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The Stability Pattern of the Cellulase Enzyme Produced by *Aspergillus flavus*

Okonkwo IF*

Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Nigeria

***Corresponding author:** Okonkwo IF, Department of Applied Microbiology and Brewing, Faculty of Bio-sciences, Nnamdi Azikiwe University, PMB 5025 Awka, Anambra State, Nigeria, Email: iphyokonkwo@yahoo.com

Research Article

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Abstract

In view of the fact that temperature plays key role in the activity of fungal cellulases, this experiment was designed to assess the stability pattern of the cellulase Enzyme produced by *Aspergillus flavus* derived from rotten wood. The characterization of the purified cellulase enzyme of *Aspergillus flavus* was carried out using Carboxymethyl cellulose, Filter paper and Cotton wool assays. The maximum and optimum temperatures were 90°C and 70°C, respectively. The enzyme retained above 50% of its original activity at 100°C for 1h and up to 80% at 80°C for 1h Enzyme decay study showed that Cellulase activity remained 100 % at 0 h for both CMC and filter paper. However, as incubation period increased, the activity decreased, but the enzyme still retained above 50 % activity in both substrates at 1h, over the range of 40-100°C. The study carried out showed that the purified *Aspergillus flavus* cellulases demonstrated useful properties among which include; high optimum temperature, increased thermal stability. They also offer additional advantage as they are more rigid than meso enzymes and moreover, are more resistant to thermal and chemical denaturation. Hence are remarkable tools for developing commercial biotechnologies and for studying protein stability.

Keywords: *Aspergillus flavus*; Cellulases; Enzyme decay; Stability pattern

Abbreviations: ANOVA: Analyzed statistically using One Way Analysis of Variance; DNMRT: Duncan's New Multiple Range Test; CMC: Carboxy methyl cellulose.

Introduction

Cellulose according to Jarvis [1] is the primary product of photosynthesis. It is also the most abundant renewable bioresource ever produced Zhang & Lynd [2]. The biodegradation of Cellulose by cellulase enzyme liberated by various microbes, represents a flow from fixed carbon reserviors to CO_2 in the atmosphere [3,4]. The degradation of Cellulose is essential in various waste treatment and agricultural processes [5-8], and so can be used to produce biobased products and bioenergy to replace depleting fossil fuels [7].

Technological innovations for conversion of less expensive forestry and agricultural wastes to fermentable sugars create formidable potentials to benefit the national interest through: improved environmental quality, export

of technologies and an energy resource supply that can be sustained [9,7] Lynd, et al. 2002. An effective conversion of lignocelluloses to fermentable sugars involves three steps: size reduction, pretreatment/fractionation, and enzymatic hydrolysis [2].

Cellulases are expensive enzymes, and a reduction in cost will be necessary for their commercial use in biorefineries. Cellulase-based techniques, which will make the biobased processing more economical include, increasing enzyme productivity, production of enzymes using less expensive substrates, production of thermo stable enzyme preparations for specific processes, and production of cellulases with higher specific activity on solid substrates. This research is therefore designed to investigate the stability pattern of the *Aspergillus flavus* enzyme system and to determine its (half life), the time it will take the enzyme activity to reduce to half

Materials and Methods

Study Area

The research was carried out at Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, In the Department of Microbiology. The town Awka is noted with a moderate climate and high temperature in the dry season and average rainfall in the rainy season. It has the mean annual temperature and precipitation of 35°C and 1117mm, respectively (NIMET, 2006).

Methods

Experimental design: The parameters employed were measured in duplicates and the mean utilized. The values were analyzed statistically using one way analysis of variance (ANOVA) and the differences separated using Duncan's New Multiple Range Test (DNMRT).

Methods

Rotten wood and compost samples were obtained around Nnamdi Azikiwe University, Awka, premises. The samples were pulverized with blender and mixed in distilled water. It was then filtered using white cloth. A drop from each filtrate was placed on CzapexDox medium to which 1 % Carboxy methyl cellulose (CMC) of low viscosity (BDH) was incorporated and spread. It was incubated at room temperature (28-30°C.) for 48 h.

The isolates were purified by successive subculture on fresh CzapexDox medium, and the cellulolytic activities of the colonies determined by point inoculation on CzapexDox-carboxy methyl cellulose medium and incubation for 72 h, while zone of clearance which is an indication of cellulolysis was detected by flooding the cultures with 0.5 % Congo- red solution for 15 min. and distaining with 1M sodium chloride for 10 min [10]. The zones were measured and result recorded.

