0273-2289/04/118/0073/$25.00

Enzyme Production and Profile by *Aspergillus niger* During Solid Substrate Fermentation Using Palm Kernel Cake as Substrate

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Received April 9, 2003; Revised September 12, 2003; Accepted September 18, 2003

Abstract

The oil palm sector is one of the major plantation industries in Malaysia. Palm kernel cake is a byproduct of extracted palm kernel oil. Mostly palm kernel cake is wasted or is mixed with other nutrients and used as animal feed, especially for ruminant animals. Recently, palm kernel cake has been identified as an important ingredient for the formulation of animal feed, and it is also exported especially to Europe, South Korea, and Japan. It can barely be consumed by nonruminant (monogastric) animals owing to the high percentages of hemicellulose and cellulose contents. Palm kernel cake must undergo suitable pretreatment in order to decrease the percentage of hemicellulose and cellulose. One of the methods employed in this study is fermentation with microorganisms, particularly fungi, to partially degrade the hemicellulose and cellulose content. This work focused on the production of enzymes by *Aspergillus niger* and profiling using palm kernel cake as carbon source.

Index Entries: Palm kernel cake; hemicellulose; solid-state fermentation; *Aspergillus niger*; mannanase.

Introduction

Palm kernel cake is the solid residue left from the extraction of oil from palm kernel; that is, it is the byproduct of kernel oil extraction. It can be

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obtained by either solvent or mechanical extraction. Palm kernel cake that is extracted using solvents is dry, gritty, and unpalatable to animals *(1)*. The commonly practiced extraction of palm kernel cake in Malaysia is done using a mechanical extraction method, and palm kernel cake has been known to be an important ingredient for the formulation of animal feeds *(2,3)*. As an excellent ruminants feed, palm kernel cake was also reported to be suitable for use in feed formulation for swine, poultry, and horses *(1–4)*. Palm kernel cake is generally used as feeding material for dairy cattle feeding in both Malaysia and Europe, but only a little is incorporated into pig and poultry rations, owing to its relatively high fiber content and lack of information on its practical utilization in these two species *(1,5,6)*. The high concentrations of hemicellulose (57.8% mannan, 3.7% xylan) and cellulose (11.6%) in palm kernel cake make it difficult to digest for poultry if only palm kernel cake is used as their diet. Therefore, palm kernel cake has to be pretreated using either chemical methods (acid or alkali hydrolysis) or biologic methods (biodegradation or enzymatic hydrolysis).

Aspergillus niger, a filamentous fungus, is one of the most used organisms in the industrial production of fermented foods, organic acids, and enzymes *(7–9)*. The use of filamentous fungi for the production of commercially important products has increased rapidly over the past half century, and the production of enzymes in submerged fermentation has been established *(10)*. Solid-state fermentation (SSF) systems have generated much interest in recent years because they offer several economical and practical advantages, including higher product concentration, improved product recovery, very simple cultivation equipment, reduced wastewater output, lower capital investment, and lower plant operation costs *(11,12)*. SSF systems have also been reported to be an effective way to produce enzymes *(10,13–15)*. The present work concentrated on the enzyme profile during SSF by *A. niger* in an SSF system.

Materials and Methods

Microorganism

A. niger was obtained from MARDI Culture Collection, Livestock Research Center, Malaysia Agricultural Research and Development Institute. Potato dextrose agar slants were used for sporulation and storage. A suspension of 1×10^8 spores/mL was prepared in sterile 0.1% Tween-80 as an inoculum.

Pregerminated A. niger

A. niger (1×10^7 spore/mL) was grown in potato dextrose broth in a 250-mL Erlenmeyer flask at 30°C agitated at 150 rpm. The inoculum used was 1 d old, and the diameter of the mycelium pellet of pregerminated *A. niger* was roughly <1 mm.

SSF of Palm Kernel Cake

Ten grams of palm kernel cake was mixed with a 50% solution of 1% urea in a 250-mL Erlenmeyer flask stoppered with a cotton plug. The mixture was sterilized at 125°C for 15 min. After cooling to room temperature, palm kernel cake was inoculated with a 10% spore suspension (1×10^7 spores/mL) or pregerminated *A. niger* and cultivated at 30°C. Sampling was done every 24 h for 10 d of fermentation time by removing a single flask from the incubator.

Extraction of Crude Enzyme

Two extraction conditions were employed: (1) agitation at 130 rpm, rotating shaking (Certmat R; B. Braun, Melsungen, Germany) for 4 h at 30°C; and (2) agitation at 150 rpm, rotating shaking (Certmat R; B. Braun) for 24 h at 10°C with 50 mL of distilled water. Solids were removed by filtration using Whatman filter paper no. 1. The clear filtrate was kept at –20°C for the enzyme assays.

