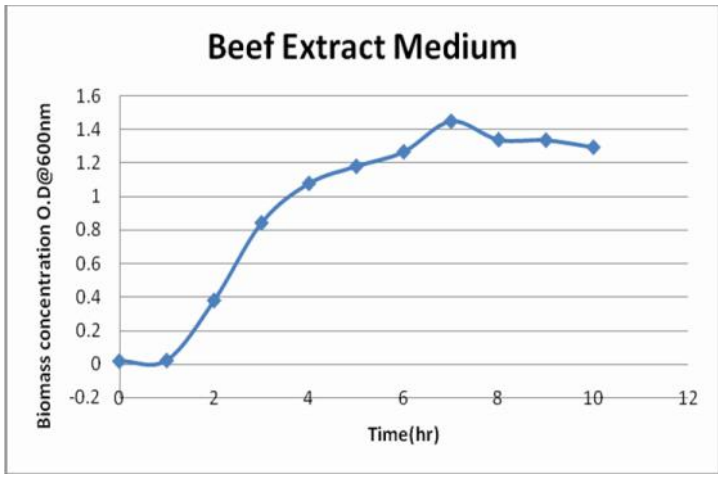
Graph 1: Growth curve using Glucose as Medium



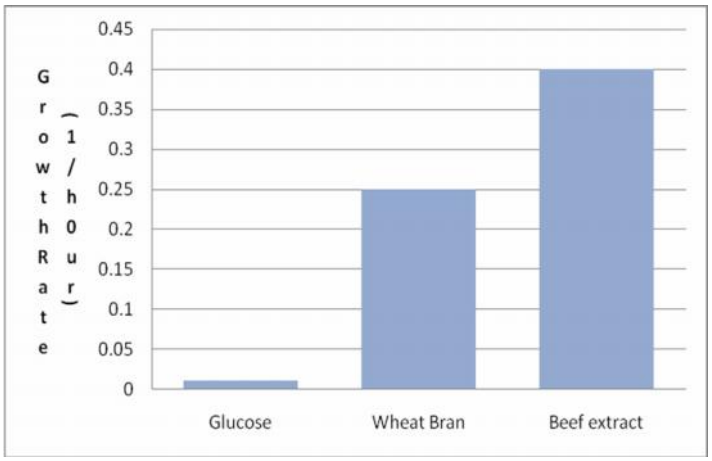
Graph 2: Growth curve using wheat bran as Medium



