

# Production of pectin lyase by solid state fermentation of sugarcane bagasse using *Aspergillus niger*

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## Abstract

Pectin lyase is an important enzyme that finds application in food processing industries, particularly in increasing the juice content of the fruits by breaking the glycosidic bonds of the long carbon chains present in the pectin molecule. In this work sugarcane bagasse was used as raw material for the production of pectin lyase by *Aspergillus niger* through solid state fermentation. Sugarcane bagasse was used in various compositions to optimize the media for the maximum productivity. The physico-chemical parameters of the production media such as pH, temperature and fermentation time were also optimized. The result shows that the maximum activity of pectin lyase 229.07 U/ml was achieved from the medium containing 50% of sugarcane bagasse. The effect of pH temperature and time on the production of pectin lyase was found to be pH6, 35°C and 84 hrs respectively. For the optimized conditions the maximum activity of Pectin lyase was found to be 290.88 U/ml.

**Key words:** Pectin Lyase, *Aspergillus niger*, Sugarcane Bagasse, Pectic Enzymes, Solid State Fermentation

## 1. Introduction

Proteins are the highly complex structures with conjugations formed with metals, carbohydrates or lipids. In a similar way all enzymes are proteins, but all proteins are not enzymes. The enzymes are called biocatalyst since they increase the rate of biochemical reactions without affecting the kinetics of the biochemical reactions. Enzymes in various forms and ingredients have been used to prepare food since before the recorded history, but the production and usage of purified enzymes has gained importance in the recent past.

Pectic enzymes (pectinases or pectinolytic enzymes) are those that catalyses the hydrolysis of pectic substances. Pectic enzymes have two classes namely Pectinesterases and Pectin depolymerases.

Pectin esterase has the ability to de-esterify pectin by the removal of methoxy residues. Pectin depolymerases readily split the main chain and it was further classified as-Polygalacturonase (PG) and pectin lyases (PL). Thus on the whole pectinases are hydrolytic enzymes, which hydrolyze the pectin molecules and are readily soluble in water. Pectinases finds its application in food industries, tea industries and textile industries. Other industries like paper and pulp industries, waste management industries, animal feed manufacturing industries, and acid fiber manufacturers also make use of pectic enzymes.

The potential to synthesize pectinases is wide spread among all microbial groups, but commercially moulds are the preferred ones, more than 90% of the enzyme can be extracted in the culture medium itself. The

various species of microorganism used are exhaustively included. The main sources of the microorganisms that produce pectinolytic enzymes are Yeasts, bacteria and large varieties of fungi and particularly *Aspergillus sp.* Few yeast have the ability for pectinases production. Endo Polygalacturonase production by yeast was first reported in 1951 using *S.fragilis* (Luh *et al.*, 1951). Other genera of yeast like *candida*, *pichia*, *saccharomyces* and *zygosaccharomyces* also have the ability to produce pectinolytic enzymes. However the main problem associated with pectinolytic enzyme production in industrial process lies in the low fermentation yield through bacterial root of production (Pilar Blanco *et al.*, 1999). Filamentous fungi are those suited microorganism or in other words biological means for the pectinases production (Maurice Raimbault *et al.*, 1998). This is due to their physiological and biochemical properties. Among the fungal sources, main role is played by the mould *Aspergillus niger*, because it produces fair amount of these enzymes and in addition it is being recognized as GRAS microorganism (Pilar Blanco *et al.*, 1999).

Substrates that are employed in the production of enzyme should be solid, as only as solid substrate can give good anchorage to the growing cells. Substrates should provide all needed nutrients to the microorganism for its growth. Other factors like particle size, moisture levels are also to be taken for consideration. Generally agro industrial wastes are employed for the pectinases production. Various substrates that are being used are- sugarcane bagasse, wheat bran, rice

bran, wheat straw, rice straw, saw dust, corn cobs, coconut coir pith, banana waste, tea waste, sugar beet pulp, apple pomade, orange peel etc. the composition or constituents of the substrates are taken in to account for, before the screening of the substrates.

Two types of fermentations can be carried out for the pectinases production. They are Solid-state fermentation - SSF and Submerged state fermentation - SMF. In comparison between these two methods, SSF present a series of advantages over submerged state fermentation. Culture conditions are similar for the filamentous microorganism as in the case of SSF. The growth of the organism is very high with large quantities of enzyme being produced. An additional feature of SSF is product obtained from submerged fermentation and the quantity of liquid waste generated is lower. Another main aspect of SSF is adequate recovery of the metabolites from the fermented solids.

