

## TAMARIND EXTRACT PRETREATMENT: VALORIZATION OF SUGARCANE BAGASSE FOR CELLULASE PRODUCTION BY *Aspergillus flavus*

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### ABSTRACT

Effective pretreatment is crucial for cellulase production from sugarcane bagasse. Pretreatment with tamarind extract could reduce the hazardous effect associated with chemical pretreatment. The present work investigated tamarind (*Tamarindus indica*) extract in combination with H<sub>2</sub>SO<sub>4</sub> and thermal pretreatment of sugarcane bagasse for cellulase production by *Aspergillus flavus*. The sugarcane bagasse was pretreated with tamarind extract pH 2 and pH 4, followed by 1% H<sub>2</sub>SO<sub>4</sub> and thermal treatment at 121°C for 15 min. The pretreatment slurry was analysed for reducing sugar while solid bagasse was analysed for weight loss. *Aspergillus flavus* grew on sugarcane bagasse under solid state fermentation and the Carboxy Methyl Cellulase (CMCase) and Filter Paper Assay (FPA) activities were compared on the various pretreatments. The pretreatments changed the visible morphology of the sugarcane bagasse observed by the swelling, fibrous appearance and colour change. Pretreatment slurry yielded highest soluble reducing sugar at 60.01 mg/ml in tamarind extract (pH 4/1% H<sub>2</sub>SO<sub>4</sub>) and highest weight loss of solids at 73.70% in tamarind extract (pH 2/1% H<sub>2</sub>SO<sub>4</sub>/thermal 121°C). *Aspergillus flavus* performed better on tamarind extract (pH 2/1% H<sub>2</sub>SO<sub>4</sub>) by producing optimal CMCase and FPA activities at 0.100 U/ml and 0.409 U/ml respectively after 3 days of fermentation. Cellulase was maximally active at temperature of 50 °C. The tamarind extract pretreatment successfully proved to be an alternative organo-chemical pretreatment of sugarcane bagasse as evidenced by the physical properties, soluble reducing sugars and cellulase activities.

**Keywords:** *Aspergillus flavus*, Cellulase, Pretreatment, Sugarcane bagasse, Tamarind extract

### INTRODUCTION

Agro-waste residues account for high share of lignocellulosic biomass worldwide. They are highly abundant and could serve as economical feed stock for the production of renewable chemicals. After extraction of soluble sugar, sugarcane factories generate high amount of the solid residues known as sugar cane bagasse. As at 2018, sugarcane bagasse production in Nigeria was reported to have increased to sixty six thousand metric tonnes from the forty nine thousand metric tonnes produced in 2015 (UNdata, 2018). Sugarcane bagasse has been reported to be composed of approximately 50% cellulose, 25% hemicellulose, 25% lignin (Huang *et al.*, 2012; Philippini *et al.*, 2019).

Sugarcane bagasse has been explored for the production of cellulase, bioethanol and metabolites (Thite and Nerurkar, 2019). In biofuel production, biorefineries relies on enzymatic saccharification followed by fermentation of the released sugars. Cellulase is the enzyme that act on the plant polysaccharide for saccharification,

however production of cellulase from pure commercial substrate is very expensive and production from plant polysaccharides yield insufficient enzyme. Such inadequacies could be attributed to the recalcitrant nature of the plant cell wall, unproductive binding of enzymes and or enzyme inactivation by inhibitory byproducts (Kumar and Wyman, 2009; Rocha *et al.*, 2012). On the other hand, the recalcitrant nature of the plant cell wall restricts the accessibility of cellulose degrading microorganisms to the cellulose matrix. Thus, partial breakdown of the cell wall matrix through pretreatment is crucial.

Several pretreatment strategies have been developed in the past. However, it is difficult to adopt a single pretreatment method for all biomass due to the diverse nature of different lignocellulosic biomass. Such pretreatment enhances ease biomass deconstruction by microorganisms. Various pretreatment methods have been reported to enhance cellulase production from sugarcane bagasse. Examples are sodium hydroxide, dilute acid steam and hydrogen

peroxide (Camassola and Dillon, 2014; Cunha *et al.*, 2014; Muhammad *et al.*, 2019). Although pretreatment using organic acids like formic acid, lactic acid and acetic acid (Xu *et al.*, 2009; Sindhu *et al.*, 2010) have been reported; these reports are, nevertheless, very few. Moreover, pretreatment of sugarcane bagasse using tamarind (*Tamarindus indica*) extract for cellulase production is yet to be explored.

