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Saccharification of Palm Kernel Cake using Thermostable Lignocellulolytic Enzymes

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Abstract: Palm kernel cake (PKC), a byproduct of the oil palm kernel extraction process, can be used as the main ingredient in the production of livestock feed. However, the high mannan and cellulose content in the PKC is a barrier to ruminant nutrient absorption efficiency. This study investigated the potential usage of endo-1,4 β-mannanase produced by *Bacillus* sp. and Cellic CTec2 enzymes to hydrolyze the mannan and cellulose contents in the PKC. Experimental screening of solid and enzyme loadings enabled the optimal liberation of C6 and C5 sugars from PKC. The highest amounts of mannose and glucose were 33.26 g/L and 47.85 g/L for 1% and 3% solid loading using endo-1,4 β-mannanase, respectively, while the highest amounts of mannose and glucose were 24.43 g/L and 42.13 g/L for 1% and 3% solid loading using Cellic CTec2. The use of enzymes for biomass hydrolysis represents an efficient and environmentally friendly approach to attaining high sugar recovery in biorefineries.

Keywords: *1,4-endo-β-mannanase, Cellic CTec2, PKC, enzymatic hydrolysis*

1. Introduction

Palm oil production in Malaysia accounts for 6.4% of the gross national income (GNI) and is critical to the country's socio-economic development. Palm oil is low-cost, high-yielding commodity, and useful in various applications. Given its versatility in a variety of applications, palm oil has grown at a 7.1% annual rate of production over the last few decades, making it one of the top 17 oils and fats sources worldwide. It not only contributes to meeting the world's demand for edible oil, but it is also widely used in oleo-chemicals as a viable alternative fuel to reduce reliance on non-renewable fossil fuels (1).

Unfortunately, the number of residues generated in the industry has increased in parallel with the increase in palm oil production. About 50-70 tonnes of biomass can be produced per hectare of oil palm planting area, accounting for 85.5% of Malaysian biomass, which consists of empty fruit bunches (EFBs), fibre, shell, wet shell, palm kernel cake (PKC), fronds, and trunks. (2). PKC, a by-product of palm oil extraction, has been used as livestock feed.

The chemical composition of PKC varies based on the type of fruit palm, the sample source, and the method of oil extraction (i.e., solvent or mechanical) (3). PKC, which makes up around 50% of the original oil palm kernel, is

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primarily made up of cell walls. Major polysaccharide chains found in such cell wall include mannan (57.8%), cellulose (11.6%), and a negligible quantity of xylan (3.7 %) (4). It consists of linear and branched mannan formed from d-mannose, d-galactose, and d-glucose.

Mannan can be classified into 4 sub-groups namely linear mannan, glucomannan, galactomannan and galactoglucomannan (5). It is the primary component in the PKC (6), and is extremely hard, insoluble, resistant to enzymatic degradation, contains a high amount of crude fibre, and mostly has a low branching structure (7). Upon hydrolysis, mannan would be broken down to mannose. This C6 sugar is an isomer of glucose and differs in the second carbon atom's conformation. Mannan-degrading enzymes of fungal origin, such as those from *Aspergillus niger*, *Sclerotium rolfsii*, *Trichoderma harzianum*, *Trichoderma konigii*, and *Trichoderma longibrachiatum* have been well researched (8).

1,4-endo-β-mannanase is a hemicellulolytic enzyme that hydrolyzes the mannan backbone by random breakage of mannosidic links to release mannobiose units with varying amounts of additional manno-oligosaccharides. It belongs to the glycosyl hydrolase family 5, 26, and 113. Based on Shukor and co-workers (3) when mannanase loading for PKC hydrolysis was raised from 0.5 to 10%, sugar concentrations rose to 9.39 g/L of mannose and 11.62 g/L of total sugar when 10% (w/w) mannanase loading was used (3).

On the other hand, Cellic CTec2 is a cellulase complex for cellulose breakdown into fermentable sugars. According to a previous study (10), enzymatic hydrolysis for 72 hours at 50°C, pH 5.0, and 150 rpm resulted in the increase in total sugars from 27.4 g/L to 29.1 g/L with 10.4 FPU/g glucan using Cellic CTec2 (10).