## Fermentation Conditions:

Various factors related to environment affect the production of pectinase. Some of them are concentration of nutrients, pH, temperature, moisture content, influence of extraction parameters on recovery of pectinases and the effects played by the inducers. Both carbon and nitrogen sources show overall effect on the productivity of pectinases (Catarina Almeida *et al.*, 2003). Pectin, glucose and sucrose when added to the media in high concentration have a repression effect on the studied enzymatic activity (Maria.F *et al.*, 2000) of the various nitrogenous matter that can be used, optimum sources are  $(\text{NH}_4)_2\text{SO}_4$ , yeast extract, Soya bean pulp powder, Soya peptone. Temperature and pH are also important parameters to be taken care. Since the system used is SSF, the conjugation of temperature and pH are highly important. The pH is regulated using a mixture of sources of nitrogen as when *Aspergillus niger* is being used pH turns to be acidic. Besides the nature of the substrate also plays a vital role in the pH maintenance. Generally the pH is maintained at 7. Temperature in SSF is maintained at 30-32°C, as it cannot be precisely controlled due to reason that SSF has solid substrates have limited heat transfer capacity. Moisture content in the substrate also plays a significant

role (Natalia Martin *et al.*, 2004). The previous studies show that it was generally maintained around 50-55 % for the production of pectinases by microbial means (Leda R. Castilho *et al.*, 2000).

## 2 Materials and Methods:

### 2.1 Microorganisms

The strain *A.niger* used in this study was isolated from citrus waste and was maintained in a medium containing pectin 0.01 g/l, Glucose 20 g/l,  $\text{N}_3\text{NO}_3$  2 g/l,  $\text{K}_2\text{HPO}_4$  1 g/l,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5 g/l, KCL 0.5 g/l,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.01 g/l, Agar 20 g/l.

### 2.2 Fermentation Medium

The production of pectin lyase was studied using different media composition M1, M2, M3 by varying amount of sugarcane bagasse and the nutrient solution as given below.

Substrate	Media Composition		
	M1	M2	M3
Sugarcane Bagasse (g)	40	50	60
Nutrient Solution (ml)	100	100	100

Dried sugarcane bagasse contains Fiber 11.8 %, Protein 6.4 %, Nitrogen 63 %, Ash 6.7 %, reducing sugar 9 % and Pectin 0.1 %. The nutrient solution consists of essential nutrients such as  $\text{NH}_4\text{NO}_3$  0.1 %,  $\text{NH}_4\text{H}_2\text{PO}_4$  0.1 % and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.1 %. The media containing M1, M2, M3 was sterilized at 121°C for 20 minutes.

### 2.3 Cultivation

Solid state fermentation was carried out using three 250 ml Erlenmeyer flask containing the media M1, M2, M3 inoculated with 10ml aliquots of *Aspergillus niger* which was obtained from a 7 day agar slant culture suspended in sterile Tween 80 solution. Using this cultivation was carried out to optimize the substrate concentration, pH and temperature.

### 2.4 Sample Extraction

The crude sample was extracted for enzymatic measurements by mixing 20g of fermented materials with 50 ml of distilled water. The mixture was then squeezed at 60 kg/cm<sup>2</sup> in a hydraulic press; the sample was then filtered through a 0.45 μm membrane

filter. The filtrate was then store as crude enzyme solution at 4°C for enzymatic measurements.

### 2.5 Analysis of Pectin Lyase:

The enzyme solution was then analyzed by continuous spectrophotometric rate determination method (Albersheim P., 1996) at 235 nm under 40°C and pH 5. When the crude enzyme solution added to pectin solution in the above said conditions the enzyme starts to break the glycosidic bonds of pectin by elimination.