Tamarind is a hardwood tree found mostly in tropical Africa (Morton and Dowling, 1987). The tree produces bean-like pods filled with seed surrounded by a fibrous pulp. The pulp of the fruit contains citric acid, tartaric acid and malic acid with pH 1.8-3.7 (Nassereddin and Yamani, 2005; Razali *et al.*, 2015). In Nigeria the pulp is used in traditional medicine and souring of “koko” produced from milled corn. It also contains polyphenols and antioxidants that have health benefits (Sandesh *et al.*, 2014; Razali *et al.*, 2015). The acidic (Tartaric acid) content of tamarind fruit could be exploited for organic acid pretreatment of agro-waste biomass. Organic acid pretreatment involves degradation of hemicelluloses and disruptions of lignin followed by solvations of the fragments (Sindhu *et al.*, 2010). Organic acid pretreatment increases accessible surface area, solubilization of hemicelluloses, solubilization of lignin, and alteration of lignin structure (Zhao *et al.*, 2009).

Cellulase are known to have various industrial applications in textile, food, brewing, pulp and paper industry, detergent as well as biorefinery. However, the major bottleneck in the commercialization of biorefinery is the cost of production of cellulases. Therefore, the use of agro-waste biomass through microbial fermentation (solid state or submerge) will reduce the cellulase production cost as well as reduce waste accumulation in the environment.

Cellulolytic enzymes are synthesized by a number of microorganisms including fungi and bacteria. Among cellulase producing organisms, fungi have been mostly reported as a well-known agents of decomposer and of the cellulosic substrate in particular (Zhang *et al.*, 2014). Fungi cellulases are commercially attractive due to the robustness and extracellular nature and their simple structure. In

this context, the aim of the present study is to examine the effect of tamarind extract pretreatment on the physical change and cellulase production from sugarcane bagasse by *Aspergillus flavus*.

## MATERIALS AND METHODS

### Collection and Processing of Sugarcane Bagasse

Fresh sugarcane was collected from Oja Oba Ilorin, Kwara State, Nigeria. The sugarcane juice was extracted to obtain the bagasse. The sugarcane bagasse was washed repeatedly with distilled water under running water until all soluble sugars have been removed. The bagasse was oven dried at 60 °C to constant weight and milled to obtain particles size of 1 mm diameter. The sieved sugar cane bagasse was stored in air tight container at room temperature until further use.

### Collection and Processing of Tamarind Fruit

Dried tamarind fruits pulp was collected from Central Market, Kaduna State, Nigeria. The tamarind extract (TE) was prepared by soaking 1600 g of tamarind fruits in 3200 ml of distilled water, for 24 hours at room temperature. The mixture was filtered through muslin cloth and stored at 4 °C until further use. The pH of the filtrate was adjusted to pH 2.0 and 4.0 in a separate beakers using NaOH or HCl.

### Collection and Maintenance of Pure Culture of *Aspergillus flavus*

*Aspergillus flavus* was collected from the culture collection of Biomass and Enzyme Technology Laboratory, Department of Microbiology, University of Ilorin, Kwara State, Nigeria. The fungi was subcultured on Potato Dextrose Agar (PDA) and transferred to PDA slant after 72 h incubation at 30 °C. It was preserved at 4 °C until further use.

### Pretreatment of Sugarcane Bagasse

Twenty grams of sugarcane bagasse was weighed into 400 ml of tamarind extract (1: 20 w/v) in 500 ml Erlenmeyer conical flask. The first set of pretreatment contained tamarind extract of pH 2.0 and pH 4.0 and then left for 24 h at room

temperature. The second level pretreatment included tamarind extract of pH 2 and pH 4, added to both was 1% H<sub>2</sub>SO<sub>4</sub> which was allowed to stay for 1 hour. While the third level pretreatment include tamarind extract pH 2 and pH 4, with 1% H<sub>2</sub>SO<sub>4</sub> for 1 h followed by thermal treatment at 121 °C for 10 min. After treatment, the pretreated samples were washed in several changes of distilled water to neutral pH and then oven dried at 50 °C for 48 h.

