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Research article

AQUEOUS TWO PHASE EXTRACTION OF FUNGAL AMYLASE AND ITS USE FOR DESIZING OF COTTON FABRICS

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ABSTRACT: The aqueous two phase system has a great potential for use in the downstream processing of fermentation products.The partitioning of fungal amylase enzyme in different concentration of PEG(30%-60%) and different molecular weight of PEG(4000,6000,8000) with 20%Sodium Sulphate system were investigated to optimized the amylase recovery.Maximum amylase activity (50U/ml) was found in 50%PEG 6000 and 20% Sodium sulphate system.Partially purified alpha amylase on desizing of cotton cloth was evaluated with varying incubation temperature from 40^0C to 80^0C for 1 hour. The highest desizing activity was found at 70^0C.The optimum temperature of partially purified enzyme was observed at 70^0C and maximum activity was found at pH7.Enzyme activity was increased with increase in substrate concentration up to 3%.
Key words : Aqueous two phase system, Amylase,Polyehylene glycol

INTRODUCTION

Aqueous two-phase systems (ATPS) result from the incompatibility or immiscibility of polymers, either between two polymers in water or a polymer solution with a salt solution. ATPS contain about 80–90% water and thereby can provide an excellent environment for cells, cell organelles and biologically active substances. Aqueous two-phase systems (ATPS) provide an alternative and efficient approach for purification of biomolecules by partitioning between two liquid phases [1] .This technique caught the attention of biochemical engineers due to its biocompatibility [2], easy scale-up[3],decrease in process time resulting in considerable saving in energy input and manpower [4],better yields and almost no environmental hazards .Polymer/salt systems are usually preferred for large scale operations due to their relatively low cost and their shorter separation time [5,6],Several salts such as potassium phosphate, sodium citrate, NaCl, KCl, NH4Cl, etc. can form an ATPS with polymers, especially PEG. These systems result in higher selectivity in partitioning, with high yields in the first extraction step.Alpha amylase is used as desizing agent for removing starch from the cotton cloth before its further processing in bleaching and dyeing [7],α-Amylase have been used for the preparation of starch syrup and dextrose, preparation of alcohol and beer. [8].Most commonly used fungi for enzyme production are Trichoderma, Aspergillums, Penicillium, Fusarium, Myrothecium and Chaetomium[9].Among these Aspergillus and Tricoderma[10,11] produces enzymes that account for approximately 20% of world enzyme market [12]. Exo-acting enzymes involved in the degradation of plant cell walls.. Strains of particularly the black aspergilli (Aspergillus niger group including well-known industrial strains of A. niger, Aspergillus aculeatus, and Aspergillus awamori) [13]

The aim of this study was to investigate the partitioning of amylase from cell free culture broth of A.niger in different PEG/Sodium sulphate aqueous two phase system and the desizing efficiency of amylase at different temperature was investigated.kinetic studies of partially purified enzyme were also carried out.

MATERIAL AND METHODS

Isolation of amylase producing strain from soil

Isolation of the Aspergillus strains was carried out with various soil samples collected from Amity campus Noida. The soil suspension was diluted up to 10^{-3}–10^{-6} times and 0.5 ml of each diluted suspension was then transferred by spread plate method with a sterile glass spreader on petri plates containing potato dextrose starch agar medium and petri plates were subsequently flooded with iodine solution.

Incubation was carried out at 30°C for 48 h. Amylase production was detected by the disappearance of the blue colour of the medium around microbial colonies.Large clear zones forming colonies were picked up and purified by streaking on PDA. These petri plates were incubated at 30°C for 2-3 days. Identification was based on cell and colonial morphological characteristics with references to the method of Rasper and Fennel [14].

Production and extraction of amylase

The strain showing larger clear zone was transferred in amylase production media which comprised of (g/L) **:** Peptone 5g, NaCl 5g, Beef extract 1.5g, Yeast extract 1.5g, Starch 10g .The pH of the media was adjusted to 5.5 and the flasks was kept for incubation at 30°C for 3 days. The suspension was filtered through Whattman filter paper and filtrate was used as enzyme source.

