

Kinetic Analysis of Crude Enzyme Extract Produced Via Solid State Fermentation of Banana Pseudo Stem Waste

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Research Article

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Abstract

Banana Pseudo stem waste after each harvest contributes about 70–80 Milli Tons Per hectore. The banana pseudo stem will be thrown as waste biomass after each harvest as it is unstable for the upcoming harvest. The biggest challenge in banana cultivation is the utilization of biomass of banana pseudostem waste into valuable products. In this study, Xylano-pectinase enzyme extract was produced from banana pseudo stem waste under solid-state fermentation by *Enterobacter cloacae* PMC04. The highest pectinase and xylanase activities obtained using banana pseudo stem as carbon source were 124.62 U/ml and 173.81 U/ml respectively. Thermodynamics stated that range 40-50°C were considered to be the optimal temperature for xylano-pectinase enzyme production and subsequent degumming of banana fibers. The crude enzyme extract were then used in the degumming of banana fibers for textile application. Textile processing of banana fiber necessitates the removal of hemicellulose substance which can be achieved by crude xylano-pectinase enzyme. It was found that crude xylano-pectinase was efficient in the removal of hemicellulose substance from the fibers. Results obtained from this study demonstrate that the proposed bioprocess could be successfully applied for the degumming of banana fibers sustainably.

Introduction

Globally, One third of food produced for human consumption is wasted and contributes to food pollution. The occurrence of food waste can be from the initial agricultural production to the final household consumption. According to Food and Agriculture Organization (FAO) the estimation of food waste is approximately 1.3 billion tonnes every year all over the world. The anaerobic decomposition of food waste causes methane emissions which is 25 times more potent than carbon dioxide at trapping heat (Motelica et al., 2020). Therefore, proper incineration and utilization of waste resource is essential to reduce the food waste in the environment. India leads in banana production with 30 million tonnes per year with major cultivators in the southern states such as Tamilnadu, Maharashtra, Gujarat, Andhra Pradesh, and Karnataka. Waste biomass generated from the banana cultivation incorporates banana pseudo stem, peduncle, and other materials (Balakrishnan et al., 2019). Banana Pseudo stem waste after each harvest contributes about 70–80 Milli Tons Per hectore. The pseudo stem, linchpin of banana fiber will be hurled as waste after harvesting of banana since it is unstable for further cultivation. The biggest challenge in banana cultivation is the utilization of biomass of banana pseudostem waste into valuable products. In this research, banana pseudo stem is utilized as solid substrate for the production of pectinase and xylanase enzyme. The production of industrial enzymes using agricultural waste substrate could improve the global market value of industrial enzyme.

Pectinase is the enzyme used to break down the pectin molecules, polysaccharides found in the cell wall of plants and natural fibers. Pectinases catalyzes the pectin degradation process either by depolymerization or desulfation reactions. The occurrence of pectinase can be pectin methyl esterases, pectin acetyl esterases, polygalacturonic acid and rhamnogalacturon (Handa et al., 2016). The microbial source of pectinases are in large demand due to their ease of multiplication, maintenance under

controlled condition and cost-effective. Xylanase is a linear class of enzyme employed in the degradation of linear polysaccharide xylan into xylose. The xylan is the major component of hemicellulose which is about 30% of the plant cellwall (Cao et al., 1992).The major role of xylanases is the conversion of industrial agricultural waste into biomass which can be further used as animal feed or biofertilizers. Solid-state fermentation possesses a series of advantages such as higher enzyme yield, negligence of foam, contaminants, and catabolite repression, ecofriendly in downstream processing, low energy, and medium requirements. The choice of agricultural residual substrate is an indispensable strategy for industrial profitability with cost-effective biosynthesis (Zeng et al., 2017).

