



# *Albizia lebbek* fruit pods as a substrate for the production of lignocellolytic enzymes by *Aspergillus niger*

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## ABSTRACT

*Aspergillus niger* isolated from the soil was investigated for its capability to produce various lignocellolytic enzymes, such as LiP, endoglucanase, FPase, xylanase by solid-state fermentation, using *Albizia lebbek* fruit pods as a substrate. The chemical composition of the fruit pods was studied, and the production pattern of the enzymes was examined by growing the fungi for 25 days. The LiP activity was low, whereas a good production of endoglucanase, FPase, and xylanase enzymes was noted. A dye decolorization capacity of the *A. niger* was also studied with Congo red. Therefore, *A. lebbek* fruit pods which are considered as waste and burnt off can be utilized for the production of holocellolytic enzymes using *A. niger*.

## 1. INTRODUCTION

Lignocellulose is the most abundant raw material on the earth. It refers to plant dry matter (biomass) and is called lignocellulosic biomass. It is made up of cellulose, hemicellulose (together called holocellulose which are carbohydrate polymers), and lignin (aromatic polymer). The cellulose and hemicellulose are tightly bound to lignin.

Lignocellulose biomass is classified into three types: virgin biomass, waste biomass, and energy crops. All terrestrial plants, such as trees, bushes, and grass are included under virgin biomass. Waste biomass from agriculture, such as sugarcane bagasse, corn stover, and straw, and from forestry (paper and saw mills) is included under waste biomass. Energy crops are those with a high yield of lignocellulose biomass that serves as a raw material for the production of second-generation biofuels (switch grass) [1–3]. There are reports that lignocellulosic biomass has an immense potential for the sustainable production of chemicals and fuels. It has ample carbon-neutral renewable source which reduces

CO<sub>2</sub> emissions and air pollution. It can be employed to produce biofuels, biomaterials, and biomolecules [4].

Fungi play a key role in degrading lignocellulose although bacterial degradation is also reported. Three groups of fungi are involved in lignocellulose degradation: soft-rot fungi, brown-rot fungi, and white-rot fungi [5]. The soft-rot fungi attack the polysaccharide component of wood but are capable of degrading lignin to some extent [6]. *Aspergillus* sp. is an example of soft-rot fungi [7]. The genus *Aspergillus* is a group of filamentous fungi with a large number of species. Fourteen different groups of *Aspergillus* were identified [8]. There are eight groups of black *Aspergillus* identified (*A. niger*, *A. tubingensis*, *A. heteromorphus*, *A. ellipticus*, *A. foetidus*, *A. carbonarius*, *A. japonicus*, and *A. aculeatus*) [9]. Of these, *A. niger* and *A. tubingensis* are important for industrial applications. The products from many of these species have obtained a generally regarded as safe status which makes them to be used in food and feed applications. A myriad of enzymes produced by *Aspergillus* are of major importance to the food and feed industry. A good fermentation capabilities and high level of protein secretion make them ideal organisms for industrial applications [10].

Many lignocellulosic materials such as sugarcane bagasse, corn stover, bran, rice straw, and wheat straw have been studied as

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lignocellulolytic enzyme inducers. Along with conventional crop biomass, invasive weeds could be used as lignocellulosic enzyme inducers [11]. Enormous natural lignocellulosic sources are utilized worldwide, but still huge quantities of lignocellulosic raw materials are not still exploited [12]. *Albizia lebeck* Benth., which is commonly called Indian siris or woman's tongue, is a deciduous tree whose parts have many medicinal values. The limited reports are available on the pods of the tree. They are not even grazed by cattle as they contain saponins. The fruits fall on the ground, and they are burnt off, which leads to environmental pollution. The fruit pods of *A. lebeck* are used as inducers for the production of lignocellulolytic enzymes by *A. niger* isolated from soil [13].

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

The fruit pods for the current study were collected from Manakondur village, Karimnagar, Telangana, washed, shade-dried cut into pieces, and made into coarse powder in a blender (1–2 mm).

### 2.2. Organism

The fungus *A. niger* was isolated from the soil in the laboratory [13].

### 2.3. Solid State Fermentation of Fruit Pods of *A. lebeck*

About 5 g of the *A. lebeck* fruit powder was moistened with 25 ml of distilled water (ALW) and basal medium, (ALBM), separately, inoculated with two mycelial discs (10 mm) per flask, and incubated at 30°C. A duplicate set of flasks was processed at 5, 10, 15, 20, and 25 days. Enzyme extraction [14] was done according to Arora *et al.* Sodium acetate buffer was added in each flask and kept on a shaker for 1 hour and filtered through a filter paper. The filtrate was then centrifuged at 10,000 rpm for 20 minutes, and the supernatant was used as the source of enzymes for assay and stored at 40°C.

