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PRODUCTION OF PECTINASE ENZYME BY *ASPERGILLUS NIGER* USING FICUS RELIGIOSA LEAVES IN SOILD STATE FERMANTATION

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Abstract:

The aim of present study was to produce pectinase enzyme using *Ficus religiosa* leaves as a substrate and the microorganism is *Aspergillus niger* - NCIM 548 in a solid state fermentation process. In the process the microorganism is cultivated on a solid substrate enriched with a high concentration of nutrients, micronutrients and materials and having large surface area. Process variables such as size of inoculum, pH, temperature, particle size and moisture content were optimized to achieve the maximum production of pectinases. The increased level of pectinase production was recorded at pH 5.0 and temperature 30°C in solid-state conditions. The optimum inoculum size was $1X10^5$ ml^{-1}, five hundred micrometer particle size and 70% moisture content of the substrate were optimum for the maximum production of pectinases in solid-state condition. Higher titres pectinases were observed when medium was supplemented with carbon (4% glucose) and nitrogen (ammonium sulphate, 0.3%) sources. Under optimum conditions, maximum production pectinase was 34.12 U/ml in solid state fermentation.

Key words: *Ficus religiosa* leaves, pectin, *A. niger*, solid-state fermentation, Pectinase

Introduction

Solid-state fermentation (SSF) is defined as a process that occurs on a non-soluble material that acts both as support and a source of nutrients, with a reduced among of water, under the action of fermenting agent[1]. Pectins

are high molecular weight acid polysaccharides, primarily made up of α-(1→ 4) linked D-galacturonicacid residues with a small number of rhamnose residues in the main chain and arabinose, galactose and xylose on its side chain [2-5]. Pectinases are widely used in biotechnological applications [6], namely, in food industry (i.e. fruit juice extraction, coffee and tea fermentation, oil extraction, improvement of chromaticity and stability of red wines), textile, paper and pulp industries and in waste-water treatment. Pectinase production has been reported from bacteria including actinomycetes [7-9], yeast [10,11] and fungi [12,13]. However, industrial production of pectinases makes use almost exclusively of *Aspergillus niger* strains [14]. The present study shows that *Aspergillus niger* NCIM 548 can produce an important pectinase enzyme with relatively good yield from *Ficus religiosa*, easily available raw material. A significant improvement in the enzyme yield was recorded when the basal medium was supplemented with different carbon and nitrogen sources.

Material and Methods:

Substrate:

Ficus religiosa leaves were obtained locally and were air dried.

Microorganism:

Aspergillus niger- NCIM 548 was obtained from National Collection for Industrial Microorganism (NCIM) Pune, maintained on potato dextrose agar medium and preserved at 4°C. The spores were harvested from 96-hour-old cultures, grown at 30°C in 0.01 % Tween 80 solution.

Fermentation:

Soild state fermentation was carried out in 250-mL flat bottom shallow glass container by taking 100 mL of medium containing (in g/L): (NH4)2SO4 0.1, MgSO4·7H2O 0.5, KH2PO4 0.5, and FeSO4· ·7H2O 0.0005. The initial moisture content of the substrate was adjusted to 60 %. The pH and inoculum size of the medium were adjusted to 5.0 and 1·107 spores/g, respectively. The flasks were incubated at 30°C.

Enzyme assay: Polygalacturonase activity was determined by measuring the release of reducing groups from Citrus pectins using a 3.5 dinitro salicylic acid (DNS) reagent assay. The reaction mixture containing 0.8ml of 1%

pectin, 67% methoxylated Braspectina in 0.2M acetate buffer, and 0.2ml of crude enzyme solution, was incubated at 50^0C for 10min. After 10min 1.0ml of DNS reagent was added and the test tubes were shaken to mix the contents, and to that solution 8ml of distilled water was added to avoid the turbidity. The absorbance was measured at 540 nm using spectrophotometer. The enzyme and substrate blanks were run parallel. One enzyme unit of endopolygalacturonase (EC 3.2.1.15) is the number of micromoles of reducing sugars measured in terms of monogalactauronic acid, produced as a result of the action of 1.0ml of enzyme extract in one minute at 30^0C temperature.

