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Bioconversion of Oil Palm Frond to Reducing Sugar in Solid State Fermentation By Applying Water-Fed Strategy

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Abstract: Tones of Oil Palm Frond (OPF) waste from oil palm industries may affects the environment when released without proper treatment. Conversion of oil palm frond fiber into reducing sugar in the Solid State Fermentation (SSF) using *Aspergillus fumigatus* culture provide alternative for usage of these biomass. In this study, effect of the particle size of oil palm frond fiber and moisture content to the production of reducing sugar in SSF were investigated. Two particle size of OPF used are 100 and 250 μm while moiture level was set at 60, 70 and 80% (w/v). The fermentation process was carried out for 10 days. Effect of moisture was further analyzed by applying water-fed strategy. All fermentation using water-fed strategy yielded higher production of sugar than compared to others. Pmax of 4.446 g L^{-1} was achieved at 70% moisture level using 250 μm OPF while maximum $Y_{p/s}$ achieved was 0.442 (g g^{-1}) in 80% moisture level using 250 μm OPF . Statistical analysis showed both particle size of oil palm frond fiber, 100 and 250 μm has no significant different towards production of reducing sugar in SSF by *Aspergillus fumigatus*. Statistically, moisture content of fiber does not significantly affect the production of reducing sugar but using water-fed strategy does gives significant difference. Sufficient moisture in SSF had increased the efficiency of fungus enzymatic system due to water holding capacity of substrate improved. Analysis two-way ANOVA suggest that presence of water fed has significant different towards production of reducing sugar in SSF and result in higher production of reducing sugar.

Key words: Oil palm frond, solid state fermentation, moisture, production, reducing

INTRODUCTION

Oil palm industries in Malaysia produced about 90 million tons of biomass per year. This include about 1.3 million tonnes of oil palm trunks, 8 million tons of Pruned and Felled fronds (OPF) and 2.4 million tons of oil palm Empty Fruit Bunches (EFB) (Alama *et al*., 2009). The Oil Palm Biomass (OPB) is classified as lignocellulose which contain 25% lignin in their cell wall. About 6.93 million tonnes of oil palm biomass (dry basis) (Bakar *et al*., 2012) is used to provide potential bioresources for the conversion into value-added products such as chemical feedstocks, biosugars, biofuels, bioplastic, cellulose and composite production (Lee *et al*., 2010). Most of these biomass can be obtained abundantly throughout the year from oil palm industries activities.

Researchers are looking for ways to efficiently increase bioconversion of cellulosic material into useful compound such as glucose, ethanol and others. The full utilization of this natural resource may be important in maintaining sustainable social development. Microbial enzymes hydrolysis are advantageous due to their specific biocatalysts such as can operate under milder reaction, not produce undesirable products and environmental friendly. Moreover, industrial enzymes from microbial sources are preferred due the short generation times of the microbes and large volumes of enzymes can be obtained within a short time. Cellulases play an important role in saccharifying cellulosic substrates for bioethanol production .

Extensive research has been done on conversion of oil palm biomass to ethanol by hydrolysis of cellulose to fermentable sugars. Biological pretreatment is the most economical method since the enzyme to degrade different substances in the materials is produced by microorganism and no chemical waste (Nazir *et al*., 2013). The use of solid state fermentation for conversion process of oil palm press fiber into usable form of

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biosugar is an ongoing study. This process could be economical by achieving high rate of bioconversion. The present study was conducted to investigate the effect of moisture content and different particle size to the production of reducing sugar from oil palm frond in solid state fermentation using *Aspergillus fumigatus*. The aims of the research is to improve yield of product through water-fed strategy implemented.

MATERIALS AND METHODS

Raw materials: The substrate used was Oil Palm Frond (OPF) chips which was obtained from Malaysian Palm Oil Board, Selangor (Ramli and Amin, 2014). Both OPF were grinded in two particle size 100 and 250 μm.

Microorganism: Microorganisms used in this experiment was *Aspergillus fumigatus* which was locally isolated from UiTM Kuala Pilah. It was previously isolated and identified using 18S rRNA characterization (Yoon *et al*., 2014).