### 2.4. Enzyme Assays

Lignin peroxidase assay was carried out using Azure B [15], and the absorbance was read at 651 nm. The xylanase assay was done according to the method of Bailey *et al.* [16], and endoglucanase and FPase activities were performed based on the methods proposed by Ghose *et al.* [17].

### 2.5. Analytical Methods

The estimation of various components in fruit pods was done by sequential fractionation [18,19]. About 1 g of substrate was suspended in 100 ml of deionized water and heated in a water bath set at 100°C for 2 hours and filtered through a tared crucible. The residue was dried at 90°C until constant weight. The loss was considered as water soluble part. The dried residue was resuspended in 0.5 M H<sub>2</sub>SO<sub>4</sub> and placed on a water bath maintained at 100°C for 2 hours and filtered, dried, and weighed. The loss was represented as hemicellulose. The residue was mixed with 72% H<sub>2</sub>SO<sub>4</sub> (10 ml) maintained at 30°C for 1 hour, thereafter diluted

**Table 1:** Biochemical constituents of *A. lebeck* fruit pods.

Biochemical constituents of <i>A. lebeck</i> fruit (g/Kg)	
Parameters	g/kg
Water solubles	83
Hemicellulose	260
Cellulose	360
Lignin	277

up to 4% H<sub>2</sub>SO<sub>4</sub>, and autoclaved at 121°C for 40 minutes. The contents were then filtered, dried, and weighed, and the loss was considered as cellulose and residue as lignin.

### 2.6. Congo Red Dye Transformation by *A. niger*

The Congo red decolorization in solid media was assessed by visual disappearance of color from the plate [19]. A dye decolorization in liquid media was measured spectrophotometrically. The decolorization rate was determined according to the following formula:

$$\text{Decolorization rate (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