Amylase assay

The activity of the alpha amylase was determined by the Bernfeld [15],procedure using soluble starch (Sigma chemical, USA) as a substrate. The reaction mixture containing 1 mL of 1% substrate (w/v) in citrate phosphate buffer (pH 6.6), 1 mL of crude enzyme, and 5 mL of the buffer was incubated for 30 mins at 40°C. The reaction was stopped by adding 2 mL of a solution of 3, 5-dinitro salicylic acid (DNS), followed by cooling to room temperature. The concentration of the reducing sugar was measured at 540 nm in an UV-Vis spectrophotometer using glucose as standard. One unit (U) of alpha amylase is defined as the amount of enzyme that releases 1 micromole of reducing sugar as glucose per minute under the assay condition and is expressed as U/mL of substrate in submerged fermentation and other studies[16].

Partitioning of amylase in different concentration of PEG SALT system

It has been studied that,the partitioning of enzyme depends on the size of the biomolecule, superficial properties, molecular load, ionic composition of the phases, polymer concentration and molecular length of the polymer[17].The atps was prepared from stock solution of PEG 6000(30%,40%,50%,60% w/volume) and 20 %(w/volume) sodium sulphate solution to achieve various concentrations with final volume of 20 ml. 5 ml of supernatant from Aniger culture was added to the ATPS.Low speed centrifugation (1000rpm) for 10 min at room temperature were used to speed up phase separation.After a gentle mixing of system components and incubation of 2 hours at 30^0C samples from top and bottom phase were then assayed for amylase activity.

Partitioning of amylase in different moleculare weight of PEG SALT system

The aqueous two phase system was prepared from stock solution of 50%PEG 6000, 50%PEG8000 and 50%PEG 4000 with 20%(w/volume) sodium sulphate solution to achieve ATPS with final volume of 20 ml. 5 ml of supernatant from Aniger culture was added to the ATPS.Low speed centrifugation at (1000rpm) for 10 min at room temperature were used to speed up phase separation.After a gentle mixing of system components and incubation of 2 hours at 30^0C .samples from top and bottom phase were then assayed for amylase activity.

Determination of optimum temperature

To study the temperature optima , the partially purified enzyme reaction mixture was incubated at different temperatures ranging from 4°C to 100°C in citrate phosphate buffer (pH6.6), and amylase activity was measured using starch (1%) solution as substrate by DNS method.

Determination of optimum PH

The pH optimum for partially purified amylase was assayed by analyzing its activity in the citrate phosphate buffer of varying pH(3,4,5,6,7) and amylase activity was measured using starch (1%) solution as substrate by DNS method.

Effect of substrate concentration

1 ml of enzyme was incubated with different concentrations of starch(1.5% ,2.0% ,2.5 .3.0% and 3.5%) in citrate phosphate buffer (pH6.6) at 40ºC for 30 minutes. Amylase activity was determined by DNS method.

Effect of temperature on desizing of cotton cloths

Five cotton cloths (5*5 inch) were taken and weighed. then cloths were kept in flask containing 1% starch solution with regular mixing for 2-3 hours so that cloths get saturated with starch .It was then dried in an oven. Water is poured over the cotton cloths to remove unbound starch.Cloths were then dried again and were weighed The starch loaded cloths were put in the flask containing amylase (20ml enzyme in 200 ml water)solution and kept in the shaker incubator at various temperature (40°C,50°C,60°C,70°C.80°C) for 2 hours after that cloths were oven dried and weighed again the desizing of cotton cloths by crude and partially purified enzyme was studied. The percentage of starch removal was calculated by appling the following formula

% of desizing = $\frac{W2-W3}{W2-W1}$*100 **,**Where,

W1 = Weight of the cotton cloth before treatment of starch
W2 = Weight of the cotton cloth after the treatment of starch
W3 = Weight after treatment of amylase
W2-W1 = Weight of starch
W2 – W3 = Weight of starch removed

RESULTS

Partitioning of amylase in different concentration of PEG-Salt system

The systems studied were slightly influenced by the PEG concentration .Partitioning of amylase found maximum in 50%PEG 6000 -20% Sodium sulphate system(40U/ml) while least in 30%PEG –Sodium sulphate system.(Table 1).