Identification of the Fungal Isolates

The isolate of choice was identified using standard reference manuals [11,12]. It was then cultivated in a medium containing the following in g/l [13] (Table 1).

(NH4)2S04	1.4
KH2P04	2
Urea	0.3
MgS047H20	0.3
CaCl2	0.3
FeS047H20	0.005
ZnS047H20	0.0014
MnS04H20	0.0016
CoCl2	0.002
Tween 80	2.0 ml
Carboxymethylcellulose	10
pH	6.8

Table 1: Medium containing the following in g/l.

This was done by inoculating aloopful of conidia into 100 ml of the sterilized medium in a 500 ml flask and incubating at 35°C on a Stuart orbital shaker model S150 at 200 rpm for 7 days. After this, the broth culture was subjected to centrifugation at 4000 rpm for 20 min using Centurion Centrifuge, and the supernatant concentrated by dialysis and then purified by Ion-exchange Chromatography and Gel filtration using Sephadex G-200. The fractions with observable protein peaks were used for enzyme activity.

Enzyme Characteristics

Temperature activity and stability: The temperature activity and stability profiles of the cellulase enzyme were carried out over a range of temperature (40-100°C). This was done by adding equal volume (0.1 ml) of purified cellulase preparation and substrates (CMC, filter paper and cotton wool) in 0.2 M phosphate buffer of pH 6.8, incubating at the test temperature for 30 min. intervals and measuring the activity by DNS method.

Determination of Half-Life of Enzyme Activity

The method of Odibo, et al. [14] was applied. The heat stability of the enzyme was determined by pre incubating

the enzyme with substrates (CMC and filter paper) in a water bath at temperature range of 40-100°C. Samples (0.1 ml each) were withdrawn at intervals of 30 min up to 3 h, and the enzyme activity determined. The time taken for the activity of the enzyme to reduce to half is taken as the half life of the enzyme.

Results and Discussion

Enzyme decay was studied by incubating at various temperature regimes (40-100°C) at interval of 30 min. Cellulase activity remained 100 % at 0 h for both CMC and filter paper (Figures 1 & 2).

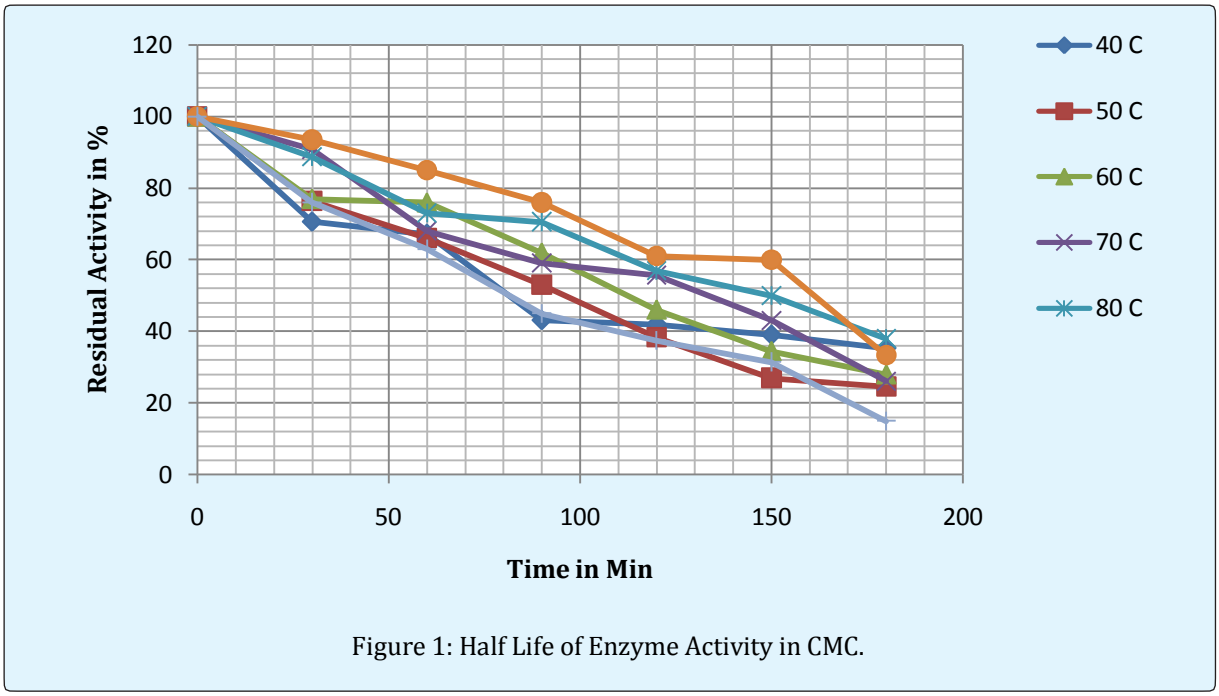

Figure 1: Half Life of Enzyme Activity in CMC.

