Effect of Microwave Pretreatment on Production of Reducing Sugar from Oil Palm Empty Fruit Bunches

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ABSTRAK

Perlakuan pendahulan biomassa merupakan tahapan terpenting dalam memproduksi produk-produk berbasis bio (*bio-based products*) secara biologis. Pada penelitian ini, energi gelombang mikro (*microwave*) digunakan selama perlakuan pendahuluan untuk meningkatkan kinerja proses sakarifikasi tandan kosong kelapa sawit (TKKS) menjadi gula-gula pereduksi. Faktor-faktor yang mempengaruhi perlakuan pendahuluan seperti daya (180-360 watt), waktu iradiasi (5-30 menit), dan *solid loading* (2,5%-7,5%) dievaluasi. Kinerja hidrolisis TKKS yang telah diberi perlakuan pendahuluan selanjutnya dianalisis dengan Cellic CTec2. Hasil penelitian menunjukkan bahwa cairan residu yang diperoleh setelah perlakuan pendahuluan menghasilkan gula pereduksi dalam jumlah yang rendah, yaitu antara 1,39 dan 3,92 mg/g-TKKS. Akan tetapi, setelah padatan residu dihidrolisis secara enzimatis, rendemen gula pereduksi meningkat secara signifikan. Menariknya, hanya pada level daya terendah (180 watt), gula pereduksi meningkat seiring dengan perpanjangan waktu iradiasi untuk semua *solid loading*. Sebaliknya, pada 360 watt, semakin lama waktu iradiasi diterapkan, semakin rendah gula pereduksi yang diperoleh untuk semua *solid loading*. Gula pereduksi tertinggi dihasilkan hingga 151 mg/g-TKKS, yaitu menggunakan 5% padatan pada 180 watt selama 25 menit. Berdasarkan hasil-hasil ini, perlakuan pendahuluan menggunakan gelombang mikro yang diikuti dengan hidrolisis enzimatis merupakan salah satu metode yang potensial untuk memproduksi gula dari TKKS.

Kata kunci: Gula pereduksi, hidrolisis enzimatis, *microwave pretreatment*, TKKS.

ABSTRACT

Pretreatment of biomass is the most crucial step in the biological production of bio-based products. In this study, microwave energy was used during the pretreatment process to enhance the saccharification performance of oil palm empty fruit bunches (OPEFB) into reducing sugar. The influential factors of pretreatment such as power level (180-360 watt), irradiation time (5-30 min), and solid loading (2.5%-7.5%) were evaluated. The performance of pretreated OPEFB hydrolysis was subsequently assessed by Cellic CTec2. The result showed that spent liquor produced after pretreatment only released a low amount of reducing sugar in the range between 1.39 and 3.92 mg/g-OPEFB. After residual solid was enzymatically hydrolyzed, a significant increase in the reducing sugar yield occurred. Interestingly, only at the lowest power level (180 watts), the reducing sugar rose along with the extension of irradiation time for all solid loadings. On the contrary, the longer irradiation time was applied, the lower reducing sugar was acquired at 360 watts for all solid loadings. The highest reducing sugar was produced up to 151 mg/g-OPEFB using 5% solid at 180 watts for 25 min. This indicated that microwave pretreatment followed by enzymatic hydrolysis was one of the potential methods to recover sugars in OPEFB.

Keywords: Enzymatic hydrolysis, microwave pretreatment, OPEFB, reducing sugar.

1. INTRODUCTION

One of the promising materials abundantly available in Indonesia is oil palm empty fruit bunches (OPEFB) generated from palm oil factories as a solid waste. This material is previously just converted to the heat and power generation or several products such as compost and mulch. Unfortunately, the rest

of this materials is still available in huge amount and needs other conversions to higher valuable products [1].