Enzymatic hydrolysis seemed to provide manifold advantages over conventional acid hydrolysis in terms of higher yields, improved selectivity, energy-saving, and utterly mild working conditions (12). Hence, this study mainly focused on assessing the two types of commercial enzymes (1,4-endo-β-mannanase and Cellic CTec2) for maximum sugar recovery from the PKC by varying the solid and enzyme loadings.

2. Materials and Methods

2.1. Collection of Palm Kernel Cake (PKC)

The palm kernel cake (PKC) was collected from Sime Darby plantation located in Selangor. The PKC was oven-dried at 80℃ to reduce the moisture content below 10% (w/w) (13). The particle size of raw PKC sample used in this study was ~500 μm, which is regarded as an ideal size in producing higher reducing sugars in enzyme hydrolysis (14). In this work, the hydrolyzability of PKC was assessed under several solid loadings (i.e. 1% and 3% w/v).

2.2. Enzymatic Hydrolysis of PKC

The 1,4-endo-β-mannanase was purchased from the Megazyme company headquartered in Bray, Ireland with an enzyme activity of 1200 U/mL. Meanwhile, Cellic® CTec2 was obtained from Novozymes A/S, Denmark with an activity of 176.19 U/mL determined following the standard procedure for measurement of cellulase activities (5). The enzyme loadings were varied from 20 U/g up to 400 U/g.

The enzymatic hydrolysis was performed in a 100 mL conical flask containing 20 mL of mixture of ammonium sulphate buffer and varying concentration of 1,4-endo-β-mannanase at 1% and 3% (w/v) solid loadings. The mixture were shaken in an incubator set at 150 rpm, and 50℃ for 72 hours. The study was repeated using Cellic CTec2 mixed with citrate buffer in a 20 mL working volume.

2.3. Sugar Liberation Analysis

The concentrations of mannose and glucose were determined using a high-performance liquid chromatography (HPLC) system equipped with a refractive index detector (RID) set at 40°C on an UltiMate 3000 LC system (California, USA). Isocratically eluted with H_2SO_4 (2.5 mM) at a flow rate of 0.6 mL/min on a Rezex ROA-Organic Acid H^+ column (300 mm×7.8 mm; Phenomenex, USA) (15).

3. Result and Discussion

3.1. Composition of PKC

The PKC used in this study consisted of 12 wt%, 62.0 wt%, and 4 wt% of cellulose, hemicellulose, and xylan, respectively, as shown in Table 1.

Table 1. Composition of PKC

Composition	Percentage
Mannan	58.0
Cellulose	12.0
Xylan	4.0
Protein	16.50
Fat	7.0
Others	2.5

In order to evaluate the effectiveness of enzymes in recovering sugars from the PKC, the mass and composition of PKC were determined for 1% and 3% loading, as outlined in Table 2.

Table 2. Mass and composition of PKC for 1% and 3% loading

Item	1% loading	3% loading
Mass of mannan and glucose (g)	0.15	0.45
Composition of mannan (%)	60	60
Moisture content in PKC (%)	6.84	0.84
Mass of PKC (g)	0.23	0.69

3.2. Enzymatic hydrolysis of 1,4-endo-β-mannanase

Enzymatic hydrolysis of PKC was initially performed using endo-mannanase. After 72 hours, the 1% enzymatically digested PKC with 350 U/g of endo-mannanase had a maximum concentration of mannose (25.43 g/L) and glucose (5.6 g/L) (Figure 1). This finding showed that 350 U/g of enzyme loading was able to provide the maximum enzymatic digestibility of hemicellulose in the PKC. However, when loaded at 400 U/g, the mannose recovery eventually levelled off.