Results and Discussion:

The experiments were conducted in triplicates and the results presented are the mean values. The maximum level of pectinase production was achieved at 72 h (fig 1). In general, any fermentation system is regulated by mainly physicochemical and nutritional factors. The nutritional parameters could be effectively monitored in the process for the maximum production of end product keeping physicochemical parameters as constant. Fig. 2 shows the effects of temperature on pectinase production; at 30^0 C the enzyme activity is maximum. Fig. 3 shows the effects of particle size on enzyme production. The maximum enzyme production was observed at mesh size 44 (36/44 that passed through no: 36 sieve but was retained by no: 44 sieve). The enzyme produced with smaller particle size due to close packing densities of substrate and reduction of void space between particles. This tend to decrease the area for heat transfer and gas exchange with the surrounding atmosphere, as a result of which oxygen diffusion will be decreasing by generation anaerobic conditions. Fig. 4 shows the effects of pH on pectinase production the solid state fermentation medium for pectinase production was prepared by taking six 250ml conical flasks adjusted with different pH ranges 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5, incubated at temperature of 30^0c and particle size 44. After 72hrs the samples were assayed for pectinase activity. The maximum production of pectinase at pH 5.0 was recorded. Fig.5 shows the effects of inoculum level on pectinase activity. Different inoculum levels 12%, 16%, 20%, 24%, 28%, 32% v/w were studied for maximum pectinase activity. The results indicate that maximum

enzyme production was observed at 20% v/w of inoculum. Further increase in inoculum level decreased the enzyme activity.

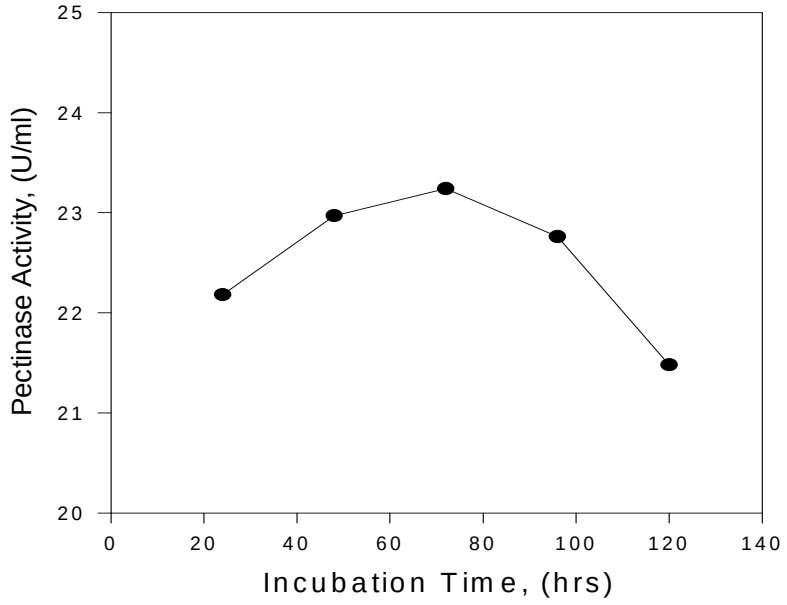

Fig 1. Effect of incubation time on pectinase activity

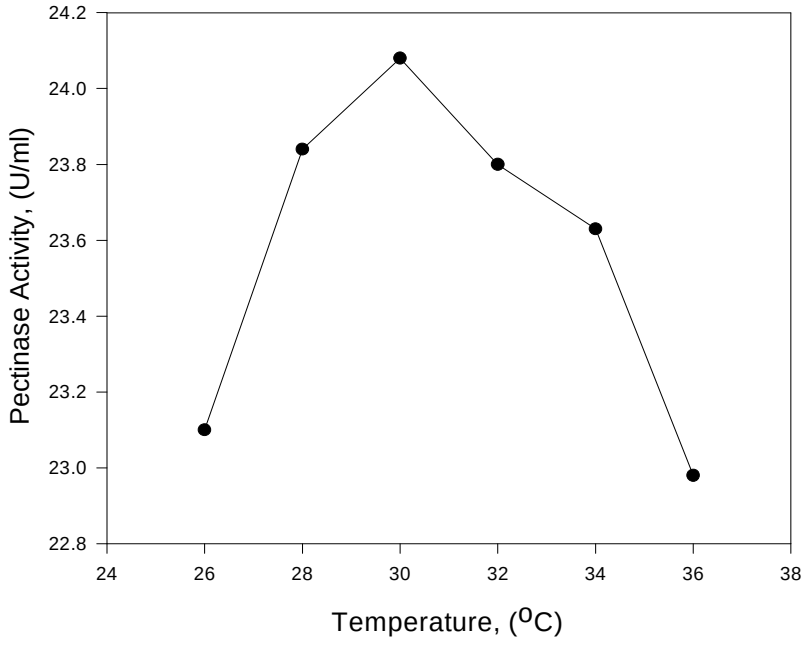

Fig2. Effect of incubation temperature on pectinase production.