Considering all these aspects, our primary interest is focused on the conversion of banana pseudo stem waste to valuable products. The research highlights the utilization of banana pseudo stem as solid substrate for enzyme production and characterize the kinetic properties of crude enzymes produced. Therefore, aim of the research is to a) produce xylanase and pectinase by solid state fermentation b)kinetic study of crude xylanase and pectinase extracts c)application of crude enzyme in fiber degumming.

Materials And Methods

Microorganisms:

The microorganism was isolated from the banana waste decomposed soil in and around Tiruchirapalli. The isolated microorganism was subjected to 16s RNA sequencing and identified as *Enterobacter cloacae* PMC04.

Raw Materials:

The banana pseudo stem was collected from NRCB (National Research centre for Banana). The banana pseudo stem were sterilized at 121°C for 20 minutes. The sterilized banana pseudo stem waste was used as solid substrate for solid state fermentation.

Solid State Fermentation

Banana pseudo stem (dried) powder 30g was taken in 500ml of Erlenmeyer flask moistened with 70% solution containing (2g/L) each of K_2HPO_4 and KH_2PO_4 . The 10ml of xylano-pectinase bacterial culture were inoculated and incubated at 35°C for 48 hours. After incubation, 1g of the fermented substrate was evacuated and extracted using 10 ml of pH 5, 0.2M citrate buffer. Using the whatman filter, the extracted enzyme was filtered and then centrifuged. The enzyme supernatant was used to analyze the pectinase activity (Pagarra et al., 2019)

Xylano-Pectinase Asssay:

1ml of standard solution containing 0.1% pectin, 0.1% xylan was added with 2ml of sodium citrate buffer and 1ml of supernatant. The mixture was subjected to 25min incubation at 45°C. After incubation, DNS

was added as a stopper and subjected to a heating bath for 10min. The addition of 4ml distilled water to attain the final volume and OD was measured at 570nm. One unit of enzyme activity corresponds to the micromoles of glucose as reducing sugar molecules produced under the reaction condition per minute (Hidayah et al., 2020)

Thermal Deactivation of Crude Enzyme Extract

Thermal studies were experimented by incubating the crude enzyme extract at specific temperature in the absence of the substrate. The standard amount of enzyme extracts were taken in test tube and placed in waterbath. The temperature was varied from 30 to 70°C for 120 minutes.

Banana Fiber Degumming

Mechanically extracted banana pseudo stem fibers were subjected to enzymatic degumming. The crude enzyme extracted from solid-state fermentation by the isolate PMC04 was used for banana fiber degumming. The banana fibers were autoclaved for 20 minutes. The parameters observed for the degumming fiber treatment are degumming temperature, degumming time, and enzymatic dosage(Jiang et al., 2017). The degummed fiber was analyzed and compared with control fibers.

Results And Discussion

The banana pseudo stem has efficient carbon source to be used as substrate for solid state fermentation. The xylano-pectinase activities produced from the fermentation of banana pseudo stem waste were analyzed and shown in Fig. 1. Enzyme production from banana pseudo stem waste. The maximum pectinase and xylanase activity was observed at 12–16 hours. The highest pectinase and xylanase activities obtained using banana pseudo stem as carbon source were 124.62 U/ml and 173.81 U/ml respectively. From the result, it is inferred that banana pseudotem has the ability to provide essential nutrient for the growth of bacteria. The higher enzyme activities are due to the higher carbon source which is essential for the growth of the bacteria (Rahul et al., 2017).

Kinetic Studies on Crude Enzymes

It is essential to understand the kinetic behavior of crude enzyme extracts produced from banana pseudo stem waste before scaling up the bioprocess. Thermal deactivation is a major problem in enzymatic degumming of banana fibers. At the initial stage of kinetic process, the enzyme activities were identified as 124.62 U/ml and 173.81 U/ml for pectinase and xylanase respectively. Deactivation of the enzymes are measured as the ratio of enzyme activity at specific time (E) to enzyme activity at initial time (E_0). The temperature was varied from 30°C to 70°C to identify the thermal deactivation of xylanase and pectinase enzyme produced from solid state fermentation of banana pseudo stem waste. The thermal deactivation is expressed as exponential decay

$$E = E_0 \times e^{-kdt}$$

E is the enzyme activity at specific time, E_0 is the initial enzyme activity, k_d is the deactivation rate constant. It was found that k_d values for crude xylano-pectinase in the range of 40-50°C was higher compared to other temperatures. The k_d values for pectinase and xylanase enzyme was illustrated in the following Table 1.