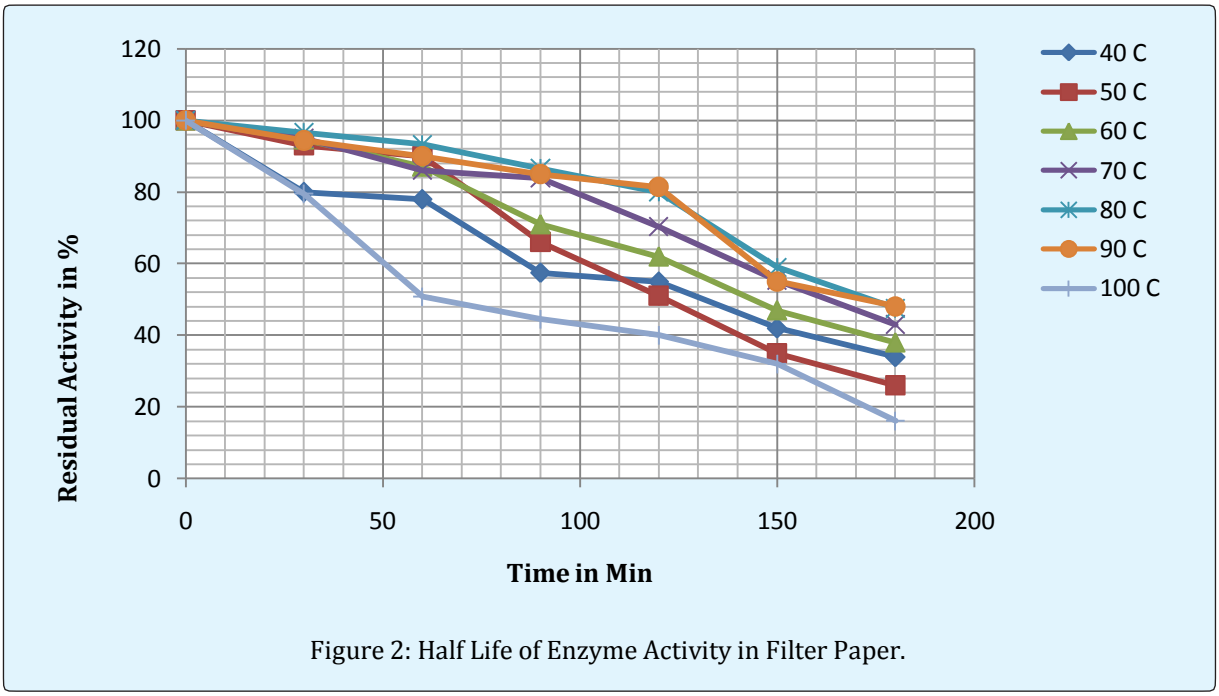

Figure 2: Half Life of Enzyme Activity in Filter Paper.

However, as incubation period increased, the activity decreased, but the enzyme still retained above 50% activity in both substrates at 1h, over the range of 40-100°C. Half life was deduced from the plot of residual activity against time Table 2, and taken as time needed for the enzyme activity to reduce by 50%.

Temperature °C	CMC	Filter Paper
40	82 Min	132 Min
50	96 Min	122 Min
60	112 Min	144 Min
70	134 Min	164 Min
80	150 Min	172 Min
90	162 Min	173 Min
100	82 Min	64 Min

Table 2: Half life of enzyme activity at various tempt in CMC and Filter Paper.

Optimum Thermal Stability

The temperature stability and relative thermal stability of the purified cellulase was statistically similar (P>0.05) at 70°C, 80°C °C for CMC and for cotton wool, though highest numerical values were obtained at 70°C, even for filter paper Appendix 1. Hence, the optimal thermal stability was achieved at 70°C; however, the enzyme retained substantial percentages of its thermal stability at 100°C as seen in Appendex1 and Figure 3for all the substrates when incubated for 30mins.

Again, the enzyme recorded minimal (P<0.05) thermal stability and relative thermal stability on filter paper compared to cotton wool and CMC. Though numerically higher values were recorded on cotton wool at various temperature regime, the values were statistically similar (P>0.05) to those obtained on CMC (Figure 3 & Appendix 2).

