International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(4): July-August 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 01-07-2012; Revised; Accepted: 08-07-2012

CELLULASE PRODUCTION FROM '*TRICHODERMA VIRIDE*' AND '*TRICHODERMA REESEI*' USING SAW DUST AND COIR WASTE AS CARBON SOURCES

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Keywords:

cellulase enzyme, *Trichoderma viride* and *Trichoderma reesei*, biowaste

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ABSTRACT

The aim is to investigate the cellulase enzyme production ability of two fungal strains such as *Trichoderma viride* and *Trichoderma reesei* in fermentation media containing biowaste viz, coir waste and saw dust. The present work was carried out to determine the cellulase production ability of fungi *Trichoderma viride* and *Trichoderma reesei* by using Saw dust and Coir waste as a carbon source. This work determines the use of biowaste to produce industrially important product like cellulase at a reduced cost. The cultures of *Trichoderma viride* and *Trichoderma reesei* were fermented in two different carbon sources individually and the cellulase production was determined at various environmental parameters like temperature and pH by using "Filter Paper Activity method".

INTRODUCTION

Cellulolytic enzymes are a group of hydrolytic enzymes (cellulases) capable of hydrolyzing cellulose to glucose. The glucose produced can be used for human and animal food or for production of chemicals. There Cellulolytic enzymes are produced by a large number of microorganisms which include fungi and bacteria. In Fungal genera like *Trichoderma* and *Aspergillus* are taught to be cellulase producing micro-organisms and crude enzymes are produced by these microorganisms that are commercially available for agricultural use. The bioconversion of various complex cellulosic waste materials such as baggase, saw dust have been reported. Likewise coir fibres are major biowaste discarded along with coir retting effluent to estuarine environment. Yet literature related to coir fibre as a carbohydrate source and cellulolytic activity by microorganisms involved in coir retting process is not studied properly[5]. Hence, the present study was carried out to determine the cellulolytic enzyme activity of fungi like, '*Trichoderma Virid*' against coir waste and saw dust as carbohydrate source. In this work, it determined the cellulose production by measuring the glucose concentration by Filter Paper Activity method , because Cellulase degrades cellulose into glucose and in the present study , the Whatman No. 1 Filter Paper was used as substrate for enzyme activity[17].

MATERIALS AND METHODS

Methods-

Pretreatment of Lignocellulosic materials

1) The substrates were sun-dried for two days so as to reduce the moisture content and make them more susceptible for crushing.
2) The substrates were ball milled at about 60 rpm for 48 h after which the substrates were individually screen analyzed in the sieve shaker and each sample was made to pass through a 0.5 mm screen.
3) The substrates were then soaked in 1 % (w/v) Sodium Hydroxide solution for 2 h at room temperature after which it was washed free of the chemicals and autoclaved at 120^0C (15 psig steam) for 1 h. The treated substrates was then filtered and washed successively with distilled water until the wash water becomes neutral[3, 5.]

Inoculum preparation

The cultures of '*Trichoderma viride*' and *Trichoderma reesei* were maintained as stock culture in Czapek-Dox agar slants. It was grown at 30°C for 5 days and stored at 4°C for regular sub

culturing. 100 ml of inoculums were prepared for culture using Czapek-Dox broth in 250 ml flasks. The inoculums were kept in shaker (200 rpm) at 35°C for 24 h before it was used for the fermentation process.

Fermentation process

To 100 ml of the optimized culture medium, a culture medium from respective species were inoculated under controlled conditions. Then it was kept in a shaker (200 rpm) at 35°C for a day. Simultaneously, separate media were prepared for coir waste as well as saw dust substrates.

Table no. 3- Optimized culture media (g/100 ml)

Components	Amount (g/100ml)
L- glutamic acid	0.03
NH_4NO_3	0.14
KH_2PO_4	0.2
$CaCl_2$	0.03
$MgSO_4$	0.03
Protease Peptone	0.75
$FeSO_4$	0.50
$MnSO_4$	0.16
$ZnSO_4$	0.14
$CaCl_2$	0.20
Tween 80	2.0%
Carbon source	3.0

Carbon source- 1) coir waste, 2) Saw dust

Optimization of pH

The optimized media were prepared using the individual substrates and the pH was set at different level such as 5, 6, 7, 8 and 9 respectively by adding 1% NaOH and concentrated HCl. Then the media were autoclaved. Later they were inoculated with a culture medium and were placed in a shaker (150 rpm) at 30°C for 5 days. After, culture filtrates were collected and used for enzyme assays. For organism and both the substrates, assay was carried out separately.