Enzyme Assays

Cellulase activities were measured by determination of filter paper assay (FPase), carboxymethycellulase (CMCase), and β-D-glucosidase activities *(16)*. Amylase activity was assayed by a modified procedure based on a method of Mulimani et al. *(17)*. Xylanase activity was determined according to the method of Bailey et al. *(18)* using 1% xylan as the substrate. Mannanse activity was determined according to George et al. *(19)* using a 0.5% solution of locust bean galactomannan as the substrate. Reducing sugar was determined by the dinitrosalicylic acid method using maltose as the standard *(20)*. Polygalacturonase activity was assayed by a modified procedure based on a method of Zheng and Shetty *(21)*. Phytase activity was determined according to El-Batal and Abdel Karem *(12)* by using sodium phytate as the substrate and measuring spectrophotometerically the inorganic phosphorus released using the Taussky-Schoor reagent as described by Harland and Harland *(22)*.

Protein and Total Reducing Sugar

Protein concentration was determined according to the method of Bradford *(23)*, using bovine serum albumin as the standard. Total reducing sugar was determined by the method of Miller *(20)*.

Results and Discussion

The fungus was grown on palm kernel cake for 10 d, and the highest production (2605.49 U/g extracted at 10°C by spore suspension as inoculum) of enzymes was around d 8, as shown in Table 1; the concentration of soluble protein was also obtained on d 8 (data not shown). The type of inoculum was found to affect the production of enzymes *(10,24,25)*.

Table 1
Total Enzyme Production and Mannanase Activity at d 8 by *A. niger* With Different Extraction Temperatures and Different Types of Inoculum

	Spore suspension as inoculum		Pregerminated *A. niger* as inoculum	
	10°C	30°C	10°C	30°C
Total enzyme activities (U/g)	2605	1894	1058	1436
Mannanase activity (U/g)	2251	1781	928	1231

The total production of enzymes (CMCase, FPase, β-glucosidase, polygalacturonase, xylanase, mannanase, amylase, and phytase) was much higher (2605 U/g extracted at 10°C and 1894 U/g extracted at 30°C) in fermentation using spore suspension as inoculum compared to pregerminated *A. niger* as inoculum. The extraction temperature was also found to affect the productivity of the enzymes, and the correlation was inversely proportional *(26–29)*. The overall enzyme activities were further improved when the extraction was done at 10°C (2.46-fold) in comparison with 30°C.

Table 1 also shows the production of mannanase by different types of inoculum and different extraction temperatures. For the first 2 d of fermentation, the production of mannanase was significantly low (data not shown). The types of inoculum affect the production of enzymes. Papagianni et al. *(10)* reported that young inoculum was not sufficient for SSF for the production of phytase by *A. niger*, and larger pellets also did not lead to successful SSF; the solid substrate was well interwoven with mycelia biomass when inoculation was by fine pellets or a mixture of fine pellets. In the present study, it was observed that the apparent color of palm kernel cake changed from dark brownish to light brownish with the growth of *A. niger* when inoculation was done by spore suspension, within 48 h after inoculation. It was found that using spore suspension as inoculum gave higher enzyme activities (2251 and 1781 U/g extracted at 10 and 30°C, respectively) in comparison to pregerminated (928.10 and 1231.10 U/g extracted at 10 and 30°C, respectively) mycelia. Spore suspension as inoculum gave higher enzyme activities because of the morphology of the growth of the fungus was totally different from that using pregerminated *A. niger* as inoculum. After 24 h of inoculation of spore suspension, a mat of mycelia was formed on the surface of the palm kernel cake and penetrated into the substrate. This can give a better surface area for the fungus to contact with the substrate and produce more enzymes for digestion of the fibers. As for pregerminated *A. niger*, the morphology was totally different: the mycelia were formed scattered throughout the substrate after 24 h of inoculation. The mycelial mat can be observed after 2 d of inoculation. In addition, the pregerminated *A. niger* needs to adapt itself to the changes in the environ-