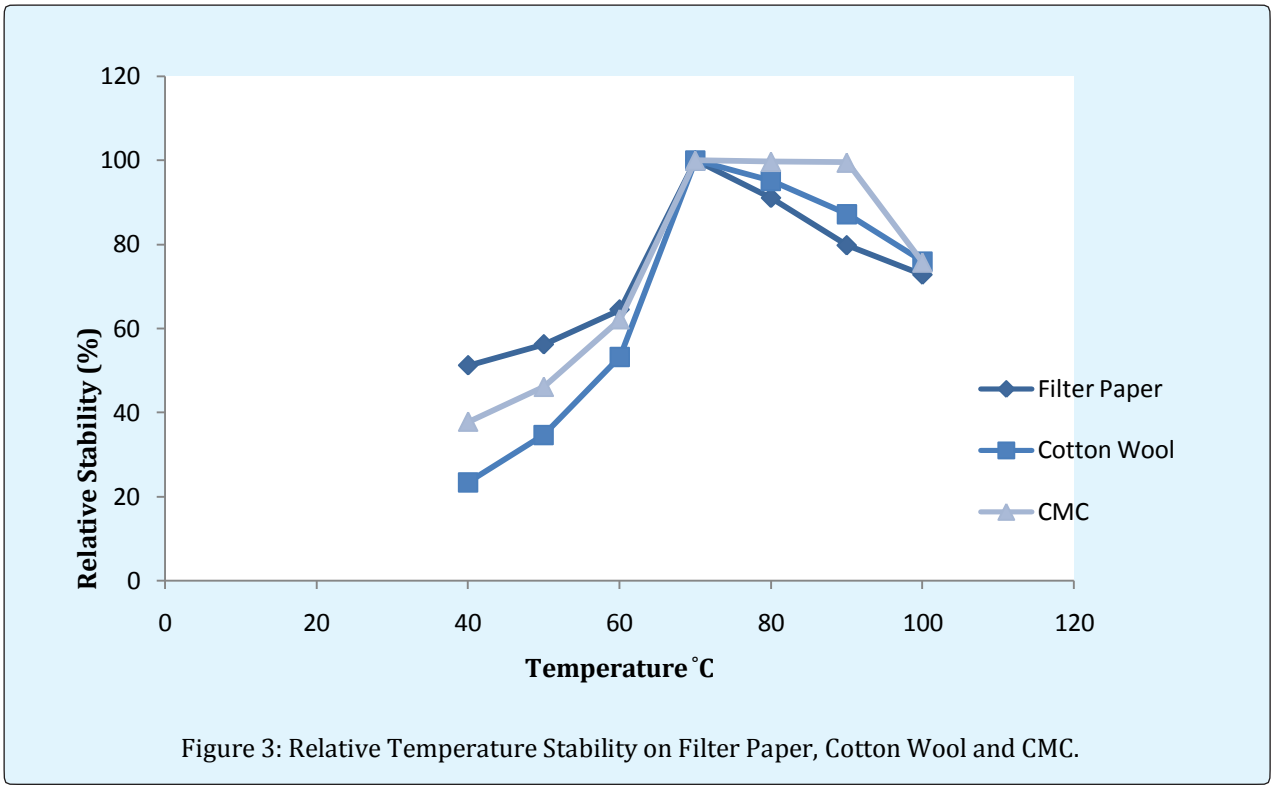

Figure 3: Relative Temperature Stability on Filter Paper, Cotton Wool and CMC.

Enzyme decay was studied by incubating at various temp regimes (40-100°C) at interval of 30 min. Cellulase activity remained 100% at 0 h for both CMC and filter paper (Figures 1 & 2). However, as incubation period increased, the activity decreased, but the enzyme still retained above 50% activity in both substrates at 1h, over the range of 40-100°C. Half life was deduced from the plot of residual activity against time (Table 1, Appendix 3 & 4), and taken as time needed for the enzyme activity to reduce by 50%. Other studies using *Aspergillus niger* Aguiar [15] have shown a residual activity of around 100% for cellulase activity at temperatures between 50°C

and 60°C, comparable to our own findings. Also half-lives of 8 h at 60°C or 1h at 70°C have been reported for some *Aspergillus niger* [15].

Conclusion

Our results strongly suggest that the cellulases in this supernatant seem to be thermophilic, which are considered beneficial for many industrial processes. The enzyme exhibited good heat stability with a half life of 60min at 100°C [16].

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