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Hydrolysis of Empty Fruit Bunch of Oil Palm Using Cellulolytic Enzymes from *Aspergillus terreus* IMI 28243

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Abstract Hydrolysis of EFB (empty fruit bunch) derived from oil palm was studied using crude enzyme from *Aspergillus terreus* IMI 282743 along with commercial enzymes from *Trichoderma reesei* and *Aspergillus niger.* Hydrolysis at 40°C and 50°C with α-cellulose or EFB gave significantly lower yield when commercial enzymes of *T. reesei* and *A. niger* were used and the hydrolysis time extended beyond 10 h. After 24 h of hydrolysis at 40°C and 50°C, the filter paper activity (Fpase) from *A. terreus* retained as much activity as *A. niger* and it was significantly higher than *T. reesei.* Glucose concentration of 0.25% and 0.5% caused significant inhibition in the crude enzyme, but in regards to the commercial enzymes it only showed a slight effect. Crude enzymes from *A. terreus* could produce the highest reducing sugars when compared to commercial enzymes from *T. reesei* or *A. niger.* Nevertheless, low yield of sugar was observed for EFB for all treatments.

Key words: Empty fruit bunch, cellullolytic enzymes, *Aspergillus terreus*

Malaysia is one of the leading producers of palm oil. In 1997, there was a total area of 2.74 million hectares of land under oil palm [2] with an yield of 19.10 tons per hectare. Crude palm oil is obtained from mesocarp of the fruits and, depending on the variety and age of the palm, the oil for bunch ratio is within the range of 25-28% [1]. Consequently, there is an abundance of empty fruit bunch which could affect the environment if it is not managed properly.

Analysis by the Forest Research Institute Malaysia has shown that empty fruit bunch (EFB) is composed of 68.3% holocellulose; 41.9% α-cellulose; 20.3% pentosan; 13.2% lignin, and 3.6% ash. Thus, EFB is a potential source of cellulose that could be used as the substrate in an enzymatic hydrolysis for the production of fermentable sugars and other products [19, 9].

In this study, EFB was used as a viable cellulosic substrate for hydrolysis with the use of crude and commercial enzyme at two different temperatures (40° and 50°C). An addition of glucose was also studied to recognize the inhibitory effect on the enzymatic hydrolysis in the presence of reducing sugar products.

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Materials and Methods

Microorganisms

A. terreus IMI282743 was identified as a local isolate [22] and maintained on potato dextrose agar (PDA) slant at an ambient temperature. The fungus was subcultured monthly to maintain stability.

Shake Flask Cultivation

The organism was subcultured on a PDA slant for 4 days at 30°C. Spores at a concentration of 10^7 were used to inoculate 250 ml enriched Mandels medium using α-cellulose (α-cellulose fiber, approximately 99.5%, Sigma Chemical Co.) as the carbon source at 1% concentration [15]. Shake flask cultures were carried out in 1 l conical flasks in a Gallenkamp orbital shaker at a speed of 150 rpm at 30°C.

Crude Enzyme

Crude enzyme was prepared by harvesting a 6 days old culture through centrifugation at 10,000 ×g for 15 min using Sorvall RC5B of the refrigerated superspeed centrifuge. The supernatant was then concentrated at 1:5 with Amicon's CH2 SP3 Hollow fibre concentrator. Commercial enzymes, *T. reesei* (celluclast), was obtained from Novo Industries A/S, Denmark and *A. niger* was from Calbiochem-Behring corp., and they were prepared in a standard assay buffer solution and desalted by passing through Sephadex G-25 before use.

Hydrolysis

The empty fruit bunch (EFB) was dried in an oven (60°C) for a day and the size was reduced by using the commercial Waring blender. Hydrolysis was carried out in a conical flask (250 ml) containing 1% of substrate and cellulase (10 U Fpase) in 100 ml 50 mM citrate buffer (pH 4.8) with sodium azide (0.01%). The flask was then shaken at 150 rpm in a Gallenkamp Orbital Shaker at 40°C or 50°C. After 24 h, the samples were determined for Fpase activity, reducing sugars, and percentage of saccharification. The percentage of saccharification [13] was estimated as equivalent reducing sugars (mg/ml) x 0.9 x 100/substrate (mg/ml). Likewise, the percentage of Fpase activity [5] after 24 h was equal to Fpase (U/ml) after 24 h/Fpase activity at time 0 x 100.

Analysis

Fpase activity was determined according to the method of Mandels *et al.* [14] using a strip of 50 mg Whatman no.1 as substrate. One U (unit) was defined as 1 µmol of glucose equivalent released as quantitated by Somogyi-Nelson [18]. The glucose released was determined by using a glucose diagnostic kit no. 510 (Sigma Chemical Co.)

RESULTS AND DISCUSSION

The glucose and reducing sugars produced during the hydrolysis showed a similar profile (Figs. 1 and 2) in which most of the sugars are produced in a period of approximately 4 to 8 h and stabilized after 24 h, except for hydrolysis by crude enzymes of *A. terreus* which was still progressing significantly for 48 h. Crude preparations yielded higher hydrolysis compared to pure enzyme where the rate of hydrolysis is low, as explained by Matsuno *et al.* [16] and Tanaka *et al.* [20] due to its limitation of synergistic activity.