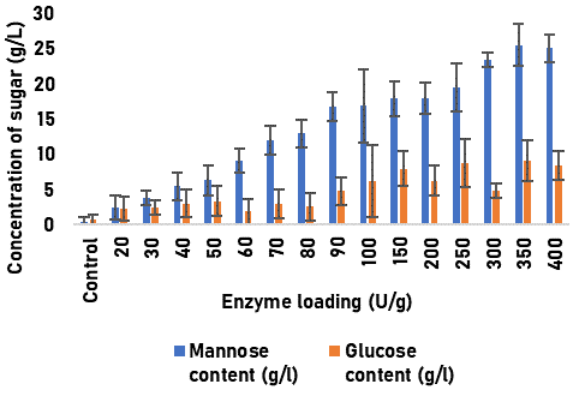

Figure 1. Concentration of sugars at 1% loading in mannanase hydrolysis

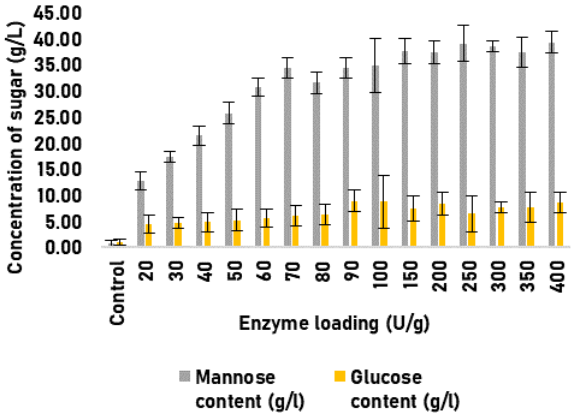

Figure 2. Concentration of sugars at 3% loading in mannanase hydrolysis

It can be deduced that the higher the enzyme loading led to a higher degradation of hemicellulose, hence promoting greater rates of monomer sugar conversions (16). However, the concentration of monomer sugar started to decrease when the enzyme-substrate ratio was saturated (17).

Nevertheless, sugar recovery at 3% solid loading was found to be the highest in 200 U/g of enzyme loading, producing a maximum mannose concentration of 37.42 g/L and 8.32 g/L of glucose. Further increase of enzyme loading beyond this threshold was no longer effective to increase the sugar recovery, as shown in Figure 2.

3.3. Enzymatic hydrolysis of Cellic CTec2

The concentrations of mannose and glucose recovered following enzymatic hydrolysis of 1% solid loading with Cellic CTec2 are shown in Figure 3.

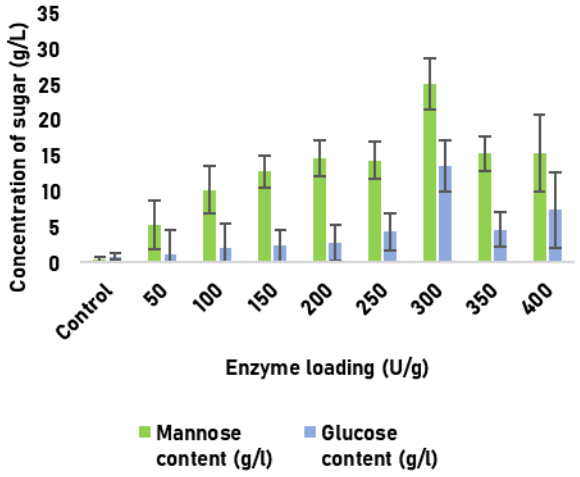

Figure 3. Concentration of sugars at 1% solid loading in CTec2 hydrolysis

In all cases, the concentration of sugars liberated increased with the higher enzyme loadings. However, Figure 3 demonstrated that the maximum sugars obtained from the 1% solid loading at 300 U/g Cellic CTec2 were 18.8 g/L and 5.63 g/L of mannose and glucose, respectively. Meanwhile, at higher solid loading of 3%, a similar trend was observed, where the maximum sugars liberation at 300 U/g of enzyme loading were 26.92 g/L and 15.21 g/L of mannose and glucose, respectively. Both of the sugars obtained in 1% and 3% solid loading with Cellic CTec2 were lower as compared to those with endo-mannanase.