Optimization of Temperature

The optimized media were prepared individually by using the substrates and autoclaved. Later it was inoculated with a culture medium and was set at different temperatures 20^{o}C, 30^{o}C, 40^{o}C and 50°C respectively. The effect of temperature on the production of cellulolytic enzyme was determined by growing the organisms at the above temperatures. Simultaneously for both the

organisms and both the substrates, separate assay was carried out. The enzyme solution obtained from these two (pH and temperature) experiments was individually optimised based on filter paper activity methods as described below.

Determination of cellulase activity

Cellulase activity was determined by filter paper activity (FPA) method of T. K. Ghose[17].

Filter Paper Activity Method (FPA)-

1) Whatman No. 1 filter Paper was used as a substrate.
2) 2ml of crystalline cellulose solution was taken in a test tube [filter paper with size 1.0 x 6.0 (50 mg) and dissolved in 0.2 M sodium acetate buffer (pH 5.5)].
3) To this tube, 0.5 ml of the culture filtrate was added (enzyme solution).
4) The mixture was incubated at 35°C for one hour and the reaction was terminated by adding 2 ml of Dinitro salicylic acid (DNS) reagent.
5) Then it was heated in a boiling water bath for 5 min. and then 1 ml of potassium sodium tartarate (40%) was added to the warm tubes.
6) The tubes were allowed to cool and the absorbance was read at 540 nm in a U.V. spectrophotometer.
7) The enzyme production was expressed as the mg glucose released per minute.

Filter Paper Unit Calculations

FPU= 0.37/ enzyme concentration to release 2.0 mg glucose

Derivation of the Filter Paper Unit-

The unit of FPU is based on the international Unit (IU).

1 IU = 1 umol/min of substrate converted

= 1 umol/min of glucose formed during the hydrolysis reaction,

= 0.18 mg/min when product is glucose.

The absolute amount of glucose released in the FPU assay at the critical dilution is 2.0 mg.

2mg glucose = 2/0.18 umol

This amount of glucose was produced in the FPU reaction

2 mg glucose = 2/0.18 x 0.5 x 60 umol/min/ml

= 0.37 umol/min/ml (IU/ml)

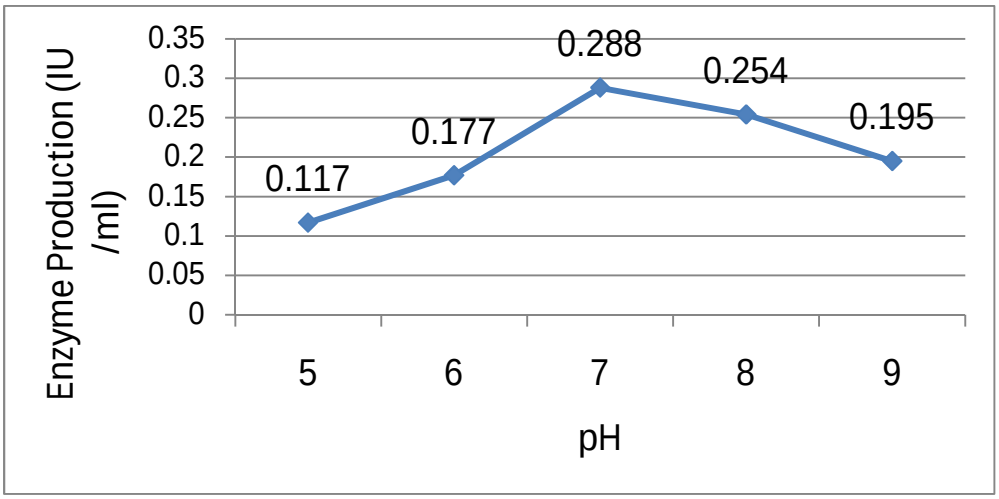
By using this derivation the units for following glucose concentrations for each samples was calculated, and graph was plotted for each organism and substrate individually.

RESULTS AND OBSERVATIONS-

***Trichoderma viride* (Coir waste)-**

From the above observations the enzyme shows its activity at pH-5 and it gradually increases upto pH-7 then at pH-8 and pH-9 it decreases gradually, so the pH-7 is the optimum pH for cellulase enzyme activity by *Trichoderma viride* fermented in coir waste.