Table 1
Deactivation rate constant for pectinase and xylanase

°C	K	K_d (hour-1) for pectinase	K_d (hour-1) for xylanase
30	303	0.0012	0.0135
35	308	0.0024	0.0170
40	313	0.0081	0.0208
45	318	0.0095	0.0286
50	323	0.0098	0.02741
55	328	0.0072	0.01554
60	333	0.0019	0.0100
65	338	0.0017	0.0087
70	343	0.0016	0.0060

The half-life ($t_{1/2}$) is the time when the enzyme activity decreases to half of its original value. The determination of half-life period is essential for the economic feasibility of banana pseudo stem waste reutilization. The longer shelf-life corresponds to the higher thermostability. The half-life can be calculated by replacing E with $E_0/2$. Table 2 illustrates the half-life period of pectinase and xylanase enzyme. The mathematical equation for half-life determination is given as

Table 2
Determination of Half-life

°C	K	t _{1/2} for pectinase (hours)	t _{1/2} for xylanase (hours)
30	303	45.22	51.33
35	308	38.19	40.764
40	313	28.64	33.317
45	318	12.89	37.258
50	323	12.67	39.8041
55	328	10.82	44.594
60	333	33.81	69.3
65	338	35.73	79.665
70	343	36.67	115.5

$$t_{1/2} = \ln(2) / K_d$$

The activation energy can be calculated from the Arrhenius equation. The Arrhenius equation can be written as

$$K_d = Ae^{-E_{ad}/RT}$$

E_{ad} is the activation energy, R is the universal gas constant and T is the absolute temperature. The activation energy for xylanase and pectinase enzyme when the temperature was in the range of 30 to 50°C is -10.314 KJ/mol and -23.52KJ/mol respectively. The activation energy increases with increase in temperature. When the temperature was in the range of 55 to 70°C, the activation energy was increased to 59.668 KJ/mol for xylanase enzyme and 47.36KJ/mol for pectinase enzyme.

The Eyring absolute rate equation can also be used to predict the thermodynamic data of pectinase and xylanase enzyme.

$$K_d = (k_B T/h) e^{(-\Delta H/RT)} e^{(\Delta S/R)}$$

$$\Delta H = E_{ad} - RT$$

In this equation, where

h is the planck constant of 6.63×10^{-34} J s,

k_B is the Boltzman constant of 1.38×10^{-23} J/K

T is the absolute temperature

ΔH is enthalpy of activation in KJ/mol

ΔS is entropy of activation in $J\ mol^{-1}K^{-1}$

ΔG is the free energy of activation

The enthalpy of activation can be calculated from the activation energies for denaturation, Further, free energy of activation can be determined from the following equation

$$\Delta G = -RT \ln \left[\frac{K_{dh}}{k_{BT}} \right]$$

$$\Delta S = (\Delta H - \Delta G) / T$$

The thermodynamic parameters of pectinase and xylanase enzyme was calculated and illustrated in the Table 3.