Inoculum preparation: The fungi were cultured in PDA agar plates and incubated at 37°C for 9 days. After growth for 9 days, the spore were harvested by using 1% v/v sterile Tween-80 solution by aseptically scrapping it out using a hockey stick. The solution was collected using a sterile pipette and placed in a conical flask as spore suspension. The cell concentration was quantified using dry cell weight method. The Whatman filter paper No.1 was used in this method. First, the filter paper was weighed to determine the weight of a single filter paper. Then, 5 mL of spore suspension prepared earlier was filtered on the filter paper. It was washed with distilled water for 3 times, let filtered for minutes. Later, the filtered spores together with filter paper was dried in the oven of 60°C overnight. The next day, the dried filtered spore with filter paper was weighted until constant weight achieved.

Solid state fermentation: The production medium was a modified Mendel basal medium with composition of 1.4 g L^{-1} $(NH_4)2SO_4$, 2.0 g L^{-1} of KH_2PO_4, 0.3 g L^{-1} urea, 0.3 g L^{-1} $CaCl_2$, 0.3 g L^{-1} $MgSO_4$, 0.005 g L^{-1} $FeSO_4$, 0.0016 g L^{-1} $MnSO_4.H_2O$, 0.0014 g L^{-1} $ZnSO_4.7H_2O$, 0.002 g L^{-1} $CoCl_2$ and 0.75 g L^{-1}peptone. The 10 g of substrate (OPF) was moistened with 45 mL production media and 5 mL spore suspension (1.02 g L^{-1}) in a 250 mL conical flask to get final moisture level of 80%. The 60, 70 and 80% of moisture level for both particle size of OPF substrate for 100 and 250 μm was prepared. The flask was incubated at 37°C. The pH of the media was set to pH (5.0). Water fed strategy was applied by adding 10 mL of distilled water was added to each sample at day 1 and another 10 mL distilled water was added to the sample in day 2. The 1 g of sample was taken for every 24 h and analyzed for reducing sugar content using DNS assay. The fermentation was run for 10 days. The same step was applied for 60 and 70% substrate moisture level.

3,5-dinitrosalicyclic acid (DNS) assay: The glucose concentrations or reducing sugar were analyzed based on prepared standard curve of glucose concentration using DNS assay. All analysis were done using UV-Vis Spectrophotometer (Lab-Tech).

For each tube, 1 g of sample was mixed with 1 mL of dinitrosalicyclic acid (DNS) reagent and two drops of 0.1 M NaOH. All tubes were incubated in 80-90°C waterbath for 5 min. After that, each tube was added with 10 mL distilled water. The test tube of the solution was inverted several times in order to mix it thoroughly. The solution was taken using a pipette and was filtered before inserted into a cuvette. Absorbance of the glucose concentration was determined using UV-V is spectrophotometer at wavelength 540 nm.

Statistical analysis: Two-way analysis is conducted using SPSS. This analysis is purposely to study the effect two independent variable on independent variable. First, the effect of different particle size of OPF and different moisture level on production of reducing sugar. Second is the effect of water fed and different moisture level on production of reducing sugar based on statistically analysis.

RESULTS AND DISCUSSION

Production of reducing sugar in SSF: Three experiments that were conducted which were production of reducing sugar using 250 μm of OPF without water fed (Fig. 1) with water fed (Fig. 2) and production of reducing sugar using 100 μm of OPF with water fed (Fig. 3).

Overall the production of reducing sugar is fluctuating for three different moisture level but in the increment trend. Production of sugar for 70% moisture gradually increased from day 1 until day 5 then plummeted down at day 6 and increase back for the following days. For 80% moisture, the graph increased from day 1-6 and fell down for the next following days. Towards the end, the production of reducing sugar seem in the decrement trendy.

