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Effect of pH, temperature and kinetics of pectinase enzyme using *Aspergillus niger* by solid-state of fermentation

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ABSTRACT

The present work emphasized the effect of pH, temperature and kinetics of pectinase enzyme which is used in food and braveries. It was carried with solid-state fermentation using soil as substrate using Aspergillus niger. The organism was subjected to different conditions such as pH, temperature and kinetics of enzyme activity. It was observed that highest enzyme activity was 340.56 µg /ml/sec at pH 4 at 96 hours of incubation. The maximum pectinase activity of 169.31 µg /ml/sec was found at 40^{0}C temperature at 96 hours incubation. Km and Vmax was calculated and found to be Vmax 0.020 µm/min/mg and Km was 2.5 µm. It showed that Aspergillus niger from the soil has the ability to produce pectinase. Hence, it may consider as a source of Industrial pectinase.

Keywords: Pectinase, solid-state fermentation, enzyme activity, *Aspergillus niger*.

INTRODUCTION

Pectinase is also well-known term for commercial enzyme preparation that breaks down pectin found in cell wall of plant [1]. This enzyme splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages. The major sources of the enzyme pectinase are plants and microorganisms. But on the basis of technical and economic point of view microbial source of pectinase has important. Microbial strains such as bacteria [2], yeast [3] and molds [4] are capable of producing pectinase enzyme. The composition of pectinase enzyme varies among species of microorganisms [5]

Pectinolytic enzymes catalyzing the degradation of pectic substances are of great industrial importance. Pectinase have extensive applications in fruit juice industries for to improve fruit juice clarity and yield [6]. Another field of application include the oil extraction [7], coffee and tea fermentation [8], animal feed [9] purification of plant viruses [10], pulp and paper industry [11], retting of flax and other vegetable fibers [12], degumming of plant fibres [13], protoplast fusion technology and waste management [14], textile industry and haze removal from wine [15], bio-scouring of cotton fibres [16], etc.

Many studies have been noticed that the enzyme preparations used in the food industry are of fungal origin [17]. Mostly useful enzymes are produced using industrial fermentation from *Aspergillus niger*[18]. Today pectinase is one of the most important enzymes in food processing industry mainly for extraction and clarification of fruit juice and wine. Solid-state fermentation is used for pectinase production because of the potential advantages such as simplicity, high productivity and concentrated product over submerged fermentation [19, 20].

In the study heat tolerance filamentous fungus *Aspergillus niger* was used for the optimization of pectinase production parameters in solid-state fermentation and also to clarify the specific fungal strain with the best pectinase enzyme production activity. The optimization was carried out by experimental designing and surface analyzing methodology.

MATERIALS AND METHODS

Collection of strain: *Aspergellus niger* was obtained from microbial culture collection and maintained on Czapek's Agar Medium. After 3 days incubation at 30^0C the agar slants were stored at 4^0C.

Chemicals: All the chemicals and solvents used were Merck Chemicals and of analytical grade.

Soil samples: The following soil samples were used in this study.
1. Trial I: Orange peel + orange pule + soil
2. Trail II: Mosambi peel + mosambi pulp +soil

Every day water was sprinkled. After two months, the soil degraded with fruit waste, which was used as an inoculum. This can be used for screening the pectinolytic fungal isolates.

Extraction of enzyme: The crude pectinase was extracted by mixing 10 gm of fermented materials with distilled water, stirred for 20 min in the shaker, filtered and then centrifuged for 20 min. The supernatant was used as the crude enzyme and then studied for enzymatic measurement by DNS method [21].

Solid state fermentation: Solid state fermentation (SSF) was carried out in 250 ml Erlenmeyer Flasks that contained 5 gm of orange peel and 5 ml of distilled water as moistening agent. The flasks were sterilized at 121^0C for 15 min and cooled to room temperature. A 1 ml of *A. niger* suspension (1 x 10^7 spores/ml) was added, mixed well incubated at 30^0C for 96 hrs. At the end of incubation period the flasks were taken out and the contents of each flask were extracts with 25 ml of sterile distilled water [22].

Enzyme production: *Aspergillus niger* was placed in a basal medium used for pectinase production, the medium consists of $NaNO_3$ 2%; K_2HPO_4 1%; $MgSO_4$ 5%; KCL 5%; $FeSO_4$ 0.001% and pectin 15%. The culture was sampled at different time during growth to determine cell density by measurement of absorbance at 575 nm.

Effect of pH on production of cellulase: The optimum pH was determined by incubating the 1 ml approximately diluted enzyme mixed with 2 ml 3% CMC in 1 ml citric buffer, pH 4.8 (Bertrand et al, 2004) buffer of different pH (4-8) for 2 hours at room temperature (40^0C). Reducing sugars thus released were estimated by the dinitrosalicyclic acid reagent method [21].

RESULTS AND DISCUSSION

The fungal strain produced clear zones around the colonies in opaque white back ground and pectin production was detected. On the basis of colony morphology and microscopic observation, the stain used in the study was confirmed as *Aspergilus niger*. The strain *A. niger* was isolated from different soil samples collected from Shrirampur area. The active procedure of pectinase has been isolated and is characterized by extremophile properties. It is agree that the optimum medium for the enhanced production of extremophile pectinase as an inducer.