The OPEFB consisted of 36.59-43.54% cellulose, 19.14-24.97% hemicellulose, and 19.83-26.53% lignin [2-5] in which those components could still be utilized to produce high-added value products. In OPEFB, the cellulose was composed of glucose, and the sugars in hemicellulose were dominated by both C-5 (arabinose, mannose, and xylose) and C-6 (glucose and galactose). To release sugars both in cellulose and hemicellulose of OPEFB, hydrolysis was needed [6]. Enzymatic hydrolysis is a more eco-friendly and selective technique to obtain high sugar yield instead of acid hydrolysis. The advantages of enzymatic hydrolysis include mild reaction conditions, high specificity, and no sugar rings modification [7]. The sugar-rich-hydrolyzate could be subsequently fermented into high added value products such as biochemicals, biomaterials, and biofuels [8].

The main challenge of enzymatic hydrolysis was the presence of lignin with a rigid structure that obstructed enzyme access in degrading cellulose and hemicellulose [9-10]. Thus, the lignin amount should be minimized from the solid fraction and destructed by the pretreatment process [11]. Assorted methods of pretreatment had been considerably reported. Heating-assisted alkaline pretreatment was mostly used to dissolve lignin [12]. The drawback of this technique, however, was that the dark liquor after such treatment could not be used for further process, and it was just discarded as the waste [13]. Meanwhile, due to the more amorphous structure of hemicellulose, heating during pretreatment could release sugars from these constituents [14]. Therefore, to recover all sugars in OPEFB, heating with solely using water was the most effective pretreatment [3].

Microwave-based (MW) pretreatment is one of the effective and efficient pretreatments for recovering sugars in OPEFB. The heat of MW pretreatment was generated from the electromagnetic wave with a range from 30 GHz to 300 MHz [15]. Several benefits of MW pretreatment were high heating efficiency, lower energy requirement, easy operation, more rapid pretreatment, and high selectivity [16]. Due to these advantages, the MW pretreatment was considered as a green technology [17].

As compared to the conventional heating, interaction between heat and solid in this pretreatment was directly conducted. The heating of conventional pretreatment method was conducted by conduction and convection and required high energy input [18]. The direct interaction of MW pretreatment caused the intermolecular collision, and ultimately organic reactions in solid were accelerated. As a result, the heating of solid became more effective [19]. During MW pretreatment, the recalcitrant structure of OPEFB lignocellulose wrecked and the crystallinity of cellulose diminished. The silicified waxy surface wrecked, the lignin removal occurred since the ether and ester linkage between lignin and carbohydrates demolished, and the reduction degree of hemicellulose increased [20,21]. Therefore, this pretreatment effectively facilitated enzyme in degrading OPEFB, so more sugars could be recovered [22].

Considerable research on MW pretreatment had been published. Various solution types were used to accelerate the process such as organic acid [23], alkaline [24], salts [20], [25], and ionic liquid [26]. However, the liquor resulted during the pretreatment could not be re-used since toxic compounds were formed and required to be removed by detoxification [13]. Due to the use of such solutions, the value of pH also altered, and neutralization was needed. To detoxify and neutralize the liquor the operating cost, however, increased [27]. Consequently, the process became less feasible to be developed on a larger scale.

This work offered a simpler technique, just using water as a solution so that all sugars could be obtained. Several influencing factors of MW pretreatment such as

irradiation time, power level, and solid loading were evaluated in this work to obtain a high yield of reducing sugars. A comparison with the conventional method, autohydrolysis, was also investigated.

2. RESEARCH METHODS
2.1. OPEFB Preparation
In this research, the OPEFB was provided by PT Condong Garut, West Java, Indonesia. The OPEFB was washed with tap water until all the adhered dirt were removed and was dried using blower oven at 60°C for one day. The dried OPEFB size was then reduced using a disc mill. Smaller OPEFB was sieved at the range between 40 and 60 mesh. The lignocellulose composition of OPEFB was analyzed following [28]. After analysis, the OPEFB used in this research contained 48.55 ± 7.64% cellulose, 28.06 ± 0.41% hemicellulose, and 23.39 ± 8.05% lignin in dry weight.

