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PROCESS OPTIMIZATION OF PECTINASE PRODUCTION BY *ASPERGILLUS AWAMORI* IN SOLID STATE FERMENTATION

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ABSTRACT

The present study deals with the process optimization and production of protease enzyme using *Artocarpus heterophyllus* as a substrate by *Aspergillus awamori* in a solid state fermentation process. Solid-state fermentation 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. 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 time, temperature, size of inoculum, pH and moisture content were optimized to get the maximum production of pectinase. The increased level of pectinase production was recorded at time 72hrs, temperature 30°C in solid-state conditions. The optimum inoculum level was 40% v/w, pH 5.0 and 70% v/w moisture content of the substrate were optimum for the maximum production of pectinase in solid-state condition. Increased yield of pectinase were observed when medium was supplemented with carbon (3% glucose) and nitrogen (ammonium sulphate, 0.4%) sources. A significant improvement in the enzyme yield was recorded when the basal medium was supplemented with different carbon and nitrogen sources.

KEYWORDS: *Aspergillus awamori, Artocarpus heterophyllus*, pectin, Solid-state fermentation.

1. INTRODUCTION

Pectinase is an enzyme that breakdown pectin, a polysaccharide found in plant cell walls. They are also known as pectic enzymes; they are pectolyase, pectozyme and polygalacturonase. Pectins are high molecular weight acid polysaccharides, primarily made up of α-(1→ 4) linked D-galacturonicacid remainder with a small number of rhamnose residues in the main chain and arabinose, galactose and xylose on its side chain.[1-4] The mechanism used to cleave a peptide bond involves making an amino acid residue that has the cysteine and threonine or a water molecule nucleophilic so that it can attack the peptide carboxyl group. Pectinase enzymes are mainly used in processes of degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples and sapota etc. Pectinases are having various biotechnological applications,[5] used in food industry such as fruit juice extraction, coffee and tea fermentation, oil extraction, improvement of chromaticity and stability of red wines, textile, paper and pulp industries and also in waste-water treatment. 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.[6] Pectinase production has been reported from bacteria including actinomycetes,[7-9] yeast,[10,11] and fungi.[12-14]

2. MATERIAL AND METHODS

2.1. Substrate: *Artocarpus heterophyllus* was collected from local market and dried naturally and powdered, packed and stored until further use.

2.2. Microorganism: *Aspergillus awamori* was used for the production of Pectinase enzyme using *Artocarpus heterophyllus* as substrate. Potato dextrose agar medium was used for the maintenance and sub culturing of the microorganism.

2.3. Preparation of Inoculum: Streaking is done from the old cultures of *Aspergillus awamori* on pure potato dextrose agar slants and incubated them at 30^0C for 3 days.

2.4. Development of Inoculum: 10ml of sterile distilled water were added to the cells from 3 days old slant, from that 1ml of suspension containing approximately 10^5-10^6 spores/ml were used as the inoculum to each flask.

2.5. Fermentation: Soild state fermantation was carried out in 250-mL conical flask by taking 100mL of medium containing (in g/L): $(NH_4)_2SO_4$ 0.1, $MgSO_4{\cdot}7H_2O$ 0.5,

KH_2PO_4 0.5 and $FeSO_4 \cdot 7H_2O$ 0.005. The initial moisture content of the substrate was adjusted to 60% v/w. The pH and inoculum level of the medium were adjusted to 5.0 and 1ml respectively. The flasks were incubated at 30°C for 72 hrs.

2.6. Enzyme assay: Polygalacturonase activity was determined by measuring the release of reducing groups from substrate 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 1ml 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 540nm 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 1ml 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 nutritional parameters could be effectively monitored in the process for the maximum production of end product keeping physicochemical parameters as constant. To determine the effect of time on enzyme production, the medium incubate at different time intervals and the maximum Pectinase activity was observed at 72hrs. After 72hrs, it was decreased due to depletion of nutrient materials. Pectinase production at different time intervals is shown in the fig.1.