Partitioning of amylase in different molecular weight PEG-Salt system

Partitioning of amylase is also effected by increasing the molecular weight of PEG.Amylase showed better partitioning in PEG 6000 as compare to PEG 4000 and PEG8000 in PEG –Sodium sulphate ATPS system.(Table 2) Maximum paritioning was found in 50% of PEG 6000 as we increased the molecular weight of PEG from 6000 to 8000 the partitioning of amylase was decreased.

Determination of optimum temperature

Optimum temperature for partially purified amylase was found at 70^0C as shown in figure 1.

Determination of optimum PH

Fungal amylase showed maximum activity when citrate phosphate buffer of pH 7 was used as shown in figure 2.

Table 1:Effect of PEG Conentration on Amylase Partitioning

PEG 6000	Amylase Activity(U/ML) IN Upper Phase
30%	18
40%	24
50%	50
60%	31

Table 2:Effect of PEG Molecular Weight on Amylase Partitioning

PEG Types (50%)	Amylase Activity(U/ML) IN Upper Phase
PEG 4000	30
PEG 6000	50
PEG 8000	25

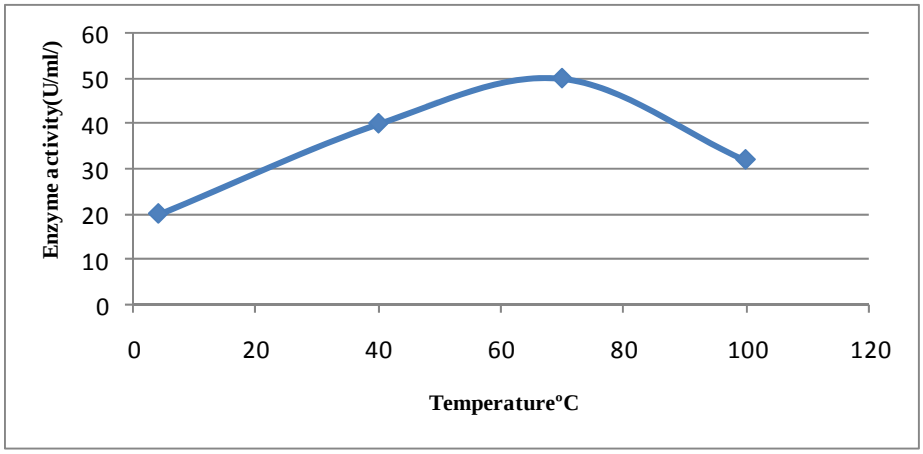

Figure 1: Effect of Temperature on Amylase Activity

Effect of substrate concentration

It is clear from the figure 3, fungal amylase activity was found optimum when starch concentration was used between 2%-3.0% .

Effect of temperature on desizing of cotton cloths

The residual activity of the partially purified alpha amylase enzyme was measured by incubating the enzyme at different temperatures (40°C,50°C,60°C,70°C.80°C). The results showed that the activity of the enzyme increased with increase in temperature. From the Table 3 it is clear that at 70ºC the percentage of desizing is very high Hence,This temperature is optimum for the desizing activity of the enzyme.