### Determination of Weight Loss

Weight loss was determined by directly weighing dried bagasse before and after each treatment, after drying in an oven at 50 °C to a constant weight.

$$\text{Weight loss} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100 \dots \text{Equation 1}$$

### Medium Formulation

The preparation was done using the composition of Mendel's medium, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.4 g; KH<sub>2</sub>PO<sub>4</sub>, 2.00 g; Urea, 0.6 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.3 g; CaCl<sub>2</sub>, 0.3 g; Peptone, 0.75 g; Tween 80, 2.0 ml. Trace element consisted FeSO<sub>4</sub> (1 × 10<sup>-3</sup>) g, MnSO<sub>4</sub>·H<sub>2</sub>O (1.6 × 10<sup>-3</sup>) g, ZnSO<sub>4</sub>·7H<sub>2</sub>O (1.4 × 10<sup>-3</sup>) g, CoCl<sub>2</sub> (2.6 × 10<sup>-3</sup>) g. These were dissolved in 1000 ml of distilled water and stored in a refrigerator for further use.

### Solid State Fermentation

The fungus was previously isolated from saw dust dump site. The ability to produce cellulase was reflected by the hydrolysis zone observed when the fungus was grown on CMC-congo red agar plate. Solid state fermentation was carried out for cellulase production by *Aspergillus flavus*. One gram of each pretreated sugarcane bagasse weighed into 200 ml conical flask, moistened with 2 ml of mineral salt medium to obtain 67% moisture content and autoclaved for 121 °C for 15 min. After sterilization, the flasks were allowed to cool at room temperature and 2 agar plugs of a 4 day old *A. flavus* was transferred aseptically to each of the fermentation flask using a 7 mm cork borer. The flasks were incubated at 30 °C for six days and sampling was carried out at 24 h interval by withdrawing each flask for enzyme extraction.

### Extraction of Crude Enzyme

To each flask, 20 ml of 0.05 M sodium citrate buffer pH 4.8 was added, placed in the shaker and agitated at 150 rpm for 30 min. The mixture was filtered through muslin cloth followed by centrifugation at 5000 rpm for 10 min. The filtrate obtained was used as crude enzyme and stored at 4 °C until further use.

### Enzyme Assay

Carboxymethylcellulase (CMCase) assay was carried out with 0.5 ml of crude enzyme sample and 0.5 ml of 1% carboxy methyl cellulose (CMC) in 0.05 M citrate buffer pH 4.8 and incubated at 50 °C for 30 min. After incubation, the reaction was stopped by the addition of 1.5 ml of dinitrosalicylic acid (DNS) reagent and boiled for 10 min. The reaction mixture was allowed to cool under running water. The reducing sugar was estimated in a spectrophotometer at 575 nm (Miller, 1959). The filter paper assay (FPA) was carried out in a reaction mixture of 0.5 ml crude enzyme added to test tube containing filter paper strip (1 × 6 cm) immersed in 1.5 ml of 0.05 M citrate buffer pH 4.8, then incubated at 50 °C for 1 h. After incubation, 1.5 ml DNS was added to stop the reaction and the mixture was boiled for 10 min at 100 °C and allowed to cool to room temperature. The released reducing sugar was determined at 575 nm (Miller, 1959). One unit of enzyme activity was defined as the amount of enzyme that liberated 1 μmol of glucose at the specified condition.

### Effect of Temperature on CMCase Activity

The effect of temperature on activity of CMCase was determined by incubating the crude enzyme with 1% CMC in 0.05 M citrate buffer (pH 5.0) at temperatures between 30°C and 60°C for 5 h. The CMCase activity was assayed by DNS method at different temperatures.

## RESULTS AND DISCUSSION

After preliminary screening, *Aspergillus flavus* produced hydrolysis zone at 1.4 mm after 3 days incubation on CMC-Congo red agar. According to previous studies *Aspergillus flavus* have been reported to produce cellulase on CMC agar stained with Grams iodine (Sutaoney *et al.*, 2020) and on CMC-Congo red agar (Gautam *et al.*, 2012).