When crude enzymes were used on α-cellulose as the substrate, the glucose content was shown to be 2.66 mg/ml which amounted to 97.5% of the total reducing sugars produced. Similarly, when EFB (empty fruit bunch) was used, the glucose content was 97.1%. In contrast with commercial enzyme from *T. reesei*, glucose production represented only 53.2% of the reducing sugar when α-cellulose was used and 52.6% when used with EFB. The reason for this might be due to the low β-glucosidase activity in *T. reesei* [17] and Celluclast from *T. ressei*, which has also been reported to have a low β-glucosidase activity [4]. A low β-glucosidase activity accumulates cellobiose, an intermediate of cellulose hydolysis which is an inhibitor of the cellulases [3, 7]. On the other hand, some enzyme activity could be lost due to the adsorption onto the undigested substrate [16]. However, since the substrate used is only 1%, the loss may not be a main

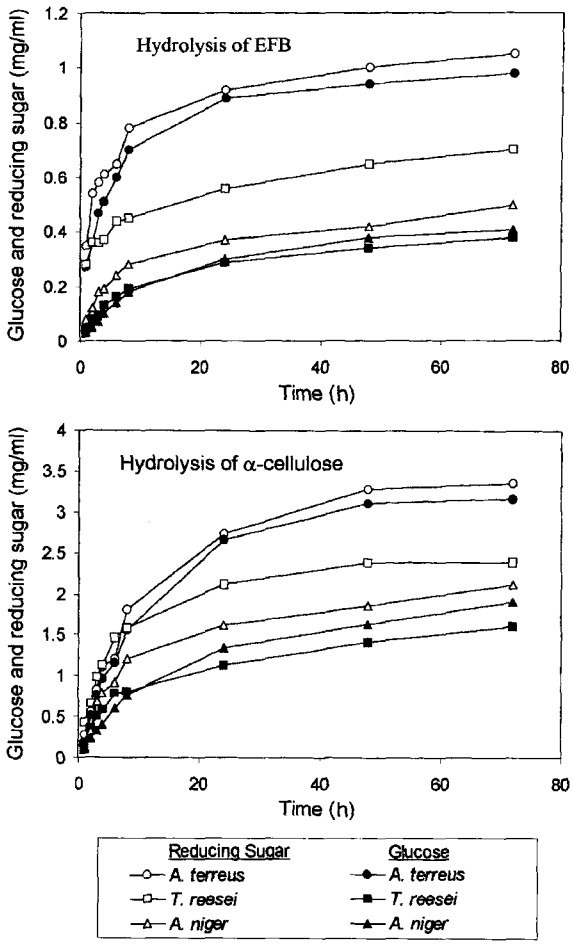

Fig. 1. Hydrolysis of EFB and α-cellulose at 50°C using crude enzyme of *A. terreus*, and commercial enzymes of *T. reesei* and *A. niger*.

contributing factor. The use of commercial enzyme from *A. niger* on α-cellulose was able to produce 82.2% of glucose from the total reducing sugar and 80.3% from EFB within 24 h.

The remaining filter paper activity was higher at 40°C as compared to 50°C (Table 1). Although sugar production was slighty higher at 50°C for *A. terreus* and *A. niger*, FPase from *A. terreus* retained as much activity as *A. niger* and showed signs of being significantly higher than *T. reesei*, suggesting a better stability for the enzymes. MacKenzie *et al.* [11] have reported a 50% reduction in the filter paper activity (FPase) in regards to enzymes of *T. reesei* when used beyond 24 h at 50°C. The temperature of 40°C has been shown to be optimal for Celluclast [5].

The effect of glucose addition on the production of reducing sugar (0.1%, 0.25%, and 0.5%) can be seen in Table 2. In all cases, the effect of glucose addition was observed to be pronounced only after 8 h of hydrolysis and was dependant on the concentration of glucose used. The time lapse of the reaction might be caused by the delay

