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THREE STAGE COUNTERCURRENT EXTRACTION OF AMYLASE BY AQUEOUS TWO PHASE POLY (ETHYLENE GLYCOL) / PHOSPHATE SYSTEMS

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ABSTRACT

Amylases are industrial important enzymes has wide application both on food and medical field. Three stage countercurrent extraction of amylase from PEG (6000) 50%(w/w) / phosphate system 40% (w/w) were carried out at pH 7 and 37 ^{0}C .The yield of enzyme is found to be high as 72% in the top phase of second stage extraction. The activity of enzyme was found to be high in PEG rich phase. Experiments performed for three stages to increase the yield. It was also observed as the yield increases at each stage the enzyme activity was found to be declining. The best activity at the first stage and the best yield and partition coefficient in the second stage was observed. This indicates as the yield increases activity decreases. Hence second stage of extraction is found to be more effective with the activity of 493.3U/ml, yield of 72% and partition coefficient of 2.57.

Keywords: amylases, countercurrent aqueous two phase system, Bacillus subtilis, poly (ethylene glycol), phosphate, three stages.

Introduction:

Amylase decomposes starch in to glucose. Every starch molecules containing reducing terminal will reduce dinitrosalycylic acid (DNS); and hence measurement of reducing sugars like glucose is possible. Amylase present in the human saliva, where it begins the chemical process of digestion.

The dietary carbohydrates should first be broken down to monosaccharides by some gastrointestinal enzymes, since only manosaccharides can be absorbed from intestinal lumen (1, 2). α-Glucosidase and α-amylase are the key enzymes involved in the digestion of carbohydrates (3). α-Amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides that are ultimately converted into monosaccharides by α-glucosidase. Liberated glucose is then absorbed by the gut and results in postprandial hyperglycemia (4, 5).

Food contain much starch but little sugar, such as rice and potato , taste slightly sweet as they are chewed because amylase turns some of their starch in to sugar in the mouth. Pancreas also makes amylase to hydrolyse dietary starch in to disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy. Plants and some bacteria also produce amylase. As diastase, amylase was the first enzyme to be discovered and isolated. All amylases are glycoside hydrolases and act on α1,4 glycosidic bonds. It will start to denature at around 60^{0}C. Amylase enzyme finds use in bread making and to break down the complex sugars such as starch in to simple sugars, yeast then feed on these simple sugars and converts it in to the waste products of alcohol and CO_2.It is also used in clothing and dish water detergents to dissolve starch from fabrics and dishes. Several sources are available for the production of amylases, such as bacteria and fungi. Bacillus spp is considered to be

the highly productive agent for the production of amylase. Large scale purification of enzymes is possible by effective downstream processing where the biological activity is preserved. Aqueous two phase extraction system is an attractive, ideal technology where clarification concentration and partial purification can be integrated in one step. Moreover, this method can be made highly selective and can be easily scale up, thus allowing wider biotechnological applications.

Materials and methods:

PEG 6000, KH_2PO_4 and other reagents are involved in this work. Microorganism Bacillus subtilis was obtained from IMTECH, Chandigarh, India.

Production of amylase:

Minimal medium was used for the fermentation of bacillus subtilis. The media contains following composition as by glucose 1% (w/v) and 0.1mM $cacl_2$ in a 110 rpm rotary shaker at 37C for six hours. The fermentation broth is centrifuged for about 15 minutes at 12,500 rpm was used as the enzyme source.

The following factors like amylase activity, specific enzyme activity, purification factor, partition coefficient, yield in top phase and yield in bottom phase was evaluated by the following formulas (1),(2),(3),(4),(5) and (6)

(1) Evaluation of Amylase activity:

Amylase activity = $A_{570\ nm/\ min}$ * 1000*(total volume of the mixture)/ 10.13*(ml of enzyme)

10.13 is assumed as extinction coefficient at 570nm for DNSassay of amylase enzyme

(2) Evaluation of Specific enzyme activity:

Specific enzyme activity = enzyme activity/mg of protein (U/mg)

(3) Evaluation of Purification factor:

Purification factor = enzyme specific activity after purification/enzyme specific activity of crude extract.