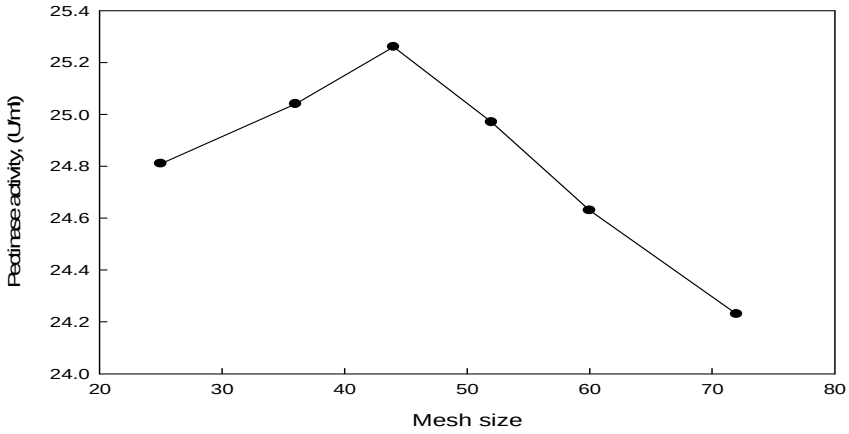

Fig 3. Effect of particle size on enzyme production.