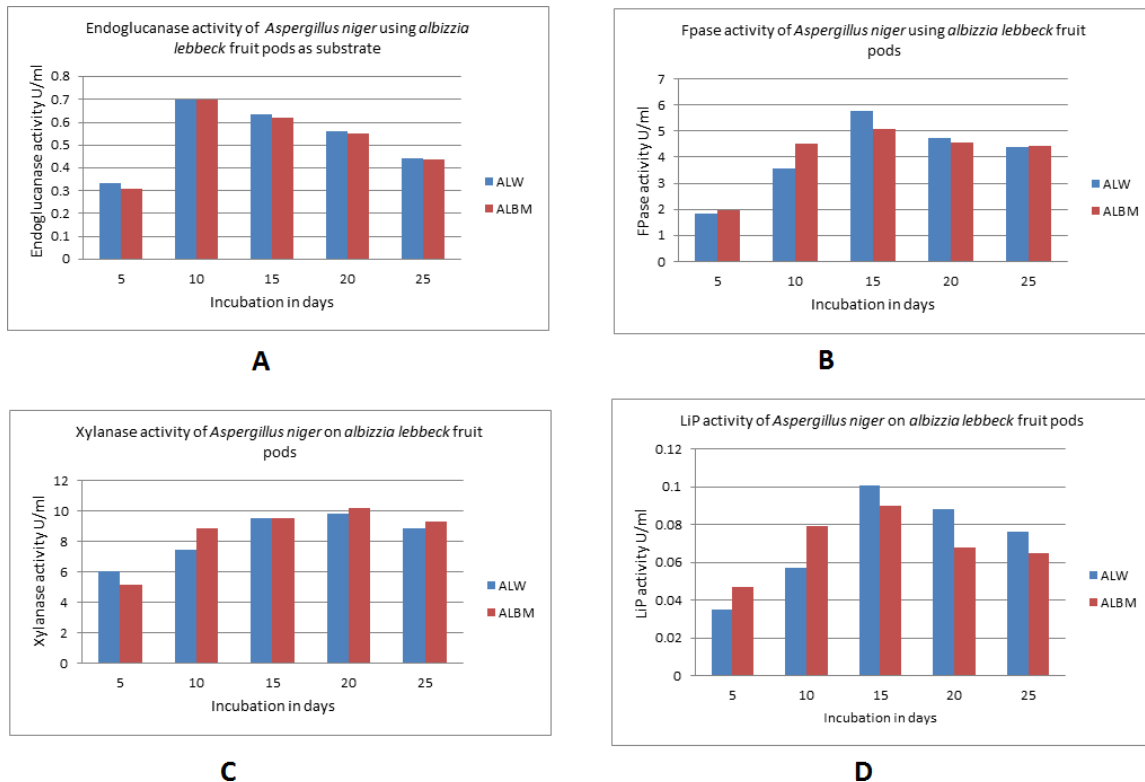
$A_0$  = Dye absorbance of control

$A_1$  = Dye absorbance after decolorization

## 3. RESULTS AND DISCUSSION

Fungi play an important role in lignocellulose degradation and are exploited for the production of the lignocellulose degrading enzymes on a large scale for their use in industry. They grow on a solid substrate with low moisture content (solid-state fermentation). A complex set of enzymes is involved in lignocellulose degradation such as ligninases, cellulases, and hemicellulases. In the present investigation, *A. niger*, a soft-rot fungus, was exploited for its ability to utilize lignocellulosic biomass of *A. lebeck* fruit pods. The fungi were isolated from degrading fruit litter in a previous work in the laboratory and tested qualitatively for the release of various lignocellulolytic enzymes [13]. *A. lebeck* fruit pods were collected, washed, shade dried, cut in to pieces, and ground to powder in a blender and sieved (1–2 mm). A solid-state fermentation of the fruit substrate was carried out in two experimental setups by moistening fruit powder with ALW and ALB. SSF was preferred as it is the attractive means to produce enzymes at low investment and operating cost and requires simple equipment, and up on all, the fungi can penetrate deep in to substrate [20,21]. Biochemical constituents of *A. lebeck* pods were done [22] and presented in Table 1.

*Aspergillus niger* was studied for its potential to produce lignocellulolytic enzymes such as LiP, endoglucanase, FPase, and xylanase. LiP breaks down lignin, whereas endoglucanase and FPase are involved in the hydrolysis of cellulose and xylanase in hemicellulose degradation. A time course study of SSF of *A. lebeck* fruit powder (ALW and ALB in duplicates) was carried out for 25 days and analyzed for the enzymes at an interval of 5 days.



**Figure 1:** (A) Endoglucanase activity of *A. niger* on *A. lebeck* fruit pods, (B) FPase activity of *A. niger* using *A. lebeck* fruit pods, (C) Xylanase activity of *A. niger* using *A. lebeck* fruit pods, (D): LiP activity of *A. niger* on *A. lebeck* fruit pods.

Endoglucanase activity of diluted extract was highest on the 10th day of incubation in both ALW and ALB (0.7 and 0.698  $\mu\text{mol}/\text{minutes}/\text{ml}$ , respectively) and gradually decreased with incubation time. Both ALW and ALB exhibited the same pattern of endoglucanase activity during the 25-day fermentation (Fig. 1A). FPase activity was maximum on the 15th day of incubation both in ALW (6.77 U/ml) and ALB (5.09 U/ml), and thereafter, it decreased gradually till 25th day of incubation (Fig. 1B). It was noted that the xylanase activity of ALW increased till 15th day of incubation and that of ALB till 20th day of incubation with the maximum xylanase activity of 9.55 U/ml (15th day) for ALW and 10.15 U/ml (20th day) for ALB (Fig. 1C). Cellulolytic activities were studied with various other substrates. The studies on rice straw degradation with *A. niger* revealed 130 U/gds of cellulase activity [23]. Soybean hulls were used as a substrate, and 3.2 U/ml of cellulase activity and 248.9 U/ml of xylanase activity were reported [24]. When *Prosopis juliflora* pods [25] were used, the cellulase activity of 1825.32 U/l and xylanase activity of 311.24 U/l was noted using *Trichoderma reesei* NCIM 1186. Endoglucanase activity of 3.42 U/g and FPase activity of 1.77 U/g by *Aspergillus niger* was reported when coir waste was used as a substrate [26]. The studies on FPase and endoglucanase production by *A. niger* using wheat bran, rice bran, groundnut powder, and saw dust were reported [27]. FPase activity of 2.9, 2.09, and 1.62 FPU/g for wheat bran, groundnut fodder, and rice bran, respectively, was observed in the same study. The highest titers of endoglucanase were recorded for wheat bran (3.24 U/g) and 1.36 U/g by groundnut fodder and 1.09 U/g by rice bran [27]. There are reports on xylanase activity of *A. niger* using sugarcane bagasse (110  $\text{U ml}^{-1}$ ) and cassava

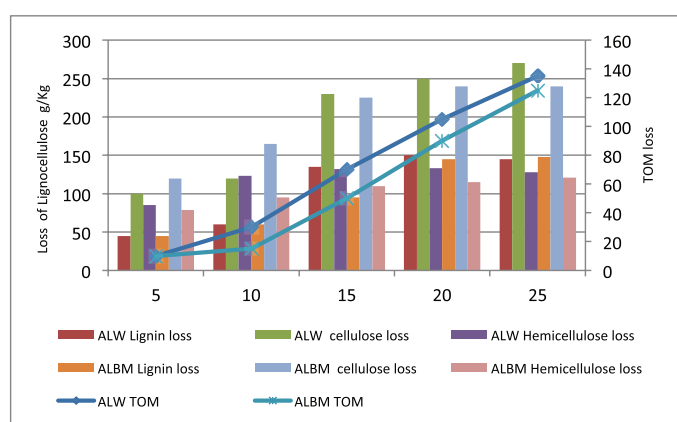
bagasse (more than 160  $\text{U ml}^{-1}$ ) [28]. It is arduous to compare the cellulolytic activities reported with other substrates as the authors demonstrated the activity in different units.