Table 3
Thermodynamic study of crude enzymes

Temperature (oC)	Pectinase			Xylanase		
	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS ($J\ mol^{-1}K^{-1}$)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS ($J\ mol^{-1}K^{-1}$)
30	10.812	56.67	-242.72	13.347	67.46	-266.689
35	10.729	61.92	-263.38	13.56	71.92	-277.532
40	11.591	74.34	-286.72	13.508	91.01	-333.923
45	11.599	44.89	-269.119	13.458	54.632	-214.119
50	11.452	48.19	-212.388	13.409	68.19	-252.628
55	45.961	40.38	-108.62	56.62	60.43	-11.615
60	45.890	40.57	15.39	56.668	67.665	33.024
65	45.182	40.81	16.311	56.71	67.108	30.763
70	45.52	40.114	16.99	56.753	67.154	30.3

Thermodynamics data states that when ΔH is positive, ΔS is negative, the process is non-spontaneous at any temperature and the reverse process is spontaneous. Therefore, deactivation rate of xylanase and pectinase could be reversible between 30 to 55°C. Further, on increasing temperature above 55°C, both ΔS and ΔH are found to be positive for both enzymes which indicates that deactivation of enzyme is

spontaneous at higher temperature from 60 to 75°C. Therefore, enzymes produced from banana pseudo stem waste are thermally stable.

Enzymatic degumming of banana fibers

The mechanically extracted banana fibers must undergo hemicellulose removal for textile application. The removal of hemicellulose substance can be done by enzymatic method. In this study, Banana fibers are treated with pectinase and xylanase enzymes which resulted in the release of galacturonide. The amount of galacturonide release for 12 hours with time interval of 1 hour was done to screen out the optimum time for fiber degumming. The degumming process was analyzed at 45°C with (15% pectinase, 15% xylanase) enzymatic dosage for 12 hours at an interval of 1 hour. The maximum galacturonide released (0.6mmol/ml) concerning fiber degumming time is identified to be maximum from 4 to 7 hours which is a relatively very period of treatment (Han et al., 2011).

SEM analysis of fibers

Scanning Electron Microscopy generates signal diversification at the outer layer of the solid specimen by high energy electrons with a focused beam. Pectin is found to be mostly entrapped in the mesial lamella of the cortical tissue. The SEM micrographs of the sample before and after treatment were analyzed. The majority of gummy substance removal can be visualized in the degummed banana fiber by 5–6 hours except other hemicellulosic substances. From the image, it is obvious that the degradation of pectin substance and removal of hemicellulosic substances in degummed banana fibers (Cheng et al., 2019). However, the minimum amount of hemicellulose substance may remain in the dislocation region of the degummed banana fibers (Mao et al., 2019). Figure 3 shows the SEM image of control and treated banana fiber.

Conclusion

The banana pseudo stem waste was used as substrate for the production of pectinase and xylanase by *Enterobacter cloacae PMC04*. The deactivation of crude enzymes could be reversible between 30 and 55°C. Further, deactivation of crude enzymes were spontaneous between 60-70°C. The results infer that combination of crude enzymes could be better strategy for degumming of banana fibers. The study clearly shows the crude pectinase and xylanase can be successfully produced from banana pseudo stem solid waste under solid state fermentation. The research also highlights an alternative approach of waste management to reduce the food waste problem.

Declarations

Author Contributions

Mira chares Subash: Conceptualization, Methodology, Data curation, Writing-Original draft preparation, Writing-Reviewing, and Editing

MuthiahPerumalsamy: Conceptualization, Methodology, Supervision, Writing-Reviewing, and Editing

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Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon request.

Ethical approval and consent to participate

This research work does not involve human participants, human data or human tissue. This research work was based on enzyme production from banana pseudostem waste.

Consent for Publication

This research work does not include any personal data in the form of videos, individual details, individual images. This work has not been submitted or published elsewhere.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