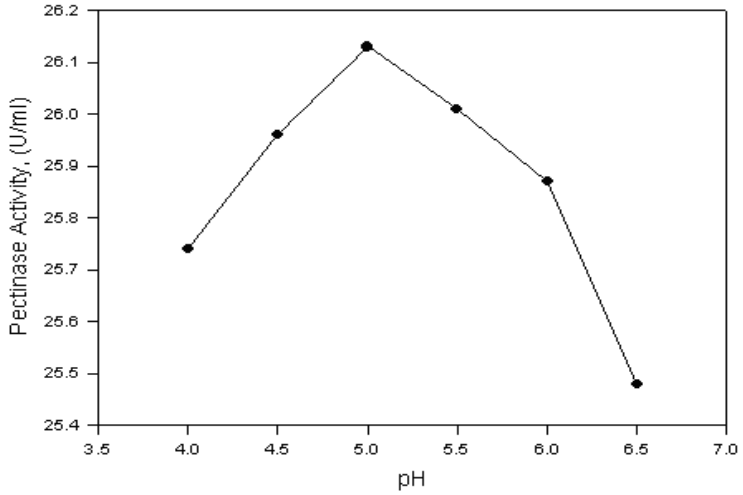

Fig 4. Effect of pH on pectinase activity

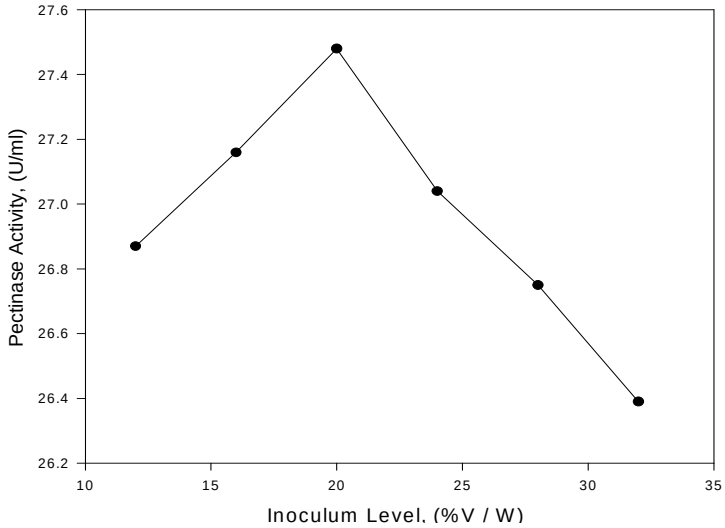

Fig 5. Effect of inoculum level on pectinase activity

Fig. 6 shows the effects of moisture content on pectinase production; Moisture content is an important parameter for the production of enzymes in solid state fermentation. High moistures results in decreased substrate porosity, which in turn prevents oxygen penetration. This may help bacterial contamination. On the other hand, low moisture content may lead to poor accessibility of nutrients resulting in poor microbial growth. Different moisture content 50%, 60%, 70%, 80%, 90%, 100%, were taken in each conical flask in the solid state fermentation and were incubated at 30^0c, pH 5.0. The maximum activity was observed at 70% of the moisture content. Table show the effect of carbon source on pectinase production, from all the substrates glucose induced the higher-level production of pectinase at 2% concentration. Fig.7 shows the effects of glucose concentration on pectinase production, at 4 (% w/w) the enzyme activity is maximum. Fig.8 shows the effect of nitrogen source (ammonium sulphate) on pectinase production. To determine the effect of Ammonium sulphate on the production of pectinase, the production medium was prepared with different concentrations of Ammonium sulphate like 0.1%, 0.2%, 0.3%, 0.4% and 0.5% w/w were dispersed in 250 ml conical flasks. After 72 hrs the enzyme activity was determined. The result indicates that maximum enzyme production was observed at 0.3% w/w of ammonium sulphate concentration.

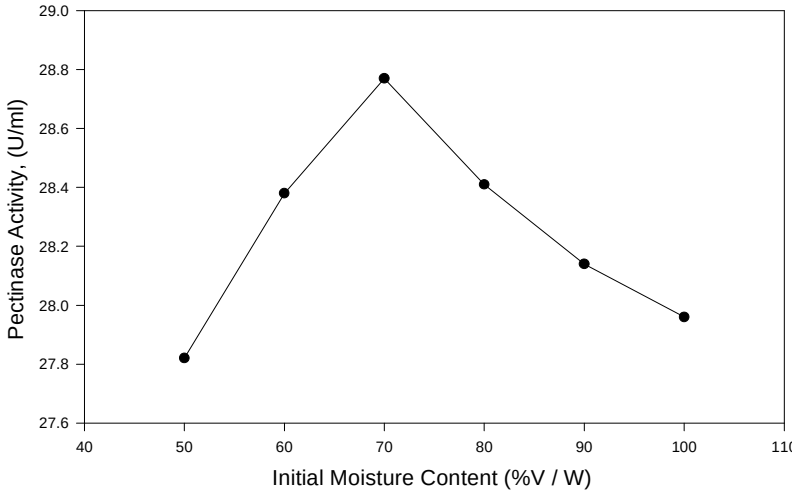

Fig. 6. Effects of moisture content on pectinase production.