### Effect of Tamarind Extract Pretreatment on Weight Loss

The weight loss obtained after each pretreatment on sugarcane bagasse is illustrated in table 1. In all the pretreatment levels applied more than 50% reduction in the initial weight of sugar cane bagasse was recorded. From the study it was observed that pretreatment T3 resulted in the

highest weight loss of 73.30% while the lowest weight loss at 51.85% was observed with pretreatment T5. This was followed by pretreatment T6 which resulted in the loss of 63.5% of its initial weight. The pretreatment T1 and T2 produced a lower weight loss at 59.0% and 58.9% respectively.

**Table 1:** Weight Loss of Tamarind Extract Pretreatments of Sugarcane Bagasse

| Code | Pretreatment                                       | Initial Weight<br>(g) | Final Weight<br>(g) | Weight Loss<br>(%) |
|------|----------------------------------------------------|-----------------------|---------------------|--------------------|
| T1   | TE pH 2.0                                          | 20                    | 8.20                | 59.00 <sup>c</sup> |
| T2   | TE pH 2.0 / 1 % H <sub>2</sub> SO <sub>4</sub>     | 20                    | 8.30                | 58.50 <sup>c</sup> |
| T3   | TE pH 2.0/1 % H <sub>2</sub> SO <sub>4</sub> /Heat | 20                    | 5.34                | 73.30 <sup>a</sup> |
| T4   | TE pH 4.0                                          | 20                    | 8.30                | 56.50 <sup>d</sup> |
| T5   | TE pH 4.0/ 1% H <sub>2</sub> SO <sub>4</sub>       | 20                    | 9.63                | 51.85 <sup>e</sup> |
| T6   | TE pH 4.0/1% H <sub>2</sub> SO <sub>4</sub> / Heat | 20                    | 7.30                | 63.50 <sup>b</sup> |

TE= Tamarind Extract

However, it should be noted that their percentage weight loss are not significantly different ( $P < 0.05$ ) from each other. On the other hand T4 recorded much lower loss in weight at 56.50%. The result is in contrast to Manzoor et al. (2012) who reported highest weight loss of 47.7% with sugarcane bagasse treated with 4% H<sub>2</sub>SO<sub>4</sub> at 121°C for 180 min. The higher weight loss observed in pretreatments T3 and T6 could be as a result of the removal of more hemicellulose and lignin portion of the sugarcane bagasse due to the three level pretreatments applied. The pretreatment method could be effective in the step wise removal of hemicellulosic portions by the tamarind extract, followed by 1% H<sub>2</sub>SO<sub>4</sub> and finally thermal treatment.

The pretreatment T1 also showed higher weight loss than T4. This shows that the lower pH (pH 2) of the tamarind extract has more effect on the removal of some portions of the lignocellulosic bagasse when compared with higher pH (pH 4).

The finding is in agreement with previous studies where the weight loss of pretreated sugarcane bagasse was attributed to the reduction in the hemicellulose and lignin portion (Timung *et al.*, 2016). Also greater weight loss indicate more lignin loss and cellulose digestibility (Manzoor *et al.*, 2012).

### Effect of Pretreatment on Hydrolysate Reducing Sugar

The result of the reducing sugar profile of hydrolysate (Figure 1) obtained after different pretreatments of the sugarcane bagasse showed that the highest reducing sugar of 60.0 mg/ml was released in pretreatment T5. This was followed by pretreatments T1 and T2 which released reducing sugars of 49.2 mg/ml and 50.5 mg/ml, respectively. On the other hand, pretreatments T3 and T4 produced the lowest reducing sugar of 19.9 mg/ml and 20.1 mg/ml, respectively. The pretreatment T6 resulted in the release of a lower reducing sugar of 35.6 mg/ml.