2.2. Pretreatment
The OPEFB was pretreated by using microwave oven SHARP R-728(S)-IN with adjustable power level and time. The OPEFB and water ratio used was varied from 2.5% to 7.5% (w/v) in a 300 mL flask. The power level was set from 180 to 360 watt for 5 to 25 minutes.

After pretreatment, the pretreated OPEFB was then separated from the liquor using cotton cloth. The spent liquor was analyzed to identify the sugar formed whereas residual solid was enzymatically hydrolyzed to improve sugar yield.

In addition, to compare the performance of the best MW pretreatment, in this study autoclave pretreatment by just using water as its solution was also carried out. This pretreatment was set at 115°C for 60 min and using 10% solid loading. The pretreatment result was further treated in the same way with the MW pretreatment for the hydrolysis step.

2.3. Enzymatic Hydrolysis
Approximately 3 g residual solid from pretreatment was dissolved in 29 mL citrate buffer pH 5 (0.2 M). After all parts of solid had been wetted, 1 mL Cellic CTec2 enzyme (Novozymes) was added. Hydrolysis was subsequently performed using an incubator shaker at room temperature with 150 rpm for 72 h. Hydrolyzate formed was further analyzed.

2.4. Analysis
Sugar content in spent liquor after pretreatment and hydrolyzate was determined by a colorimetric technique using a 3.5-dinitro salicylic acid (DNS) solution and spectrophotometer for analytical instrumentation [29]. The concentration of reducing sugar was measured following this method, but the data in this work was presented in sugar yield. Thus, the yield of sugars was calculated by equation 1 as follows:

$$Yield(g - reducing\ sugar/g - OPEFB) = \frac{Reducing\ Sugar\ Concentration\left(\frac{g}{L}\right) \times Volume(L)}{Initial\ OPEFB\ (g)} \quad (1)$$

A one-way variance analysis (ANOVA) with a 95% confidence level was used to analyze the significance of the data. IBM SPSS Statistic 25.0 was used as the computation tool of the data.

3. RESULTS AND DISCUSSION
3.1. Reducing Sugar Yield in Spent Liquor
Table 1 showed the yield of reducing sugar secreted after microwave pretreatment at different process conditions. According to the given table, at the lowest solid loading (2.5%), the reducing sugar yield statistically showed insignificant change when the pretreatment process applied longer duration and higher power level of microwave, expressed as the p-value of more than 0.05. It was perhaps because of just a little amount of solid loading, so the probability of solid exposure to the microwave was also low.

Table 1. Reducing sugar in spent liquor after microwave pretreatment

	Reducing Sugar (mg/g-OPEFB)	
	Power Level 180 watts	Power Level 360 watts
2.5% Solid Loading		
Pretreatment time (min)		
5	1.89 ± 0.01^{abcd}	2.39 ± 0.17^{abcd}
15	1.39 ± 0.12^{a}	1.84 ± 0.06^{abc}
25	2.29 ± 0.16^{abcd}	2.07 ± 0.00^{abcd}
5% Solid Loading		
Pretreatment time (min)		
5	2.66 ± 0.02^{bcdef}	3.68 ± 0.24^{fg}
15	1.46 ± 0.03^{a}	2.21 ± 0.04^{abcd}
25	3.92 ± 0.32^{g}	2.62 ± 0.38^{bcde}
7.5% Solid Loading		
Pretreatment time (min)		
5	2.90 ± 0.85^{defg}	3.50 ± 0.22^{efg}
15	1.69 ± 0.16^{ab}	2.75 ± 0.16^{cdef}
25	1.67 ± 0.15^{ab}	2.85 ± 0.07^{cdef}

Note: The different superscript letters showed that there was a significant difference in the mean value ($p < 0.05$)

On the other hand, the pretreatment time and power level had a significant effect on the yield of reducing sugar for 5% solid loading ($p<0.05$). The rise of the time from 5 min to 15 min could significantly decline the reducing sugar yield at 180 and 360 watts ($p<0.05$). After the time was extended for 25 min, the reducing sugar significantly increased for both power levels ($p<0.05$). The fluctuating trend was probably because of an unstable process. Pretreatment performed at a more prolonged duration should release more sugars. The decrease of reducing sugar might be caused by the decomposition of the sugars to by-products such as furan [13]. The longer pretreatment time was employed, the more sugars were converted to furan. The reducing sugar could rise with the extension of time since, in this condition, the rate of sugar decomposition perhaps became lower than the sugar released [30].