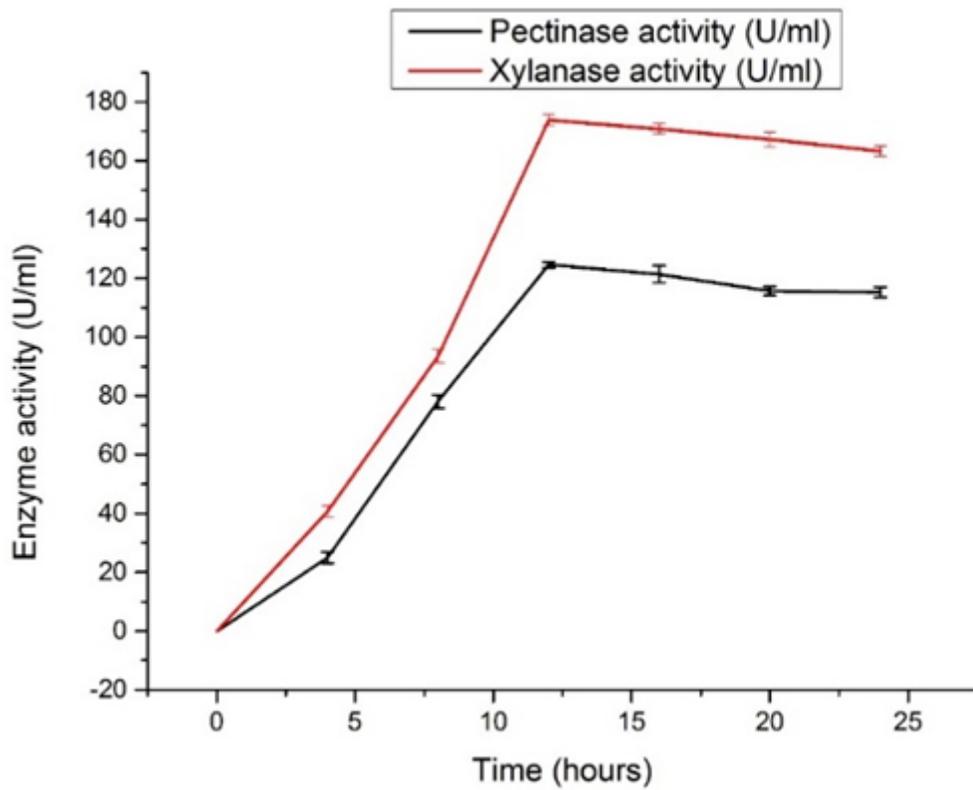


Figure 1

Enzyme production from banana pseudo stem waste.

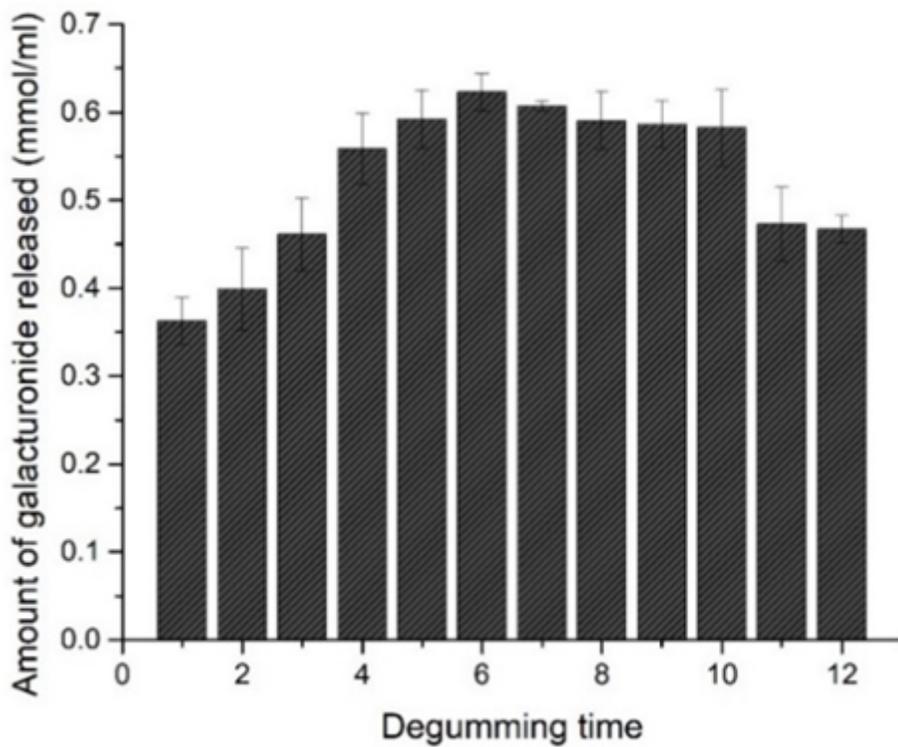


Figure 2

Degumming of banana fiber with crude xylano-pectinase enzyme

Control (Untreated fiber)