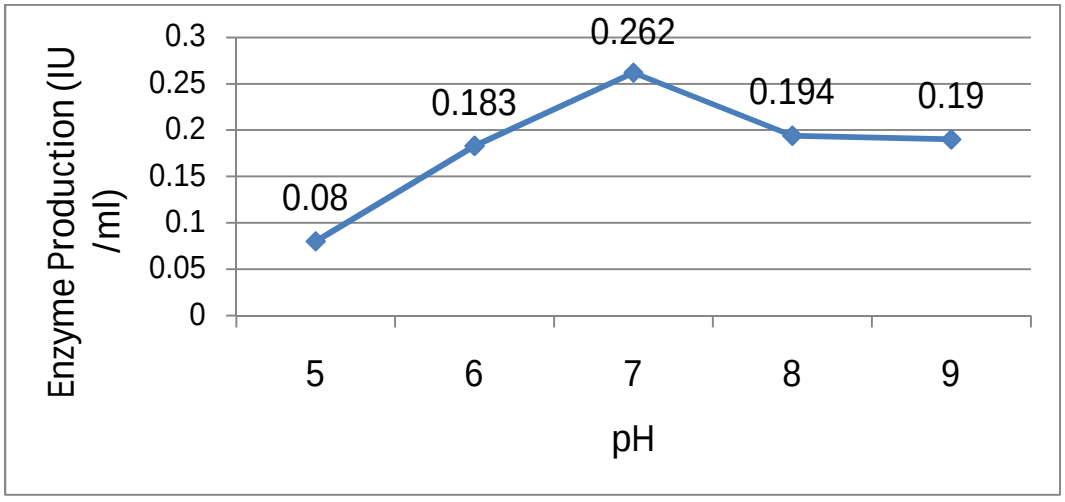
Chart no.1- Effect of pH on cellulase production by *Trichoderma viride* using coir waste as substrate.



***Trichoderma viride* (Saw Dust)-**

From the above observations the enzyme shows its activity at pH-5 and it gradually increases upto pH-7 then at pH-8 and pH-9 it decreases gradually, so the pH-7 is the optimum pH for cellulase enzyme activity by ***Trichoderma viride*** fermented in saw dust.

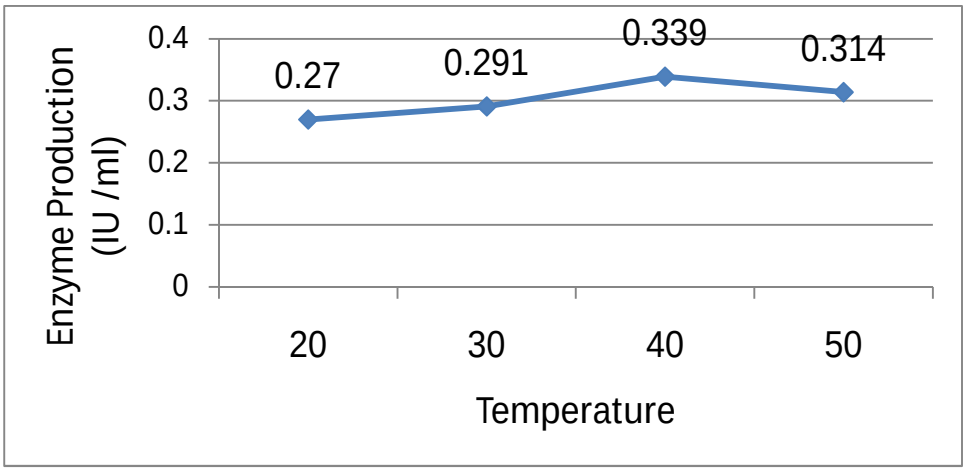
Chart no.2 - Effect of pH on cellulase production by *Trichoderma viride* using saw dust as substrate.



***Trichoderma viride* (Coir Waste)**

From the above observations the enzyme shows its activity at temperature 20°C and it gradually increases upto temperature 40°C, then at temperature 50°C it decreases gradually, so the temperature 40°C is the optimum temperature for cellulase enzyme activity by *Trichoderma viride* fermented in coir waste.