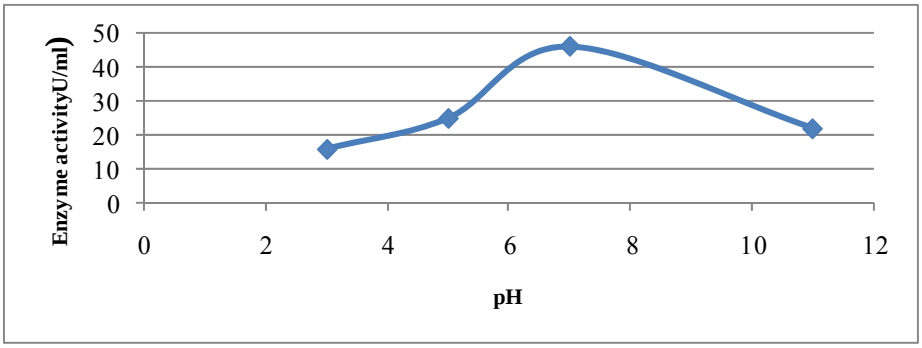

Figure 2:Effect of pH on Amylase Activity

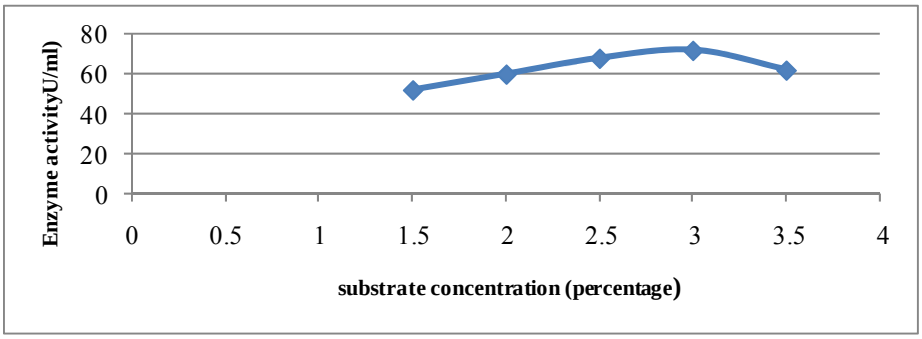

Figure 3 : Effect of Substrate Concentration on Amylase Activity

Table 3: Effect of Temperature on Desizing Activity of Amylase

	Temperature (ºC)	W1	W2	W3	W2-W3	W2- W1	Desizing %
Cloth 1	40ºC	0.543	0.598	0.560	0.013	0.055	23.36
Cloth 2	50ºC	0.569	0.643	0.619	0.014	0.074	41.89
Cloth 3	60ºC	0.566	0.601	0.584	0.017	0.035	48.50
Cloth 4	70ºC	0.553	0.608	0.573	0.035	0.055	63.63
Cloth 5	80ºC	0.561	0.610	0.581	0.029	0.049	55.10

DISCUSSION

Partitioning of fungal amylase were studied by varying different factors like PEG molecular weight and its concentration in atps system. The optimum temperature, pH ,effect of substrate concentration and desizing temperatute were studied for fungal amylase. Partitioning of amylase found maximum (50U/ml) in 50%PEG 6000 -20% Sodium sulphate system.The reason being that the interfacial tension is dependent on the polymer and salt composition. When the polymer concentration is increased, the composition of the phase system is removed from the critical point and the interfacial tension is increased [18]. As a result, the biomolecules will favour more to the top or bottom phase. It has been found that increasing the concentration of PEG 1500 from 12 to 18% (w/w) resulted in increase partitioning of polyphenol oxidase to the bottom phase. [19].

Maximum partitioning was found in 50% of PEG 6000 as we increased the molecular weight of PEG from 6000 to 8000 the partitioning of amylase was decreased. This effect was studied earlier as high molecular weight polymer acquire a more compact conformation with intramolecular hydrophobic bonds and hindered the partition of biomolecule into the top phase [20].On increasing the molecular weight of PEG ,the chain length of polymer increases which in turn causes the increase of the excluded volume, which mean less space available for the protein in the PEG rich phase. Similar behavior was observed when atps were used for the purification of penicillin acylase from Escherichia coli and cutinase from E. coli WK-6 recombinant, respectively, using poly (ethylene glycol)-sodium citrate and poly (ethylene glycol)- hydroxypropyl starch[21,22].

Partitioning of α-galactosidase from Aspergillus oryzae was found maximum in bottom phase when molecular weight of PEG was increased . [23]. The optimum temperature for amylases from fungal and yeast sources has generally been found to be between 30°C and 70°C. Furthermore, the optimum pH values for amylases from most bacteria and fungi have been reported in the acidic to neutral range [24,25].In general the activity of enzyme increased on increasing substrate concentration. It has been studied that the optimal concentration of starch for maximum α-amylase activity was between 2 and 3% [26].The desizing activity of fungal amylase was found maximum at 70^0C. It has been studied that the alpha amylases remove selectively the size and do not attack the fibres [27,28]. Industrial processes involving amylase for textile desizing are carried out at 95^oC for 90 min [29,30].

CONCLUSION

Hence partially purified fungal amylase by Aqueous two phase system could be potentially useful for desizing at lower temperatures (70^oC) leading to energy savings as well as being environment-friendly. The results are significant as they indicate that the enzyme will be active even under the presence of high temperatures and pH, thereby favoring its use under harsh conditions of textile desizing processing.

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