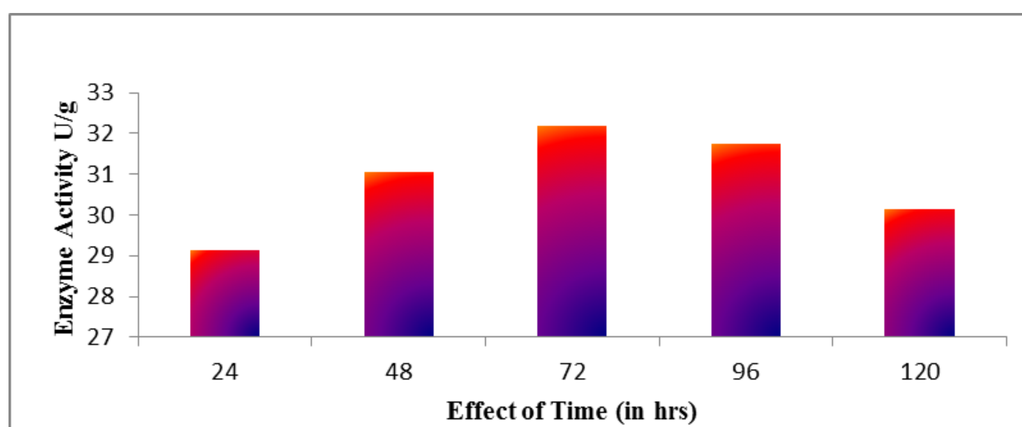

Fig. 1: Effect of time on enzyme production.

The temperature of the substrate is very critical in SSF as it ultimately affects the growth of the microorganism. The maximum amount of Pectinase was observed at 30^0c temperature Fig.2.

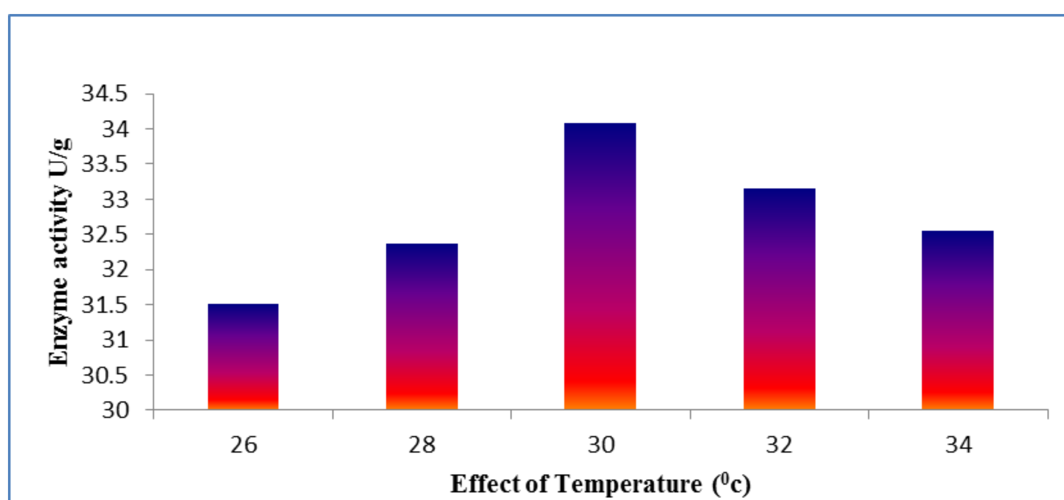

Fig. 2: Effect of temperature on enzyme production.

To determine the effect of pH, the nutrient medium was adjusted with different pH ranges 3, 4, 5, 6, and 7.0. The maximum production of Pectinase was recorded at pH 5 fig.3.