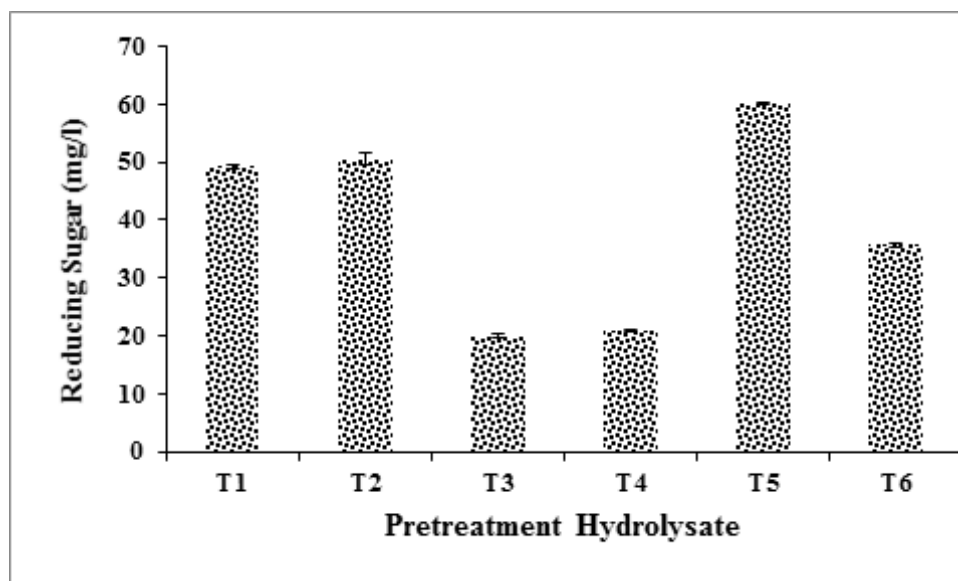


**Figure 1:** Reducing Sugar Profile of Pretreatment Hydrolysate

T1: TE pH 2.0; T2: TE pH 2.0/1% H<sub>2</sub>SO<sub>4</sub>; T3: TE pH 2.0/ 1% H<sub>2</sub>SO<sub>4</sub> /Heat; T4: TE pH 4.0; T5: TE pH 4.0/1% H<sub>2</sub>SO<sub>4</sub>; T6: TE pH 4.0/ 1% H<sub>2</sub>SO<sub>4</sub>/Heat  
TE= Tamarind Extract

It could be deduced from this result that the addition of 1% H<sub>2</sub>SO<sub>4</sub> had a higher hydrolysis effect on sugarcane bagasse, while the effect of temperature on the release of reducing sugar is lower than that of the acid. The use of 1% H<sub>2</sub>SO<sub>4</sub> treatment of lignocellulosic materials has been found to cause swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure.

#### Effect of Tamarind Extract Pretreatment on CMCase Activity

Fermentation of pretreated sugarcane bagasse was carried out under solid state fermentation, the incubation period commenced from day 0 to day 6, as shown in figure 2. Based on the incubation period it was observed that the maximum cellulase production by *Aspergillus flavus* was achieved by day 3 in all the pretreatment followed by a decline until the 6<sup>th</sup> day of fermentation. The result shows that the CMCase activity varies with respect to the different pretreatments. The highest CMCase activity (0.100 U/ml) was recorded in pretreatment T2 while the lowest activity (0.011 U/ml) was observed in pretreatment T6.