There are no reports on LiP activity by *A. niger*. In the present study, LiP activity was noted (Fig. 1D). Attempt to perform LiP activity was made as the fungi decolorized Azure B while qualitative screening was done in a previous study [13]. The LiP (0.344 U/ml for ALW and 0.306 U/ml for ALB) production was very low when compared to LiP produced by white-rot fungi such as *Phanerochaete chrysosporium* which produced 1.80 U/ml using the same *A. lebeck* fruit pod as a substrate [29]. The study on LiP production using *Achras zapota* [7] was reported to be 2,100 U/ml. The degradation of Azure B in the qualitative studies may be attributed to other lignin degrading enzymes (manganese peroxidase, versatile peroxidase, or dye-decolorizing peroxidase), which are not covered under the purview of this study.

There is an increase in the water solubles with incubation period which indicates the degradation of lignocellulosic components into low molecular weight components. A loss in total organic matter (TOM), cellulose, hemicellulose, and lignin, and water solubles was analyzed according to the method of Datta [17] (Table 2). A comparison between TOM loss and loss in cellulose, hemicellulose, and lignin is shown in Figure 2. A strong correlation existed between TOM loss and lignin, cellulose, and hemicellulose loss. A strong positive correlation of 0.921 (ALW) and 0.964 (ALB) was noted between lignin loss and TOM loss. A correlation of 0.959 (ALW) and 0.863 (ALB) was observed between cellulose loss and TOM loss. Hemicellulose loss and TOM loss when compared had shown a correlation of 0.713

**Table 2:** Variations in *A. lebeck* fruit composition during fermentation for 25 days.

S. no	Parameters	Days of incubation				
		5	10	15	20	25
a1	ALW					
	TOM	10	30	70	105	135
	Water solubles	65	55	65	60	80
	Hemicellulose	85	123	132	133	128
	Cellulose	100	120	230	250	270
2	ALB					
	Lignin loss	45	60	135	150	145
	TOM	10	15	50	90	125
	Water solubles	60	55	70	65	90
	Hemicellulose	79	95	110	115	121
	Cellulose	120	165	225	240	240
	Lignin loss	45	60	95	145	148



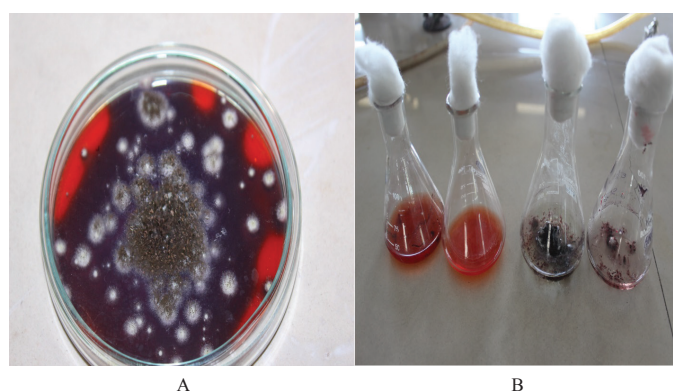
**Figure 2:** Comparison of TOM loss and lignin, cellulose, and hemicellulose loss.

(ALW) and 0.905 (ALB). It can be noted that lignin, cellulose, and hemicellulose loss contributed to TOM loss. Although LiP activity was low in *A. niger*, it degraded lignin efficiently, which indicates that other ligninolytic enzymes (MnP, Vp, etc.) may be responsible for lignin degradation. On the whole, *A. niger* exhibited a good cellulase and xylanase activity using *A. lebeck* fruit pods as a substrate.

The Congo red decolorization by *A. niger* was preliminarily evaluated in solid media. Decolorization was determined as the disappearance of the red color during fungal growth (Fig. 3A). The complete disappearance of the red color was achieved on the 6th day of fungal growth. Decolorization in liquid media was performed using Czapek–Dox medium. The total removal of dye was attained after 7 days of incubation (Fig. 3B). The decolorization capacity of the organism may be due to dye- decolorizing peroxidases, a new family of heme peroxidases. These enzymes have been demonstrated to perform lignin degradation [30].

#### 4. CONCLUSION

*Albizia lebeck* fruit pods used in the current study can be an attractive carbon source for enzyme production under solid-state fermentation by *A. niger*. Further, the pods can be subjected to pretreatment with acid or alkali and analyze the production of the enzymes.



**Figure 3:** (A) Decolorization of Congo red on solid media and (B) liquid media.

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#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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