Graph 3: Growth curve using beef extract as Medium



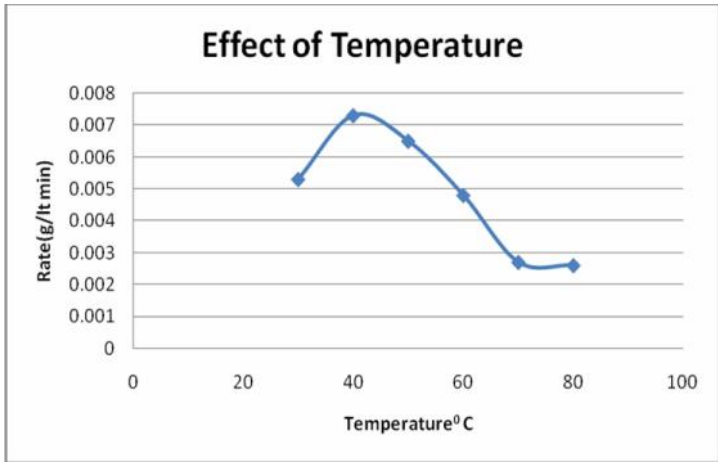
Graph 4: Optimisation of Medium



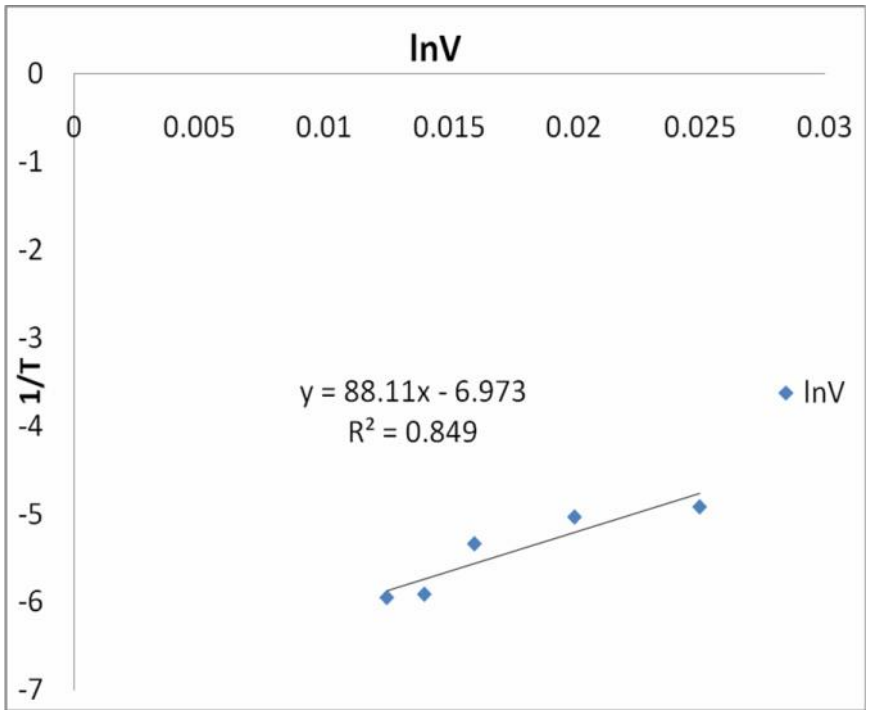
Effect of kinetic studies on amylase:

From the following figures amylase is proved to be optimum at 40 ^{0}C and at a pH of 6. The activation energy and half life of the enzyme were found to be 15.84 KJ/moles and 1.772 minutes respectively. The optimum reaction time for the enzyme was found to be 20 minutes.

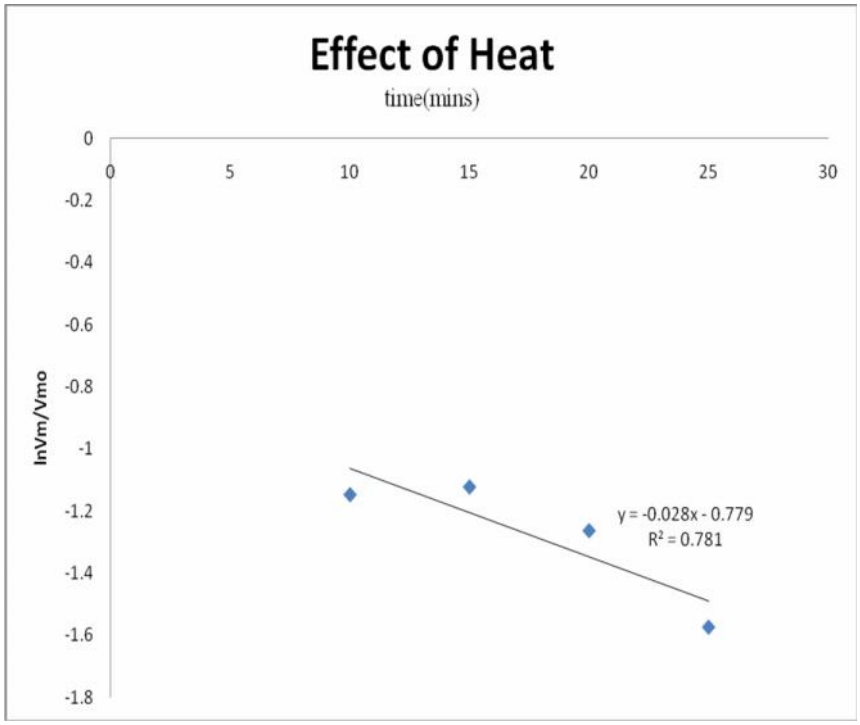
Graph 5: Evaluation of Optimum Temperature