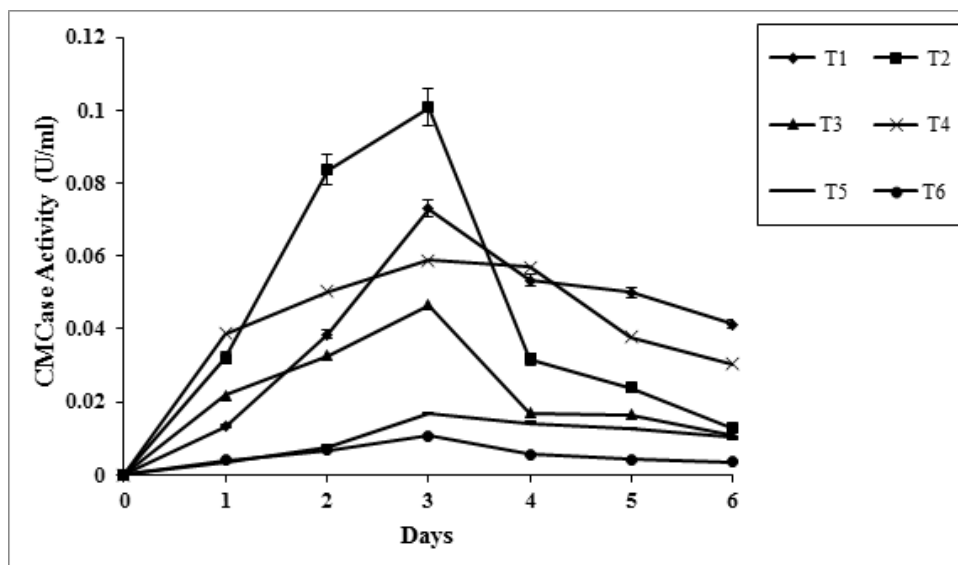


**Figure 2:** CMCase Activity of *Aspergillus flavus* Grown on Tamarind Extract Pretreated Sugarcane Bagasse

T1: TE pH 2.0; T2: TE pH 2.0/1% H<sub>2</sub>SO<sub>4</sub>; T3: TE pH 2.0/ 1% H<sub>2</sub>SO<sub>4</sub> /Heat; T4: TE pH 4.0; T5: TE pH 4.0/1% H<sub>2</sub>SO<sub>4</sub>; T6: TE pH 4.0/ 1% H<sub>2</sub>SO<sub>4</sub>/Heat

TE= Tamarind Extract

With respect to the effect of Tamarind extract pH on CMCase activity, it was observed that a higher CMCase activity (0.100 U/ml) was achieved at pH 2.0 (T1) as compared to lower CMCase activity (0.073 U/ml) observed at pH 4 (T4). The addition of 1% H<sub>2</sub>SO<sub>4</sub> as a second level pretreatment resulted in higher CMCase activity (0.100 U/ml) of Tamarind extract pretreatment at pH 2 (T2), but resulted in a detrimental effect on the CMCase activity (0.016 U/ml) of the pretreatment at pH 4. The addition of thermal pretreatment resulted in a lower CMCase activity. Although, the thermal pretreatment improved CMCase activity in T3 (0.046 U/ml) than T6 (0.011 U/ml).

The results shows that the addition of 1% H<sub>2</sub>SO<sub>4</sub> has a positive effect on the tamarind extract pretreated sugarcane bagasse than H<sub>2</sub>SO<sub>4</sub> pretreatment followed by thermal pretreatment. The addition of thermal pretreatment could have resulted in the formation of some inhibitory products which retarded the CMCase activity. The formation of fermentation inhibitors such as furfural and 5-hydroxymethyl furfural (5-HMF) could increase due to increase acid concentration, temperature and time (Ezeji *et al.*, 2007;

Taherzadeh and Karimi 2007; Kamoldeen *et al.*, 2017). In a similar study, sulphuric acid pretreatment resulted in higher values of enzyme production than sulphuric acid pretreatment followed by autoclaving of banana peduncle waste (Arooj *et al.*, 2017). The addition of thermal pretreatment could have also damaged part of the cellulose component due to harshness. It was reported by Kazeem *et al.* (2017) that the pretreatment of rice husk caused crack in the cellulose matrix which was responsible for lower enzymatic saccharification. The deformation of cellulose and redeposition of lignin could also lead to unproductive binding of the enzyme to the substrate.

#### Effect of Tamarind Extract Pretreatments on Filter Paper Activity Assay of Cellulase (FPA)

*Aspergillus flavus* produced FPA activity from all the pretreated sugar cane bagasse (Figure 3). However, the optimal production of FPA activity occurred in different fermentation days based on the type of pretreatment. Majorly, the optimal FPA activity was achieved at day 3 in pretreatments T2, T3, T4 and T6 except for pretreatments T1 and T5 which recorded maximum FPase activity at day 4 which was followed by a decrease in activity till the 6<sup>th</sup> day of

fermentation. It should be noted that *A. flavus* produced higher FPA activity than CMCase activity. This is very uncommon among cellulase producing fungi. Among all the pretreated substrates the highest FPA activity of 0.409 U/ml was observed for T1 on day 4. However, there is

no significant difference ( $P < 0.05$ ) in FPA activity from T1 and T2 at day 3 and day 4 fermentation time. Samples T5, T6 and T4 recorded highest activity at 0.372 U/ml 0.227 U/ml 0.216 U/ml, respectively.