### Pectin + H<sub>2</sub>O → 4 deoxy-6-methyl-4, 5-galacturonic acid oligomers

Due to this action the solution will become turbid. The increase in  $A_{235\text{nm}}$  was recorded for 5 minutes,  $A_{235\text{nm}}/\text{minute}$  using the maximum linear rate for both the test and blank was obtained.

$$\text{Units/ml enzyme} = \frac{((\text{Da}_{235\text{nm}} / \text{min Test} - \text{Da}_{235\text{nm}} / \text{min Blank}) (2.4) (\text{df}))}{(1.0) (0.4)}$$

2.4 = Total volume (in milliliters) of assay

df = dilution factor

1.0 = Change in  $A_{235\text{nm}}$  per minute at 40°C as per the unit definition

0.4 = Volume (in milliliters) of enzyme used

One unit of enzymatic activity (U) was defined as the amount of enzyme which released 1 μmol of unsaturated uronide per minute, based on the molar extinction coefficient (5500) of the unsaturated products. The enzyme production was expressed in units per ml of initial dry solid substrate (U/ml).

## 3 Results and Discussion

### 3.1 Optimization of Substrate

The production of pectin lyase is studied using different media composition M1, M2, M3 by varying amount of sugarcane bagasse as given below. The results of fermentation after 7 days at a pH of 7 at 30°C, the results were given in the figure 3.1.