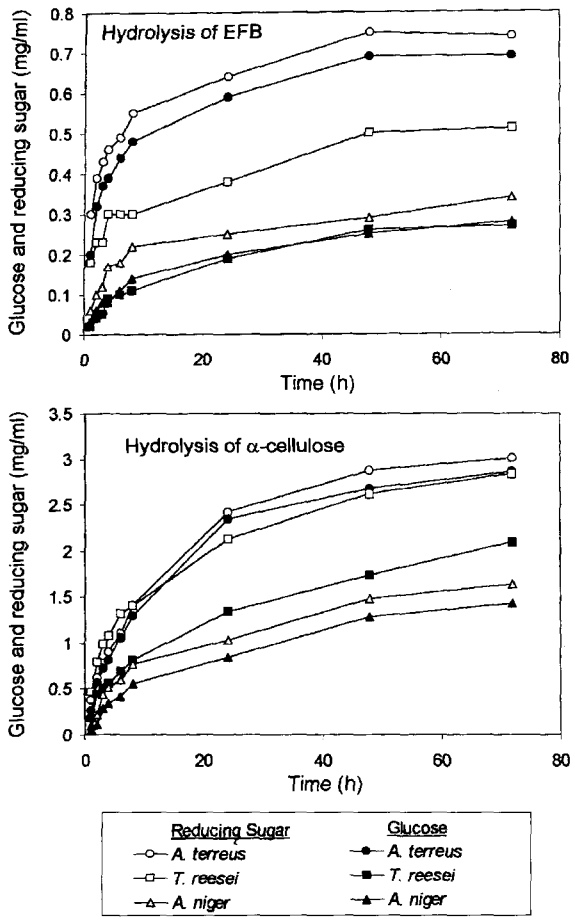

Fig. 2. Hydrolysis of EFB and α-cellulose at 40°C using crude enzyme of *A. terreus*, and commercial enzymes of *T. reesei* and *A. niger*.

Table 1. The effect of temperature on the percentage of Filter paper activity and the production of reducing sugars after 24 h hydrolysis.

Hydrolysis temperature	Enzyme source	% of Fpase activity	Reducing sugar production (mg/ml)	
			α-cellulose	EFB
40°C	*A. terreus*	83.3	2.43	0.64
	T. reesei	56.2	2.13	0.39
	A. niger	87.6	1.03	0.25
50°C	*A. terreus*	66.7	2.73	0.92
	T. reesei	31.2	2.12	0.56
	A. niger	69.2	1.63	0.37

Table 2. The effect of glucose addition after 24 h hydrolysis on the percentage of reducing sugar production.

Enzyme	α-Cellulose			EFB		
	Glucose conc. (%)			Glucose conc. (%)		
	0.1%	0.25%	0.5%	0.1%	0.25%	0.5%
Crude enzymes of *A. terreus*	98.3%	64.4%	30.5%	96.7%	68.7%	45.0%
Commercial enzymes of *T. reesei*	83.8%	75.0%	72.0%	82.0%	70.8%	67.7%
Commercial enzymes of *A. niger*	97.8%	80.2%	78.3%	89.0%	77.8%	75.1%

effect on the enzyme that brought about changes in the structure of the substrate which make it more susceptible to inhibition [10, 16]. Howell and Mangat [8] has reported that the effect of end-product inhibition is active after 8–12 h of hydrolysis. Glucose not only inhibits hydrolysis, but also promotes desorption by specifically binding to cellulase [16].

When α-cellulose was used with crude enzyme and the commercial enzymes from *T. reesei* and *A. niger*, the percentage of saccharification (Table 3) was found to be 24.58, 19.09, and 14.67, respectively, while for EFB it was 8.25, 5.00, and 3.30, respectively. The presence of 10.5% lignin in EFB might be the reason for the low percentage of saccharification [12]. The addition of 0.1% glucose for 2 h of hydrolysis showed a minimal effect on the percentage of saccharification of the *Aspergillus* as compared to *Trichoderma* (Table 3). However, the addition of 0.25% and 0.5% for the same period of time seems to have a significant inhibition on the crude enzyme compared to the commercial enzymes. It has been reported that glucose inhibits β-glucosidase activity [6, 21], and a system with low β-glucosidase activity like Celluclast [4] is more prone to the inhibition, as explained earlier [3, 7]. However, according to the result (Table 3), this seems to be true only when 0.1 or a higher percentage of glucose was used, but at a higher concentration level the crude enzyme

Table 3. The effect of glucose on the percentage of saccharification of α-cellulose and EFB by crude enzyme of *A. terreus* (*), commercial enzyme of *T. reesei* (+), and commercial enzyme of *A. niger* (#) for 2 h of hydrolysis.

Glucose conc. (%)	Percentage of saccharification					
	α-cellulose*	EFB*	α-cellulose+	EFB+	α-cellulose#	EFB#
0	24.58	8.25	19.09	5.00	14.67	3.30
0.1	24.17	7.98	16.00	4.10	14.35	2.94
0.25	15.83	5.67	14.31	3.54	11.76	2.56
0.5	7.50	3.72	13.75	3.39	11.49	2.48

appears to be more sensitive. This suggests that the enzyme is less tolerant to glucose only at higher than 0.1%. The accumulation of glucose is a factor that has to be reckoned with during the saccharification of substrates.

CONCLUSIONS

The present study conducted at 40°C and 50°C demonstrated that the enzyme preparation from *Aspergillus terreus* IMI 282743 gave an improved hydrolysis of either α-cellulose or EFB, as compared to commercial enzymes isolated either from *Trichoderma reesei* or *Aspergillus niger*. However, the addition of glucose higher than 0.1% could hinder the hydrolytic activities more than the commercial enzymes. The presence of higher β-glucosidase activity in the *A. terreus* preparation could explain the reason for the sensitivity.

Acknowledgments

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