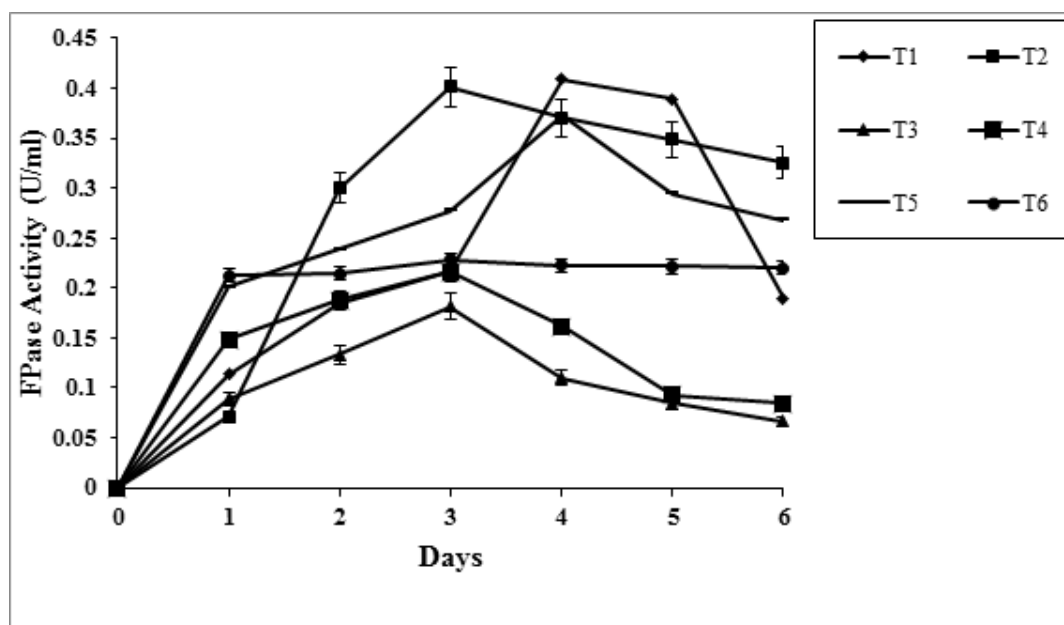


**Figure 3:** FPase Activity of *Aspergillus flavus* Grown on Tamarind Extract Pretreated Sugarcane Bagasse

T1: TE pH 2.0; T2: TE pH 2.0/1%  $H_2SO_4$ ; T3: TE pH 2.0/ 1%  $H_2SO_4$  /Heat; T4: TE pH 4.0; T5: TE pH 4.0/1%  $H_2SO_4$ ; T6: TE pH 4.0/ 1%  $H_2SO_4$ /Heat  
TE= Tamarind Extract

Among all the pretreated bagasse, T3 was observed to produce the lowest FPA activity at 0.181 U/ml. The extent of FPA activity in relation to pH varied. Higher FPA activity of 0.409 U/ml was observed in TE pH 2 (T1) as compared to 0.216 U/ml observed for TE pH4. Similarly, the addition of 1%  $H_2SO_4$  to the TE was more favorable for higher FPA activity at pH 2 (T2, 0.402 U/ml) than pH 4 (T5, 0.372 U/ml). The addition of the third layer of pretreatment which included heating at 121 °C for 15 min resulted in lower production of FPA activity at both pH 2 and pH 4. The decrease of FPA activity after the optimum period might be due to loss of moisture and inactivation by pH during fermentation as reported by Melo *et al.* (2007) and Ijaz *et al.* (2011). Dhillon *et al.* (2004) also reported that prolonged incubation would lead to depletion of nutrients, cell death and proteolytic digestion.

It may also be due to the denaturation of enzyme at various pH which is a common phenomenon during fermentation due to the release of acidic by-products in the fermentation media.