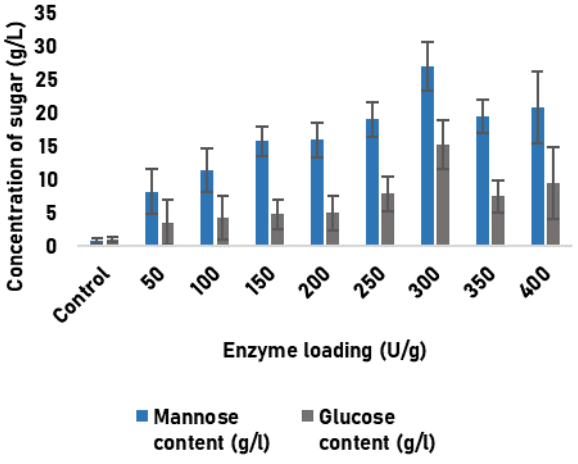

Figure 4. Concentration of sugars at 3% solid loading in CTec2 hydrolysis.

3.4. Comparison between Endo-Mannanase and Cellic

CTec2 Hydrolysis

The saccharification performance of endo-mannanase and Cellic CTec2 enzymes were evaluated on the cellulose and hemicellulose degradation of PKC. The statistically highest sugars that can be obtained at 1% solid loading were 31.03 g/L using 350 U/g of endo-mannanase ($P < 0.050$). For comparison, the maximum total sugar recorded in 300 U/g of Cellic CTec2 was 24.43 g/L, as shown in Table 3.

At 1% solid loading, the enzyme endo-mannanase had better enzymatic digestibility to PKC, resulting in greater glucose and mannose conversion than Cellic CTec2.

Meanwhile, the highest sugar concentration at 3% solid loading using 1,4-endo-β-mannanase and Cellic CTec2 were 45.74 g/L and 42.13 g/L, respectively (Table 4). This finding ascertained that the endo-mannanase had a better saccharification performance than Cellic CTec2 in hydrolysing PKC. One of the reason leading to difference in saccharaification performance was due to the difference in enzyme activity.

The activity of Cellic CTec2 enzyme calculated prior to the study was 176 U/mL while the activity of 1,4-endo-β-mannanase was 1200 U/mL. Higher activity of enzyme promotes better enzymatic digestibility on hemicellulose of PKC. According to a previous report, the maximum activity of the recombinant thermostable β-mannanase enzyme can reach up to 5435 U/mL, which can be obtained by high-density, feeding batch cultivation after 168 hours of induction with methanol in a 50-L bioreactor (18). The optimum pH and temperature for the β-mannanase enzyme are pH 6.0 and 60℃, respectively, and the β-mannanase enzyme is stable and active at 20℃ to 100℃.

Hence, the use of enzymes represents an efficient and environmentally friendly approach for high sugar recovery from PKC.

Table 3. Total simple sugar produced at 1% loading

Enzyme Concentration (U/g)	Sugars produced using endo-mannanase (g/L)	Sugars produced using Cellic CTec2 (g/L)
Control	0.90	1.53
50	9.46	6.49
100	22.84	12.33
150	22.68	15.06
200	23.98	17.38
250	28.10	18.67
300	28.04	24.43
350	31.03	19.94
400	33.26	22.73

Table 4. Total simple sugar produced at 3% loading

Enzyme Concentration (U/g)	Sugars produced using endo-mannanase (g/L)	Sugars produced using Cellic CTec2 (g/L)
Control	1.63	1.59
50	30.9	11.59
100	43.46	15.45
150	44.95	20.37
200	45.74	20.79
250	45.42	26.77
300	46.25	42.13
350	45.01	26.83
400	47.85	30.15

4. Conclusion

In this study, enzymatic hydrolysis was used to recover the simple sugars in the PKC. Enzyme hydrolysis was chosen as it was proven to have a high recovery of sugars with excellent specificity compared to conventional acids or other chemical hydrolyses. However, enzyme hydrolysis will be costly due to a shortage of enzyme production. It was found that the simple sugars present in the PKC were mannose and glucose. As for the enzyme hydrolysis using endo-mannanase, the highest total mannose and glucose at 1% and 3% loading were 31.03 g/L and 45.74 g/L, respectively. This shows that the amount of simple sugar produced at 3% loading was higher compared to 1% loading. For the enzyme hydrolysis using Cellic CTec2, the highest total mannose and glucose at 1% and 3% loading were 24.43 g/L and 42.13 g/L, respectively, thus highlighting the potential applicability of enzyme hydrolysis in producing high recovery of sugars for further biochemical processing.

5. Acknowledgements

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