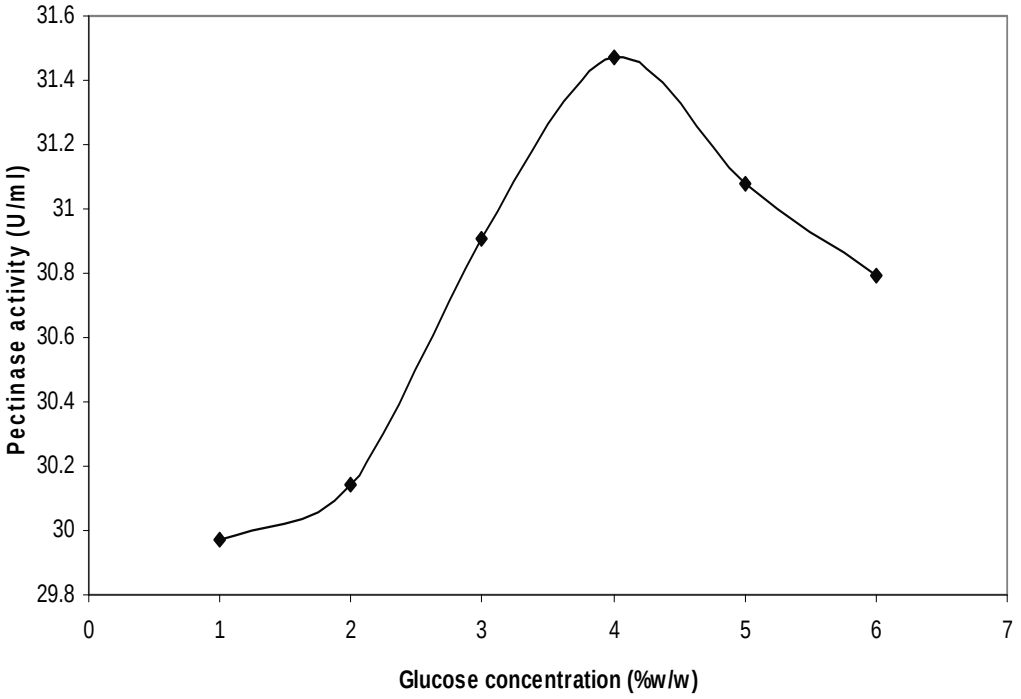

Fig 7. Effect of glucose concentration on pectinase activity

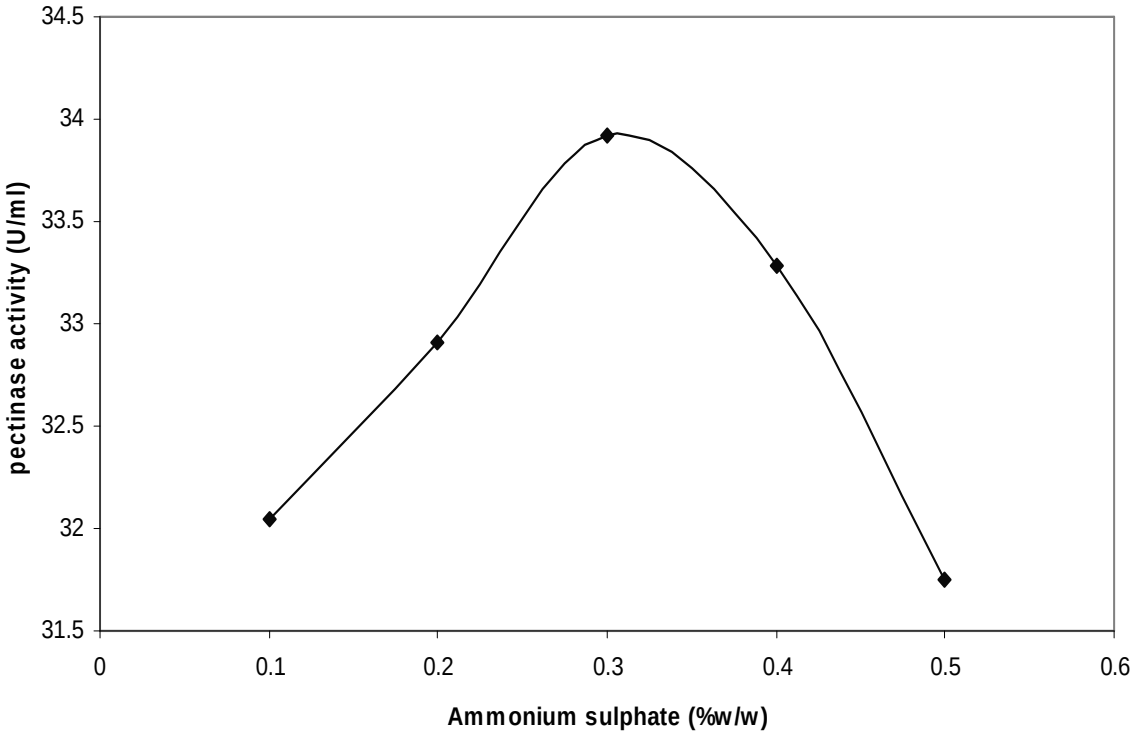

Fig 8. Effect of ammonium sulphate on pectinase activity

Table-1: Shows the Effect of Carbon source on Pectinase Production.

Carbon source (2 %w/w)	Enzyme activity (U/ml)
Glucose	29.41
Sucrose	28.74
Mannose	28.11
Lactose	27.87
Maltose	26.96

Conclusions:

The potential of pectinases solely as food enzymes is well known in the biotechnological industries because of their myriad applications. The production of pectinase enzyme from agro wastes by fungi in solid-state system could not only be cost effective but it could also offer several process merits. In the present study pectinase enzyme was produced from *Aspergillus niger* NCIM 548 using *ficus religiosa* as substrate. Various parameters like incubation time, temperature, particle size, pH, inoculum level, moisture content, carbon source, nitrogen source were studied.

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