In Fig. 2, the trend of the graph indicates that the production of reducing sugar increase linearly. The production of reducing sugar for all three moisture level, 60, 70 and 80% increase steadily from day 1 until day

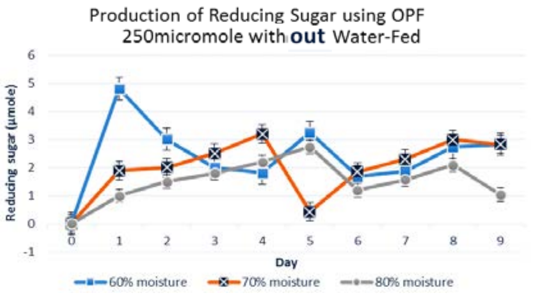

Fig. 1: Production of reducing sugar using 250 µm OPF without water-fed strategy

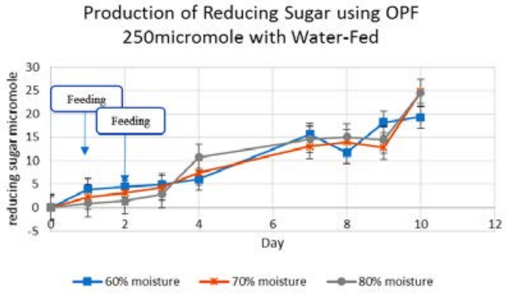

Fig. 2: Production of reducing sugar using 250 µm OPF with water-fed strategy

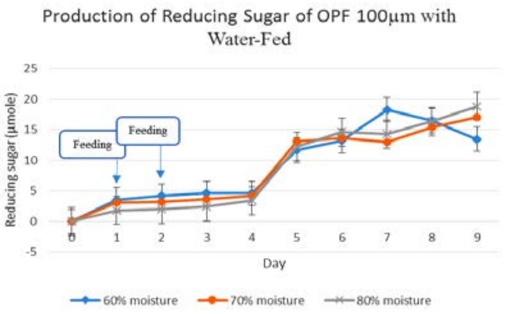

Fig. 3: Production of reducing sugar using 100 µm OPF with water-fed strategy

5 and then sharply rise up from day 5-9. For the 60% moisture level, the production of sugar increase at day 8 and fall back at day 9. While the others in the increment trend.

In Fig. 3, the trend show that production of sugar increased linearly along incubation period. For the 80% of moisture level, the sugar produced slowly for the first 4 days and then rose up at day 5 until day 9. For the 60% and 70% moisture level, production of sugar slightly increase at day 1-5. Continue up to day 7, the graph of 60% moisture fell down and then rose back for the next following days. In contrast sugar produce steeply for the next following day at 70% of moisture.

The reducing sugar production was examined based on product maximum (P_{max}) and yield of sugar ($Y_{p/s}$). The maximum sugar produced was 24.780+0.564 µmole or 4.446+0.360 g L^{-1} at 70% moisture level for the 250 µm OPF with water fed and the highest $Y_{p/s}$ was 0.442 g g^{-1} at 80% moisture level for 250 µm OPF with water-fed. This result show an improvement compared to a study using hydrolysis of OPF and EFB for conversion to reducing sugar that gives 27.4 and 24.8% yield, respectively (Jamroo *et al*., 2015).

Based on comparison, larger particle size of 250 µm of OPF produce more reducing sugar compared to 100 µm reducing sugar that produce less sugar. That was only 18.857+0.670 µmole or 3.394+0.221 g L^{-1}.

On the contrary, the comparison also was made to know which moisture level is the best to produce sugar for the both particle size of sugar. The highest sugar for 250 µm particle size was produced at 70% moisture level and 100 µm produced was at 80% moisture level. Comparison on these two result, the best condition to produce reducing sugar was at 70% moisture level for 250 µm of OPF.

The result is supported by study done by Yoon *et al*. (2014), the best moisture level to produce sugar is between range 60-80%. The moisture content lower than 60% and above than 80% is less favorable for both cellulose productions in SSF and fungal growth. So in between range 60-80% moisture level, 70% moisture is the best condition to produce sugar.

The overall trend of this graph production of reducing sugar for 250 µm OPF without water fed (Fig. 1) showed inconsistent production of reducing sugar. This is slightly lower compared to the production of reducing sugar for 250 µm OPF with water fed (Fig. 3). It is suggested that during incubation period in SSF, the substrate keep losing its moisture and become dry. Insufficient of moisture level will reduce the enzyme activity in fungus thus result in low production of reducing sugar. This is supported by study done by (Pandey, 2003), when content of moisture is below than required level, the solubility of substrate nutrients is limited and it does effect the effective nutrients uptake by the fungi.