(4) Evaluation of Partition coefficient:

Partition coefficient (K) = Concentration of enzyme in top phase /Concentration enzyme in bottom phase

(5) Evaluation of Yield:

Yield in top phase (Y_T) = $\{C_TV_T/C_TV_T+C_BV_B\}$ *100

Yield in bottom phase

(Y_B) = $\{C_BV_B/C_TV_T+C_BV_B\}$ *100

V_T is volume of top phase

V_B is volume of bottom phase

Quantification of protein:

Amount of protein in the samples assessed by Bradford (1976)

Results and Discussion:

Effect of three stage ATP extraction on the yield of amylase:

Three stage extraction was conducted, by using PEG 6000 50%(W/W) and 40%(w/w) phosphate. The maximum yield of 72% was obtained in the second stage of the extraction and as the stage increases it was found yield decreases.

Effect of three stage ATP extraction on the activity of amylase:

As the experiments conducted and the enzyme activity was assessed it was found to be 493.3 Units/ml in the first stage

extraction. It was also observed as the yield increases the activity decreases.

As the experiments conducted, the partition coefficient was found to be highest as 2.57 in the second stage of Extraction.

Effect of three stage ATP extraction on the partition coefficient:

	Specific enzyme activity Units/mg	Purification factor	Enzyme activity Units/ml	Partition coefficient	Yield %
Crude extract	387.6	-	74.80	-	100
First stage	2976	8.50	493.3	1.964	67
Second stage	100.53	0.359	458.7	2.57	72
Third stage	81.55	0.041	41.26	2.185	69

Table 1. Amylase activity, specific enzyme activity, partition coefficient, purification factor and yield of amylase in the top phase of PEG6000/salt system.

Table 2. Amylase activity, specific enzyme activity, partition coefficient, purification factor and yield of amylase in the bottom phase of PEG6000/salt system**.**

	Specific enzyme activity Units/mg	Purification factor	Enzyme activity Units/ml	Partition coefficient	Yield %
Crude extract	387.6	-	74.80	-	100
First stage	5181.9	13.36	437.42	1.964	33.73
Second stage	240.74	0.720	429.02	2.57	28.00
Third stage	170.18	0.490	386.58	2.185	30.25

Figure 1 – Effect of Temperature on Amylase Activity

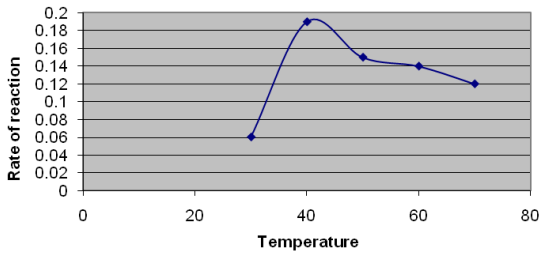


Figure 2 – lnV vs 1/T

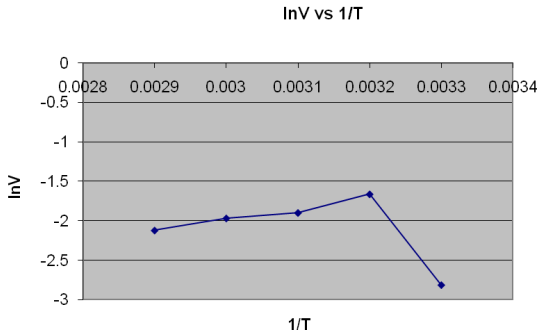
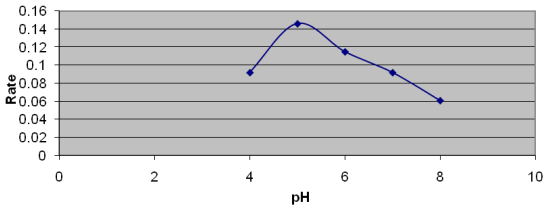


Figure 3 – Effect of pH on Amylase activity.



The DNS assay was performed at various temperatures using sucrose as the substrate and the optimum temperature was found to be 40^0C

Figure 2-The graph of lnV vs 1/T gives the activation energy (Ea) = 5100 KJ/Kmole

Figure 3-Effect of pH on amylase activity:

DNS assay was performed at various PH, and the optimum temperature was found to be pH 5.

CONCLUSIONS:

The countercurrent extraction of amylase by aqueous two phase system with simple reagents and stages of process confirmed to be effective.

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