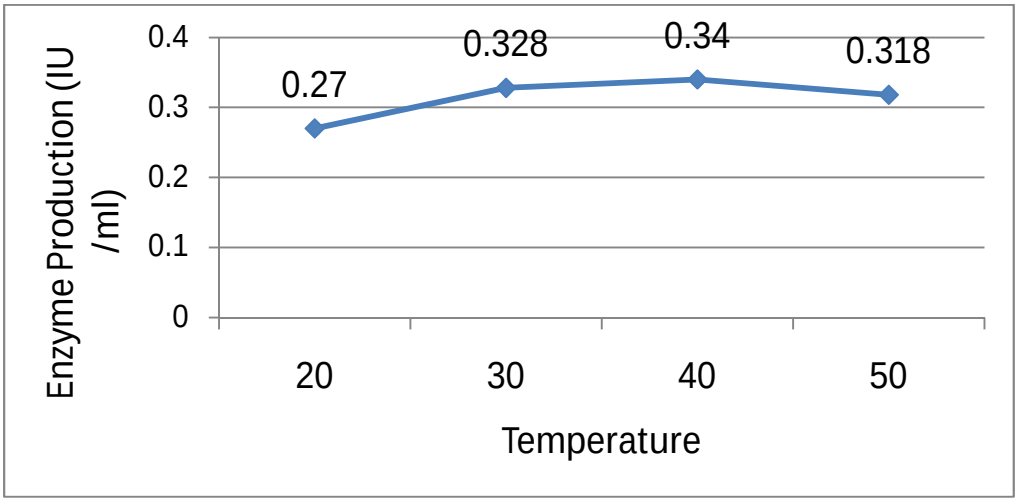
Chart no. –3- Effect of temperature on cellulase production by *Trichoderma viride* using coir waste as substrate.



***Trichoderma viride* (Saw dust)-**

From the above observations the enzyme shows its activity at temperature 20°C and it gradually increases upto temperature 40°C, then at temperature 50°C it decreases gradually, so the temperature 40°C is the optimum temperature for cellulase enzyme activity by *Trichoderma viride* fermented in saw dust.

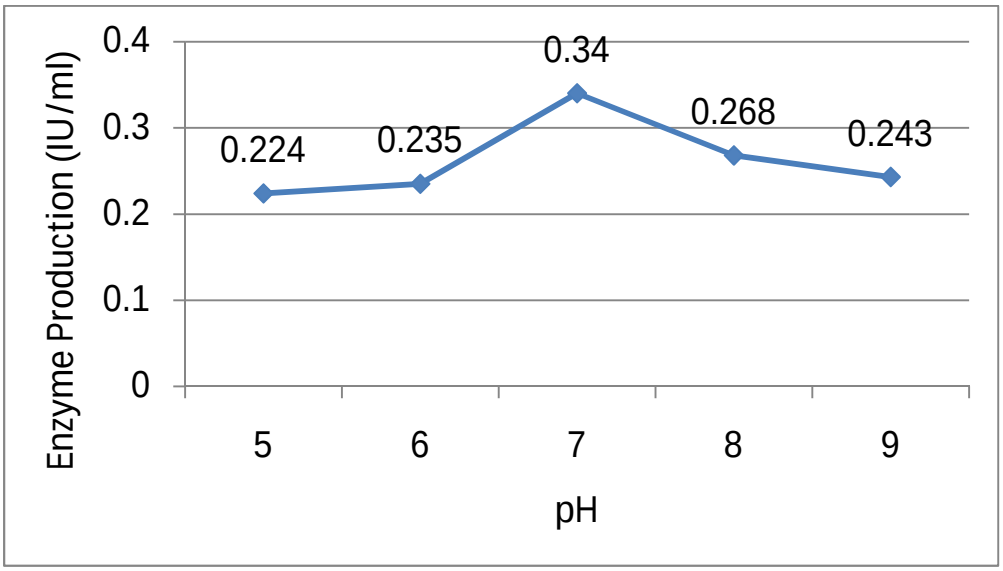
Chart no -4 - Effect of temperature on cellulase production by *Trichoderma viride* using saw dust as substrate.



***Trichoderma reesei* (Coir Waste)**

From the above observations the enzyme shows its activity at pH-5 and it gradually increases upto pH-7 then at pH-8 and pH-9 it decreases gradually, so the pH-7 is the optimum pH for cellulase enzyme activity by ***Trichoderma viride*** fermented in coir waste.