On the other hand, by applying water-fed strategy sufficient moisture is maintained for the fungus to conduct the SSF process. The moisture content is closely related to the lignocellulosic structure of substrate. The porosity and specific surface area of the solid particle of OPF will influence the efficiency of air diffusion and water

Table 1: Effect of moisture concentration in feeding medium on reducing sugar production in SSF

Particle size (μm)	Moisture level (%)	Pmax (μmole)	g L^{-1}	$Y_{p/s}$ (g/g)
100 with water fed	80	18.857±0.670	3.394±0.221	0.3390
	70	17.057±0.561	3.070±0.480	0.2050
	60	18.310±0.510	3.300±0.567	0.1650
250 without water fed	80	2.730±0.2300	0.491±0.100	0.0491
	70	3.193±0.2300	0.575±0.100	0.0383
	60	4.803±0.2400	0.865±0.120	0.0432
250 with water fed	80	24.563±0.460	4.421±0.380	0.4420
	70	24.780±0.564	4.446±0.360	0.2960
	60	19.308±0.420	3.475±0.420	0.1730

Table 2: Table of two-way ANOVA for interaction between particle size and moisture level on production of sugar tests of between-subjects effects

Source	Type III sum of squares	df	Mean square	F-value	Sig.
Corrected Model	68.621[a]	5	13.724	0.306	0.908
Intercept	9125.201	1	9125.201	203.631	0.000
Particlesize	66.585	1	66.585	1.486	0.226
Moisture* particlesize	1.207	2	0.603	0.013	0.987
Error	4033.113	90	44.812		
Total	13226.934	96			

[a]$R^2 = 0.017$ (Adjusted $R^2 = -0.038$)

Table 3: Two-way ANOVA interaction between interaction between presence and absence of water fed and moisture level on production of sugar

Source	Type III sum of squares	df	Mean square	F-value	Sig.
Corrected Model	1404.431[a]	6	234.072	7.224	0.000
Intercept	1290.129	1	1290.129	39.815	0.000
Moisture	1.636	2	.818	.025	0.975
Treatment	1390.548	2	695.274	21.457	0.000
Moisture* treatment	2.256	2	1.128	.035	0.966
Error	2462.638	76	32.403		
Total	8030.237	83			
Corrected Total	3867.069	82			

$R^2 = 0.363$ (Adjusted $R^2 = 0.313$)

holding capacity of the substrate (Pandey, 2003). Thus, result in higher production of reducing sugar.

Based on statistically analysis result, the moisture ($p = 0.991$), particle size ($p = 0.226$) and interaction between moisture and particle size (moisture x particlesize) was $p = 0.987$ which is bigger than $\alpha = 0.05$. This indicates that there was no significant different between the effect of different particle size of OPF and different moisture level on production of reducing sugar. This is probably because the range of particle size used in this experiment is not suitable to make a difference (Table 1-3).

Based on statistically analysis result, the moisture ($p = 0.975$) and interaction between moisture and particle size (moisture* water fed treatment) was $p = 0.966$ which is bigger than $\alpha = 0.05$. This indicates that there was no significant different between the effect of different moisture level on production of reducing sugar. The range of moisture content used in this study probably unsuitable to make big difference. However, the water-fed treatment does effect production of sugar as ($p = 0.000$) is smaller than $\alpha = 0.05$. This suggest the water fed strategy in SSF give increment in sugar production.

CONCLUSION

In conclusion, the best condition to produce reducing sugar was at 70% moisture level for 250 μm of OPF. However based on statistic analysis, both particle size; 100 and 250 μm does not affect the production of reducing sugar in SSF by *Aspergillus fumigatus*. This is due to the range of particle size used in this experiment is not significantly large enough to make a difference. Large range of particle size of OPF likely from 100-800 μm can be attempted to give significant comparison on production of sugar.

This study has proved that the water-fed strategy in SSF media do affect the production of reducing sugar in Solid State Fermentation of Oil Palm Frond by *Aspergillus fumigatus*. The presence of water fed at day 1-2 provide sufficient moisture for the fungus to conduct the SSF processthus result in higher production of reducing sugar.

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