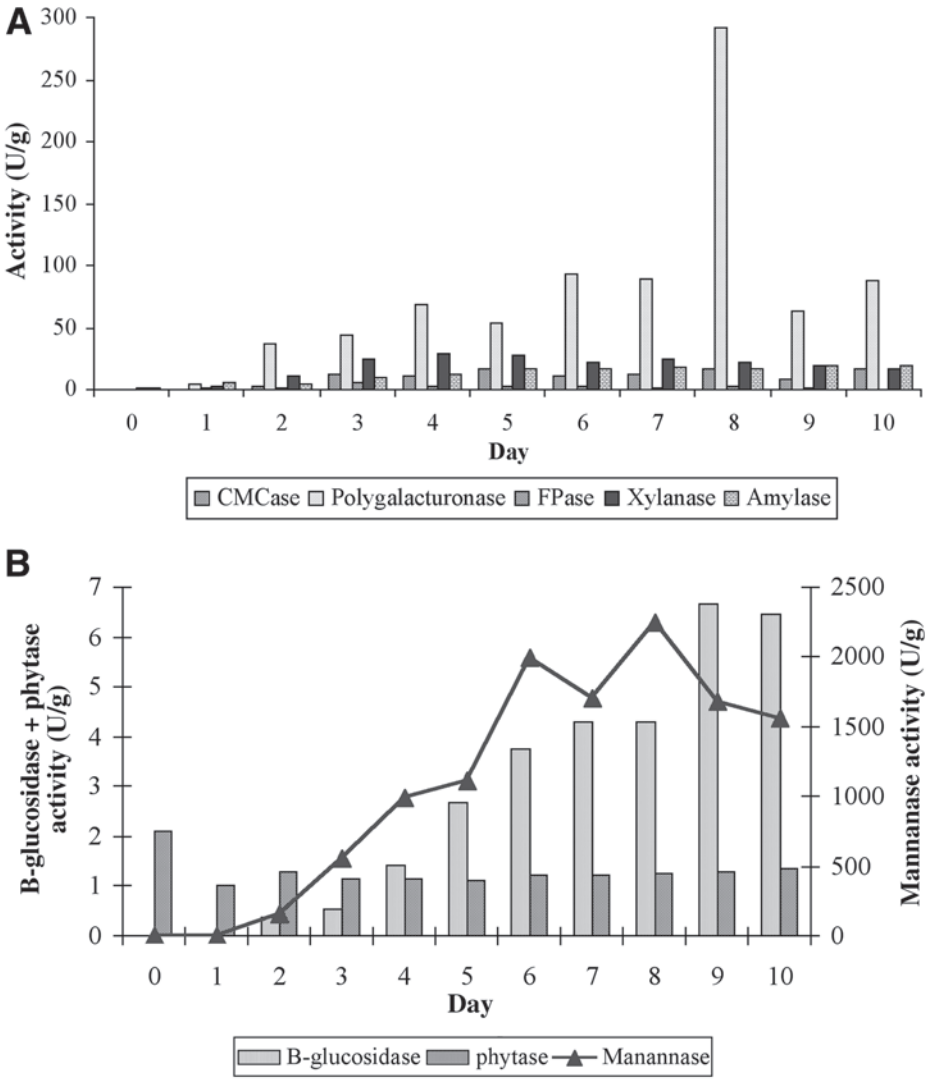

Fig. 1. **(A)** Enzyme profiling by spore suspension and extracted at 10°C, 150 rpm (rotating agitation), 24 h for CMCase, polygalacturonase, FPase, xylanase, and amylase; **(B)** enzyme profiling by spore suspension and extracted at 10°C, 150 rpm (rotating agitation), 24 h for β-glucosidase, phytase, and mannanase.

ment, i.e., from liquid culture to solid state. Therefore, the production of enzymes is slightly low as compared to spore suspension as inoculum.

From the eight enzymes that were screened and profiled, mannanase was the most abundant enzyme produced by *A. niger* during the SSF (i.e., 2252 U/g at d 8) (Fig. 1). This is owing to the fact that the palm kernel cake cell wall is composed of mainly linear and highly crystalline mannan and a small quantity of galactomannan *(30)*. According to Noraini et al. *(31)*, *A. niger* has the highest mannanase activity, 41.91 U/mL, in liquid

culture. SSF systems also have been reported to be an effective way to produce enzymes *(10,13–15)*. Polygalacturonase was the second highest enzyme (292.92 U/g at d 8) produced by *A. niger* extracted at 10°C, as shown in Fig. 1A.

Conclusion

A. niger was found to be able to grow on the cheap byproduct of the oil palm industry—palm kernel cake. Mannanase is the most abundant enzyme that was produced by *A. niger*. Spore suspension gave a higher enzyme activity in comparison to pregerminated *A. niger* and was used as inoculum for further experiments. The extraction temperature (i.e., 10 and 30°C) affected the extractability of enzymes.

Acknowledgment

We acknowledge financial support from the National Biotechnology Directorate of Malaysia.

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