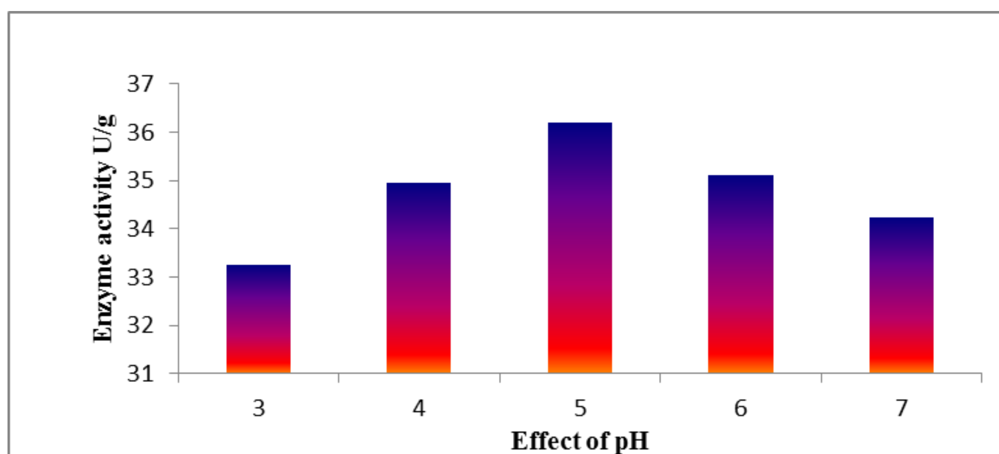

Fig. 3: Effect of pH on enzyme production.

Different inoculum levels were prepared for the production of enzyme 20%, 30%, 40%, 50%, 60% v/w. The maximum enzyme production was observed at 40% v/w of inoculum fig.4.

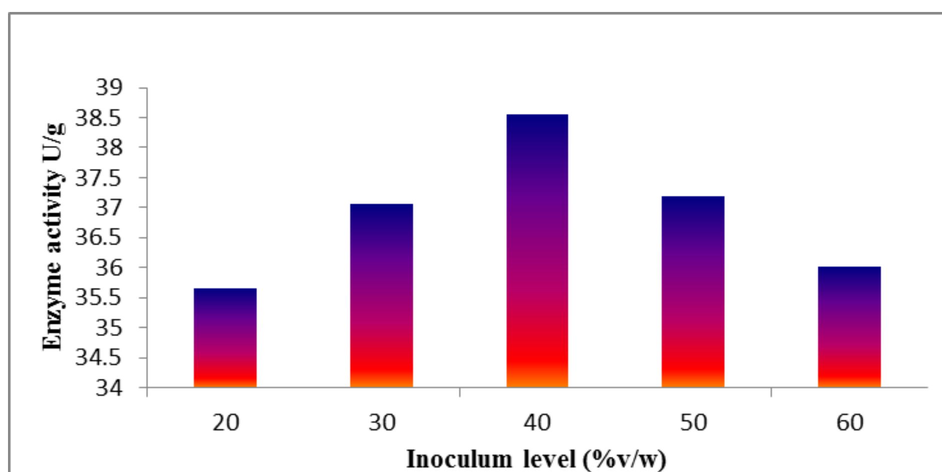

Fig. 4: Effect of inoculum level on enzyme production.

Moisture content is an important parameter for the production of enzymes in SSF. High moisture content results in decreased substrate porosity, which in turn prevents oxygen penetration. This may help bacterial contamination. Different moisture content 40%, 50%, 60%, 70%, 80%, 90%, and 100% v/w were taken in each conical flask. The maximum activity was observed at 70% v/w of the moisture content fig.5.

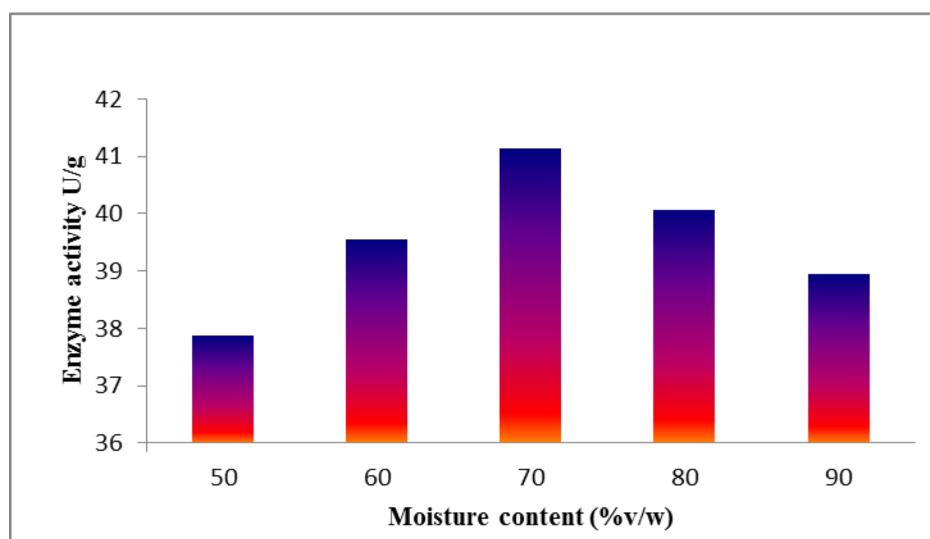

Fig. 5: Effect of Moisture content on enzyme production.