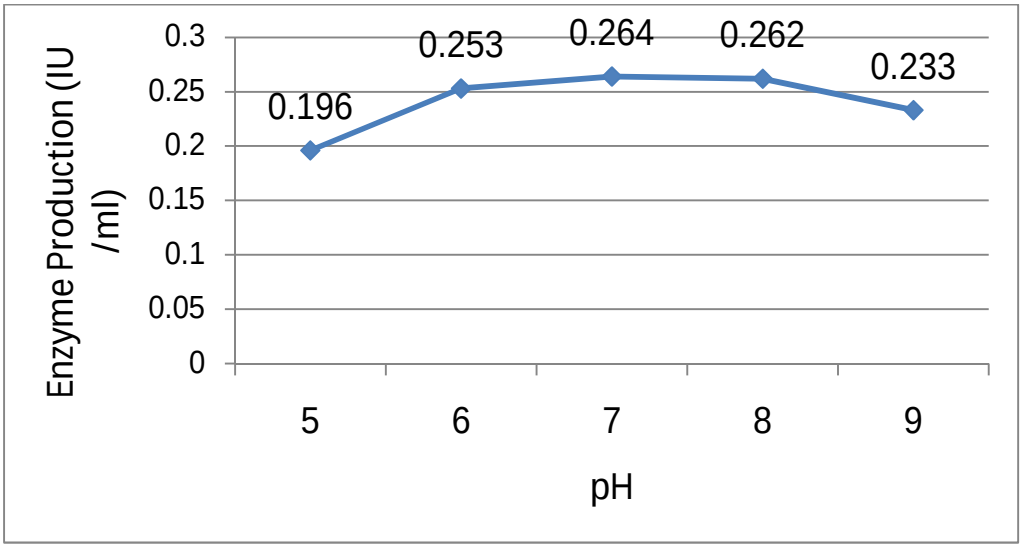
Chart no - 4 - Effect of pH on cellulase production by *Trichoderma reesei using* coir waste as substrate.



***Trichoderma reesei* (Saw Dust)**

From the above observations the enzyme shows its activity at pH-5 and it gradually increases upto pH-7 then at pH-8 and pH-9 it decreases gradually, so the pH-7 is the optimum pH for cellulase enzyme activity by *A. fumigatus* fermented in saw dust.

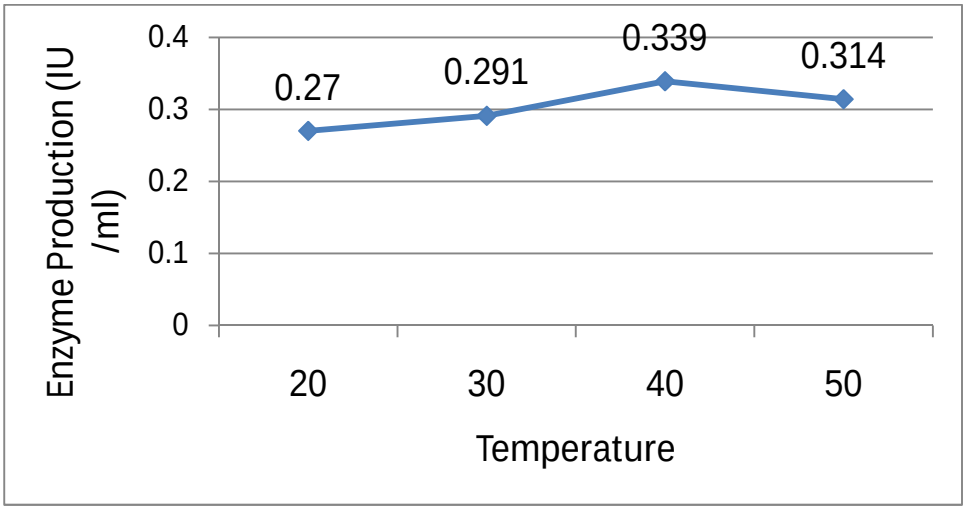
Chart no -5- Effect of pH on cellulase production by *Trichoderma reesei* using saw dust as substrate.



***Trichoderma reesei* (Coir Waste)**

From the above observations the enzyme shows its activity at temperature 20°C and it gradually increases upto temperature 40°C, then at temperature 50°C it decreases gradually, so the temperature 40°C is the optimum temperature for cellulase enzyme activity by ***Trichoderma reesei*** fermented in coir waste.