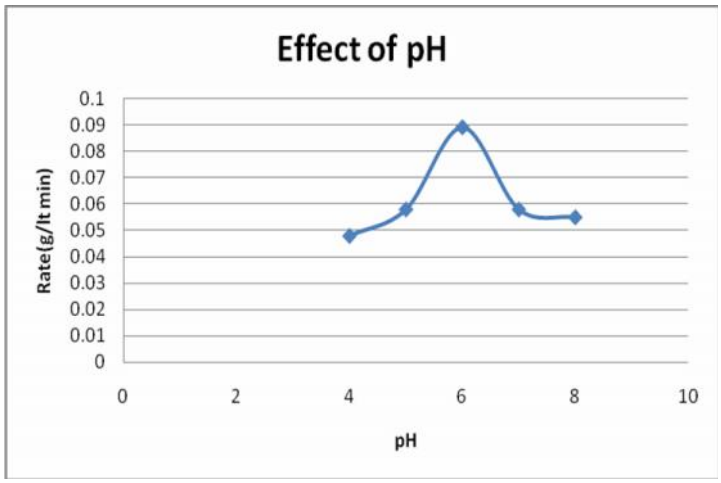
Graph 6: Evaluation of Activation Energy



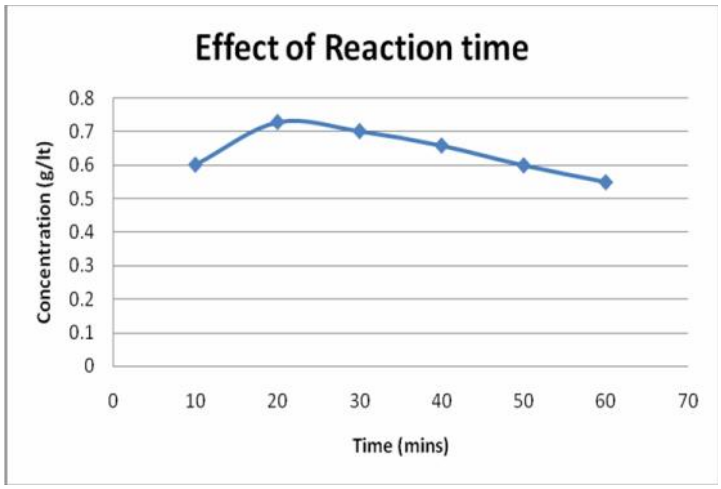
Graph 7: Evaluation of Halflife period



Graph 8: Evaluation of Optimum pH



Graph 9: Evaluation of Optimum Reaction Time



Effect of phase partitioning on amylase enzyme:

Amylase activity was measured by performing enzyme assay with starch as a substrate. DNS is used to detect the starch conversion and the same is expressed in Unit.One unit was defined as the amount of enzyme required to form 1 mole of glucose per minute.

Table 2: Effect of enzyme activity on both Top and Bottom phase respectively.

						Protein concentration (mg)		**Enzyme Activity (g/lt min)**	
S. No	**PEG (%w/w)**	**Sodium Citrate %(w/w)**	**Sodium Phosphate %(w/w)**	**K_2HPO_4 %(w/w)**	**KH_2PO_4 %(w/w)**	**Top phase**	**Bottom phase**	**Top phase**	**Bottom phase**
1	50	25				0.180	0.175	0.117	0.107
2	50		25			0.486	0.018	0.334	0.244
3	50	30				0.512	0.253	0.610	0.154
4	50		30			0.500	0.167	0.624	0.570
5	40		25			0.298	0.067	0.202	0.142
6	40	25				0.419	0.200	0.600	0.381
7	40		30			0.380	0.083	0.612	0.264
8	40	30				0.612	0.086	0.812	0.600
9	50			25		0.356	0.300	0.580	0.269
10	50				25	0.043	0.011	0.360	0.290

Conclusion:

Thus the extraction of amylase by ATP was studied by simple reagents. This work shows the optimum activity of 376.4 U were obtained in top phase of 40%PEG and 30% sodium citrate system

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