Five different carbon sources were screened for the production of Pectinase enzyme which includes sucrose, maltose, glucose, fructose, and lactose. These are enriched with % w/w. The results indicate that glucose supplementation gave marginally improved enzyme than other supplementations. Production medium was prepared with different concentrations of glucose like 1, 2, 3, 4, 5 and 6 %w/w. The result indicates that maximum enzyme production was observed at 3% w/w of glucose concentration fig.6.

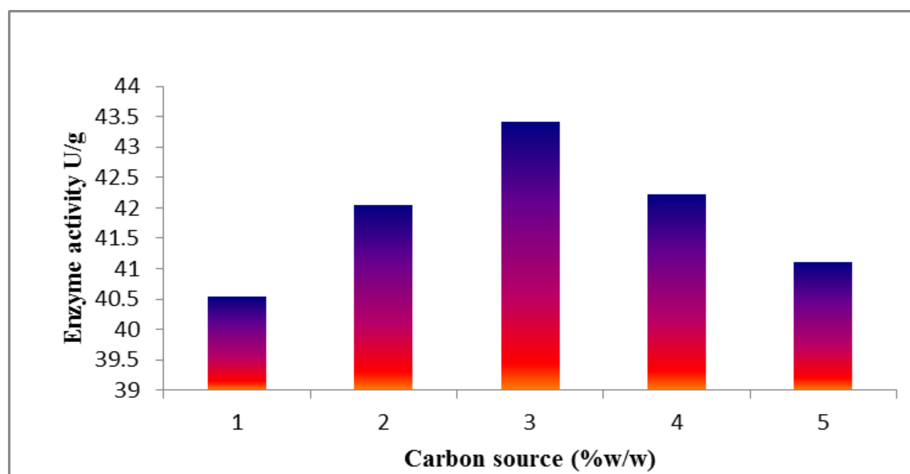

Fig. 6: Effect of carbon source on enzyme production.

To determine the effect of Pectinase on the production of enzyme, 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 250ml conical flasks. The result indicates that maximum enzyme production was observed at 0.4% w/w concentration fig.7.

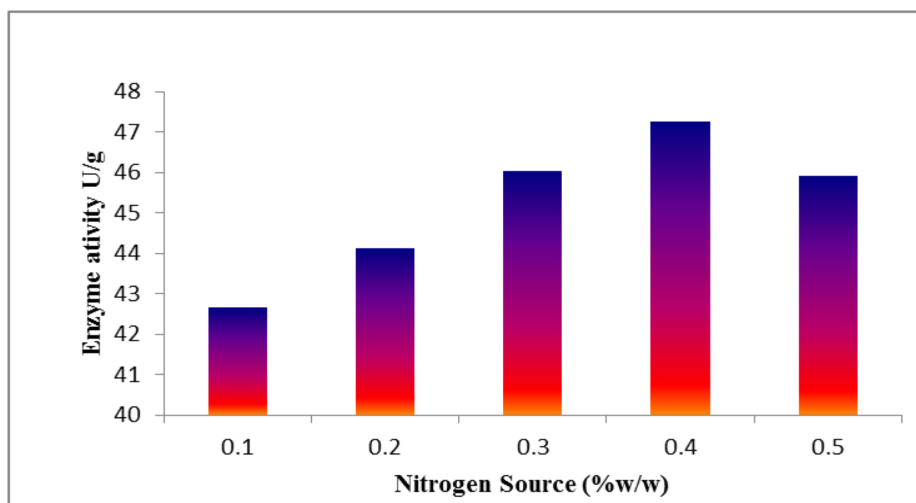

Fig. 7: Effect of nitrogen source on enzyme production.

CONCLUSION

Finally the author concluded that *Aspergillus awamori* is a promising agent for industrial application since it gave a significant Pectinase (47.25 U/g) activity in *Artocarpus heterophyllus* under solid state fermentation. As *Artocarpus heterophyllus* is low cost substrate, easily available raw material and showing suitability for solid state cultivation of microbes, the lab-scale study on protease production from *Artocarpus heterophyllus* as major substrate might give the basic information of further development for large scale protease production.

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