The increase in enzyme production was probably due to the availability of cellulose in the medium while a decrease in production beyond optimum concentration was probably as a result of an inhibiting effect of accumulated cellobiose and cellodextrin in the growth medium (Omojasola and Jilani, 2008; Abubakar and Oloyede, 2013). It could be deduced from the result that the second level pretreatment which included 1%  $H_2SO_4$  had no effect on increasing FPA activity but had positive effect on the CMCase activity. Therefore, pretreatment with tamarind extract only is enough to produce FPA activity- rich cellulase which could be tailored to a particular industrial application.

The application of tamarind extract pretreatment only could have produced little or no inhibitory products which resulted in a more favorable fermentation by the fungus.

#### Effect of Temperature on CMCase Activity

The effect of temperature on CMCase activity of the crude enzyme is shown in figure 4. The temperature profile of the CMCase shows optimal temperature at 50 °C for CMCase. The CMCase activity increased gradually as the temperature also increased and reached maximum at 50 °C which later declined at 60 °C. This is in line with the findings of Abubakar and Oloyede (2013), who reported that cellulase of *A. niger* was

found to be more active at temperature of 45-50 °C. According to previous studies, Sadhu *et al.* (2013) reported that the optimum temperature of cellulase was at 50 °C. Cellulase from *Chaetomium* sp. exhibited maximum activity at 20 °C (Al-Kharousi *et al.*, 2015). In contrast to this the maximum cellulase activity produced from *Penicillium* sp CR-316 was attained at 65 °C (Picart *et al.*, 2007). The decrease in enzyme activity at higher temperatures could be due denaturation of the enzyme. Likewise, as the temperature rises, reacting molecules have more and more kinetic energy, this increases the chances of successful collision and so the rate increases.

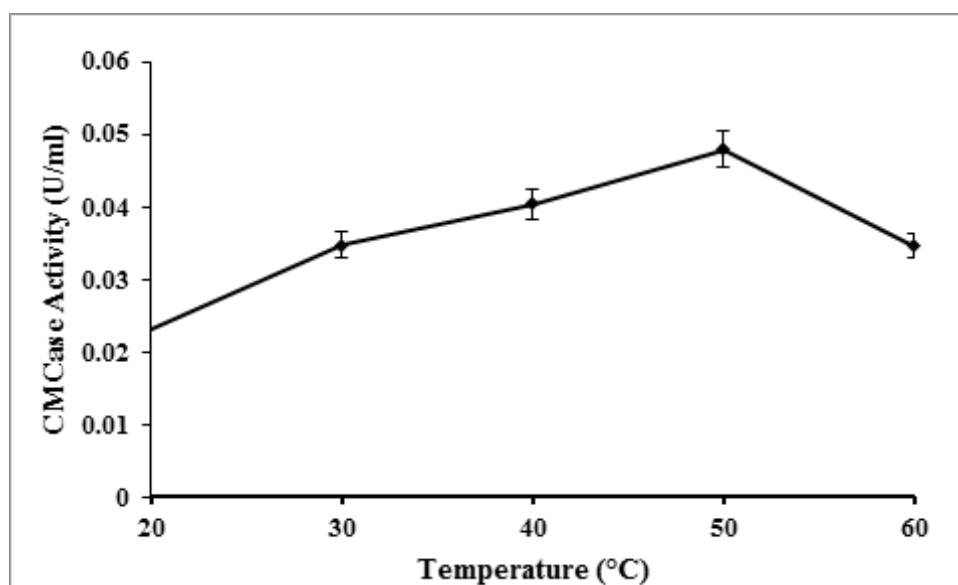


**Figure 4:** Effect of Temperature on the Activity of Crude CMCase Produced from *A. flavus* Grown on T2 Pretreated Sugarcane Bagasse

#### CONCLUSION

Generally, the pretreatment of sugar cane bagasse with tamarind extract at pH 2 (T1) yielded higher CMCase and FPase activities than pretreatment at pH 4 (T4). Tamarind extract pretreated sugarcane bagasse at pH 2/1% H<sub>2</sub>SO<sub>4</sub> (T2) enhanced the production of CMCase than FPase when compared with other pretreatments. Therefore, tamarind extract could be applied as a low cost pretreatment method of sugarcane bagasse and opens the window for possible application to other agro-waste biomass. Further studies on the chemical and morphological changes will give a better understanding of the mechanism of

pretreatment.

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