Effect of pH on pectinase production: The effect of pH on pectinase was studied by conducting experiment at different pH level as 3, 4, 5, 6 keeping temperature at 40^0C. It was revealed that pH was increased from 3 to 4, the pectinase activity was also increased. pH beyond 5 reduced the pectinase activity. It showed that the performance of *A. niger* to lower pH for its growth and metabolism. It was revealed that highest enzymatic activity was 340.56 µg /ml/sec at pH 4 at 96 hours of incubation. The low or high pH value inactivates the enzyme and may affect it production. The effect of pH on bio-enzyme synthesis has been reported by many workers. The pH 4.0 to 5.5 was reported for *A. terrreus* and *A. niger* [23], pH 4.0 to 5.0 was reported for *Sclerotiumrolfsii* [24] and pH 5.0 to 6.0 reported *for Rhizopusorzae* [25].

Table 1.Effect of pH on pectinase enzyme activity

Sr No.	Time (Hour)	Enzyme activity (µg/ml/sec) 10^{-4}			
		pH 4	pH 5	pH 6	pH 7
1	24	13.21	71.23	62.21	59.31
2	48	41.31	113.71	101.12	98.24
3	72	80.56	285.24	209.57	170.95
4	96	105.86	340.56	234.51	201.58
5	120	63.39	21.58	138.17	171.53

Table 2.Effect of temperature on pectinase enzyme activity

Sr. N0.	Temperature (^{0}C)	Velocity (µm/min/mg) 10^{-4}
1	30	2.2
2	35	3.3
3	40	8.5
4	45	9.5
5	50	2.0
6	55	1.0

Table 3.Production of total pectinase

Sr. No.	Time (hour)	Enzyme activity (µg/ml/sec)		
		35^{0}C	40^{0}C	45^{0}C
1	24	13.27	22.25	20.11
2	48	21.34	50.12	48.54
3	72	58.29	89.17	88.91
4	96	101.33	169.31	121.32
5	120	92.22	141.32	109.13
6	144	81.11	128.21	98.17

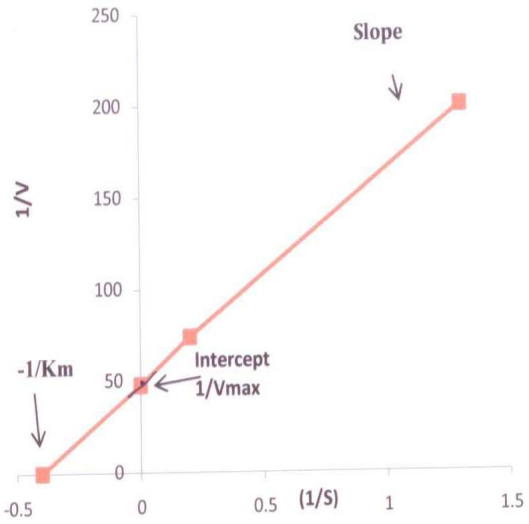

Fig: 1: Showing Vmax and Km Values

Effect of temperature on pectinase production: In the present study the effect of temperature on pectinase production was studies by using soil substrate. The experiment was conducted at different temperature level as 35^{0}C, 40^{0}C and 45^{0}C temperature maintaining all other constant conditions for the fermentation period from 24, 48, 72, 96, 120 and 144 hours. The temperature increases from 30 to 40 the pectinase activity found to be increased. The maximum pectinase activity of 169.31 µg /ml/sec was found at 40^{0}C at 96 hours incubation. It is conformity to those of Baladhndayutham and Thangavelu [26].Further increase in temperature declined the enzyme activity. Hence, the 40^{0}C temperature was studied for further study. The decline in enzyme activity at higher temperature may be due to enzyme denaturation.

The kinetics of pectinase enzyme production using *A. niger* under optimum conditions of temperature and pH was studied. The kinetics parameters were evaluated. It was noticed in the study that Km and Vmax was calculated, a plot of 1/[V] v/s 1/[S] was shown a straight line indicating double reciprocal line since reciprocal of [V] and [S] was used for plotting. The linear graph can be extrapolated at saturated substrate concentration. On the basis of graph Km and Vmax was calculated and found to be Vmax as 0.020 µg/min/mg and Km as 2.5 µm (Fig. 1).

For the production of pectinase enzyme *A. niger* exhibited its highest enzymatic activity on the 4th day of incubation and biomass production after cultivating in mineral salt medium for 7 days. Although it is different from findings by Welling [27] who had reported maximum enzymatic activity after 9 hours for *Bacillus* sp. *Asparagus niger* had its highest enzymatic activity in 6 days, after cultivation in mineral salt medium for 7 days.

The optimum temperature for pectinase and cellulase produced by *A. niger* was 50^{0}C and 60^{0}C with pentose and cellulose activity respectively. Temperature beyond 50^{0}C led to decrease in pectin cellulose yield. This is in contrast to the finding of Devi [28,29] who had reported optimum temperature of 45^{0}C for Aspergillus sp. for pectinase production. Oyeleke [5] focused that increase in temperature led to increase in enzyme activity but there was limit to the increase in activity because higher temperature led to a sharp decrease in activity. This could be due to the denaturing of protein structure.

For the cellulose production *A. niger* was cultivated in mineral salt medium for 7 days at room temperature and it was showed its highest enzymatic activity after 4 days with an activity of 1.9 x 10^{-4} µg/ml/sec. This is similar to that of findings of Umbrin [30] who reported maximum cellulase productivity after 4 days in the solid state fermentation of *A. niger* by *Vignamungo.*

The selected isolates in the study have to be thoroughly characterized before these would be utilized for fruit juice clarification, haze removal, etc. The ability of enzyme such as antibacterial, antifungal, anthelmintic, anti-inflammatory, antidiabetics, anticancer, etc. are proposed in the future plan of work. For the use of enzyme at large scale one can plan to study the hybrid produced by the *Aspergillus niger* processing different properties. It is also indeed that immobilizations enhances the activity of enzymes and protect them from shear and stress, so in future plan it can be immobilize and activity can be checked.

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