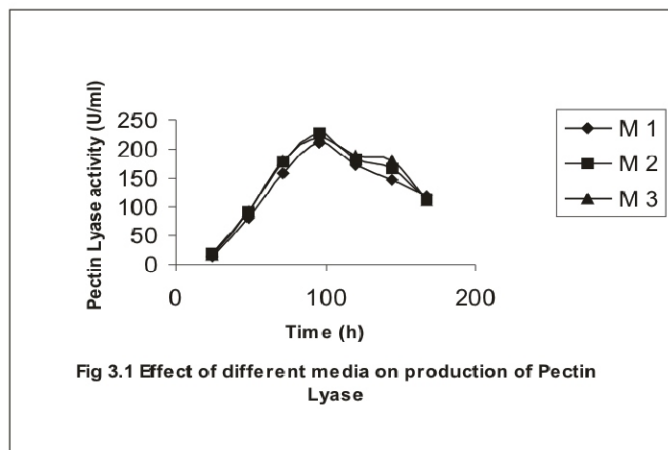


Fig 3.1 Effect of different media on production of Pectin Lyase

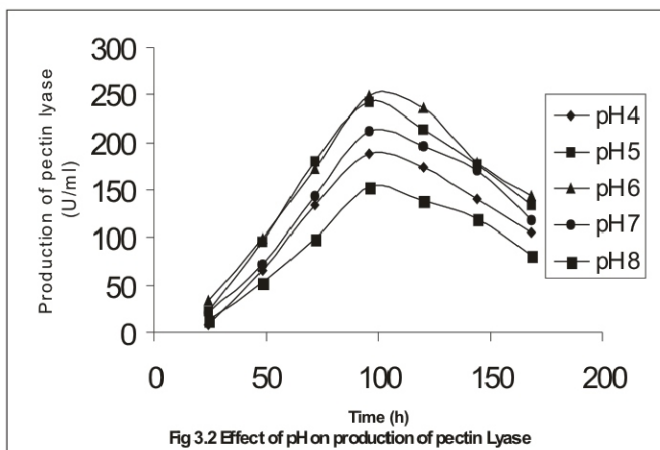


Fig 3.2 Effect of pH on production of pectin Lyase

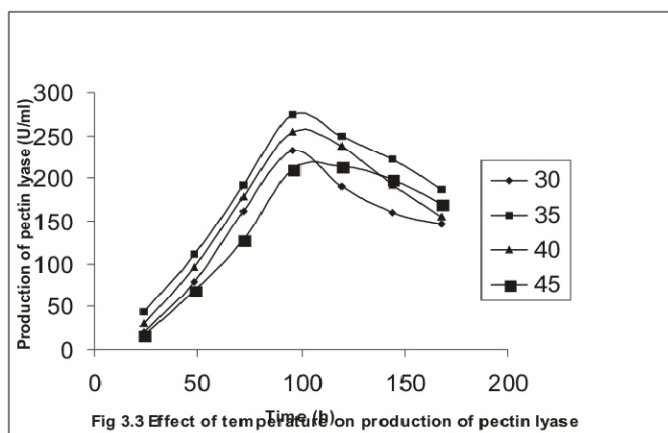


Fig 3.3 Effect of temperature on production of pectin lyase

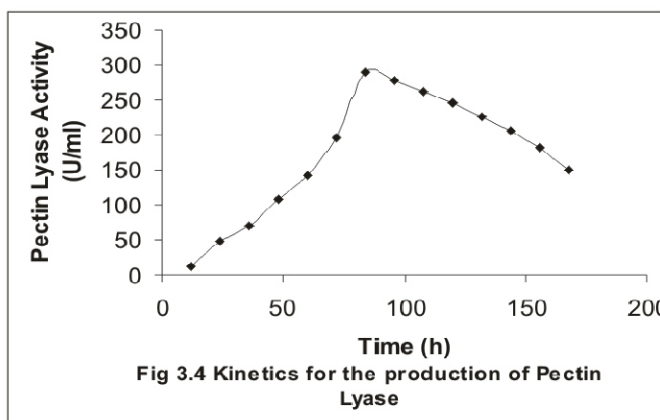


Fig 3.4 Kinetics for the production of Pectin Lyase

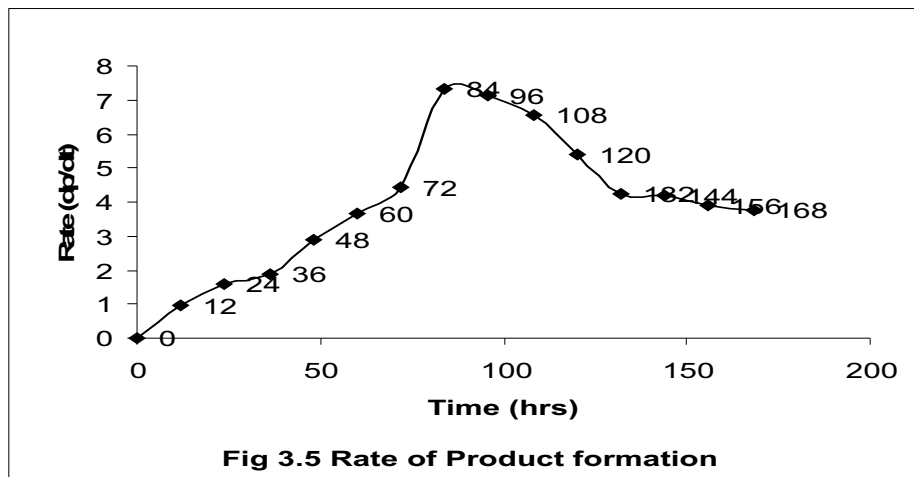


Fig 3.5 Rate of Product formation

From the graph it can be seen that the production of pectinases was maximum in the medium M2. This infers that even the substrate concentration also makes an important role in the production of enzyme. For this case it is optimum at 50g/100 ml of the nutrient medium. The further optimization of pH and temperature for pectin lyase production is carried out at the particular

substrate concentration. The results are given in the table and even represented graphically.

### 3.2 Optimization of pH

pH of the medium plays a vital role in the most of microbial process. The effect of pH and optimization of it was studied by varying the pH from 4 to 8 for the production of pectin lyase.

The pectin lyase production was analyzed for every 24 hours of fermentation. The results are shown in the fig 3.2

From the results the optimum pH was found to be 6 with the maximum activity of pectin lyase of 249.72 U/ml.

### 3.3 Optimization of temperature:

For this optimization the substrate concentration and the pH kept constant and the temperature is varied between 30°C - 45°C. The process carried out for 7 days. Samples were analyzed every 24 hours and were assayed to find the amount of pectin produced.

From the results the optimum temperature was found to be 35°C with the maximum activity of pectin lyase of 274.51 U/ml.

### 3.4 Rate of Pectin Lyase formation

With the optimized conditions the substrate, temperature and pH the SSF is carried out and results were obtained for samples collected at

an interval of 12 hours and assayed for the pectin lyase activity. The pectin lyase activity with respect to time was plotted in the fig3.4.

From the graph it is clear that at a time of 84 hours the pectin lyase production is maximum and after that period the production rate decreases which shows that, optimum HRT is 84 hours. Using *Aspergillus niger* as the culture.

The rate of production found by P vs. T plot, i.e. dp/dt plot. The value of dp/dt is obtained and is plotted against fermentation time.

Thus to conclude the substrate concentration 50g/100ml of medium with pH6, temperature 35°C and HRT is around 84 hours. Thus the maximum activity 290.88 U/ml. Pectin lyase is obtained at the above said fermentative conditions.

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