Degummed fiber

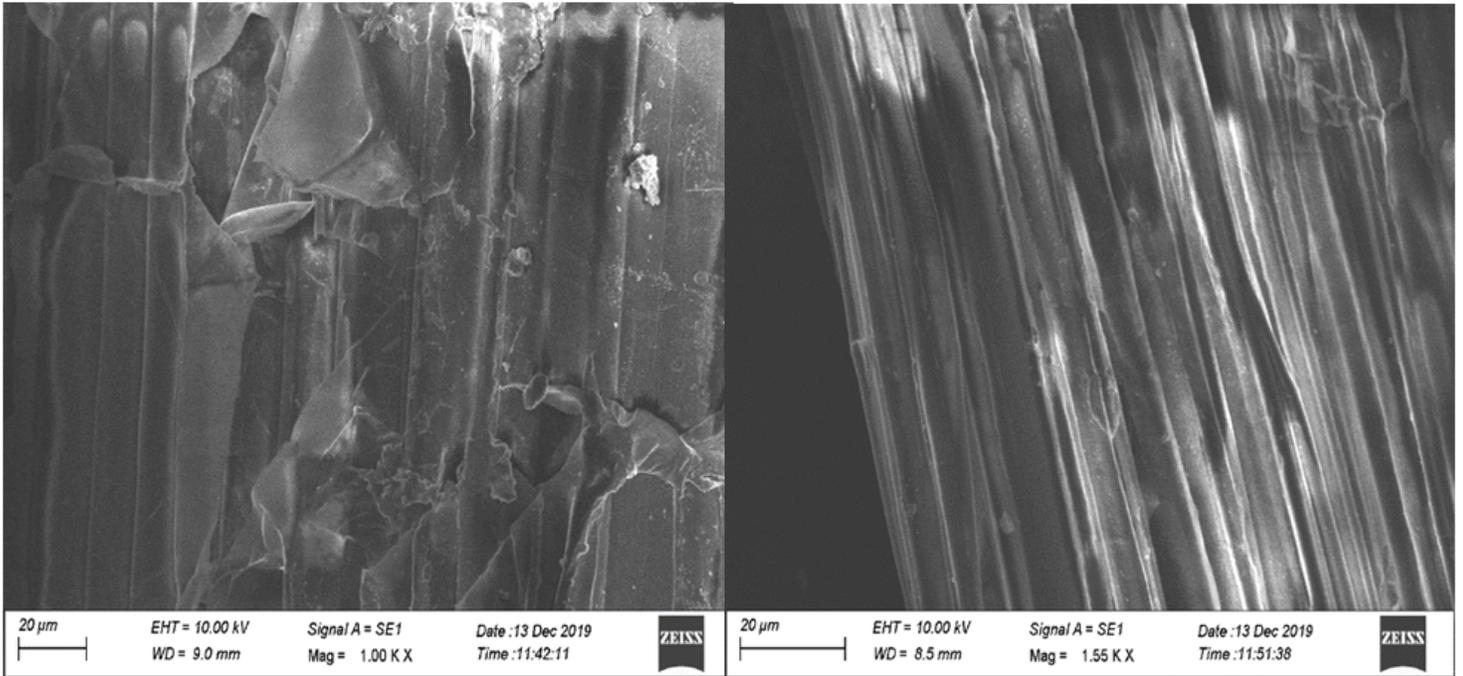


Figure 3

SEM image of degummed banana fiber