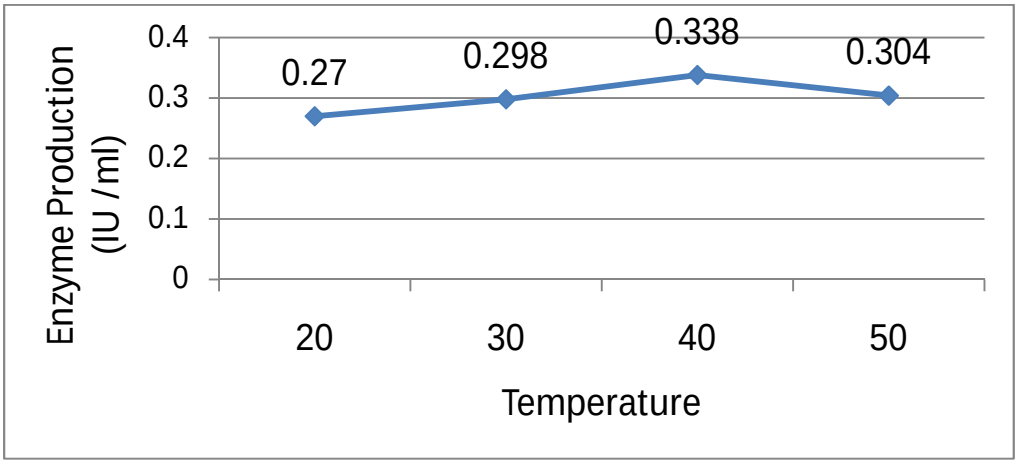
Chart no. –6- Effect of temperature on cellulase production by *Trichoderma reesei* using coir waste as substrate.



***Trichoderma reesei* (Saw dust)**

From the above observations the enzyme shows its activity at temperature 20°C and it gradually increases upto temperature 40°C, then at temperature 50°C it decreases gradually, so the temperature 40°C is the optimum temperature for cellulase enzyme activity by ***Trichoderma reesei*** fermented in saw dust.

Chart no -7- Effect of temperature on cellulase production by *Trichoderma reesei* using saw dust as substrate.



DISCUSSION

Major causes to study the commercial production of cellulases are the yield stability and cost of cellulase production. Therefore, research should also aim at exploiting the commercial potential of existing and new cellulase in nature. Agricultural residues such as corn stove, wheat straw, rice straw, baggase etc. were used in cellulase production. Although, the raw materials are cheaper, pre-treatment is generally required to improve the utilizability of lignocellulosic materials and the cost is considerable in view of the above facts, in the present study, the natural waste materials such as coir waste as well as saw dust waste have effectively utilized as major carbon source for the production of cellulase enzyme by fungal strains. A capacity to degrade cellulose is a character distributed among a wide variety of aerobic, facultative aerobic, anaerobic bacteria. The characters are restricted to a few species among several major taxa. The important cellulolytic fungus like *Trichoderma* sp. *Penicillium* Sp.; *Sporotrichium* Sp; *Aspergillus* sp etc have been reported to have cellulolytic activity. In the present study, two fungal strains such as *Trichoderma viride* and *Trichoderma reesei* were selected as the major cellulytic fungal strains for cellulase enzyme production. In the present study, the effect on environmental factors such as pH and temperature against *Trichoderma viride* and *Trichoderma reesei* were analysed. The optimum temperature of cellulase enzyme was found to be around 40°C. This value is lower than that of commercial cellulase production (60°C). On the other hand, endoglucanase was reported to be stable at 50°C and the enzyme showed major peaks at pH 4.5 and 7.5. The result is probably due to the presence of two isoenzymes or subunits in enzyme preparation. It was reported that optimal pH for Carboxymethyl cellulases was found to be 6.0 to 7.0.2 In the Filter Paper Assay method by using coir waste as substrate, high level of enzyme production was obtained at pH 7 (0.340 IU ml-1. While sawdust used as substrate, high level of production was obtained at pH 6 (0.264 IU ml-1.

Regarding the temperature influence on production of enzymes, In Filter Paper Assay method, when coir waste used as substrate, high level of enzyme production was obtained at 40°C - 0.033 IU ml-1.The Filter Paper Activity method is the standard method for determination of cellulase activity. In this method the Whatman No.-1 filter paper is used as a substrate for enzyme activity. The principle behind this method is, the cellulase enzyme have an ability to degrade cellulose into glucose, here the Whatman No. 1 filter paper is acts as a source of cellulose. The cellulase enzyme activity was determined by measuring amount of glucose produced in this reaction.

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