Three main factors responsible in the decomposition of reducing sugars to furan during microwave pretreatment process were solid loading, irradiation time, and power level [31]. The lower solid loading was used, the higher percentage of hemicellulose degradation was obtained. Low solid loading, however, could not generate more reducing sugar yield. If the process employed higher solid loading, the reducing sugar yield also could decrease because just little water content on high solid loading was insufficient to absorb more energy from microwave to degrade lignocellulose material.

The use of high power level also contributed to furan formation because this parameter would correspond to the elevation of the process temperature [31]. The high temperature in the pretreatment solution led to hemicellulose dissolution, and more acetic acids detected. Acetyl groups substituted hydroxyl groups of sugars in hemicellulose structure [32]. The concentration of acetic acids increased as a consequence of the rise of temperature and irradiation time. More acetic acids, along with more severe condition, caused reducing sugar dehydration into furan, so its yield declined [33]. Thus, to avoid furan formation and sugar yield reduction, the

high power level was usually applied at short irradiation time and vice versa.

For 7.5% solid loading, the increase of time from 5 to 15 min significantly influenced the reducing sugar obtained ($p<0.05$) to be lower. As mentioned above, the extended pretreatment time led to the sugar conversion to furan, so its yield reduced as a result. In addition, more solid loading (lower water content) restricted the energy absorption generated from microwave to the water. This restriction caused low deconstruction of lignocellulose fraction. The high rate of sugar decomposition along with the limitation of energy absorption caused the decrease of the reducing sugar yield.

Nevertheless, when the pretreatment used longer duration up to 25 min, the reducing sugar obtained remained constant from 1.69 ± 0.16 to 1.67 ± 0.15 ($p>0.05$). This showed that a longer time to emit microwave irradiation was as just a little influence in lignocellulose degradation. On the other hand, the power level increase from 180 to 360 watts was able to elevate the reducing sugar yield for all pretreatment times and 7.5% solid loading. This indicated that the more energy provided from the increase of power level was able to depolymerize the lignocellulose structure. Hence, more sugars were formed.

Overall, this process maximally produced 3.92 mg-reducing sugar/g-OPEFB at 180 watts power level for 25 min and using 5% solid loading. This amount, however, was insufficient to be used as a fermentation substrate. Thus, a subsequent process aimed to increase the yield of reducing sugar was needed, which was enzymatic hydrolysis.

3.2. Reducing Sugar Yield in Hydrolyzate

Reducing sugar obtained after enzymatic hydrolysis was used to evaluate pretreatment performance. The amount of reducing sugar in the hydrolyzate was illustrated in Fig. 1. According to this figure, at the lowest power level of MW pretreatment (180 watts), the increase of MW pretreatment time could release more reducing sugar. Nevertheless, when the MW pretreatment time was extended from 15 to 25 min, the reducing sugar amount declined significantly ($P<0.05$) for both 2.5% and 7.5% solid loading. Interestingly, solid loading of 5% showed a different trend. By using the solid loading at this concentration, the reducing sugar significantly rose from 95.23 mg/g-OPEFB to 150.74 mg/g ($p<0.05$).

At the lowest solid loading (2.5%) and 180 watts, the increase of MW pretreatment time from 5 to 15 min could produce more reducing sugar from 42.51 mg/g to 100.08 mg/g after the pretreated OPEFB was enzymatically hydrolyzed. However, when the pretreatment was performed until 25 min, the amount of sugar diminished to 40.15 mg/g. It could be caused by less available cellulose and hemicellulose in OPEFB. This amount was insufficient to enhance the sugar yield. In other words, the releasing rate of reducing sugars was not faster than the degradation rate of reducing sugars to Furan during the pretreatment process.
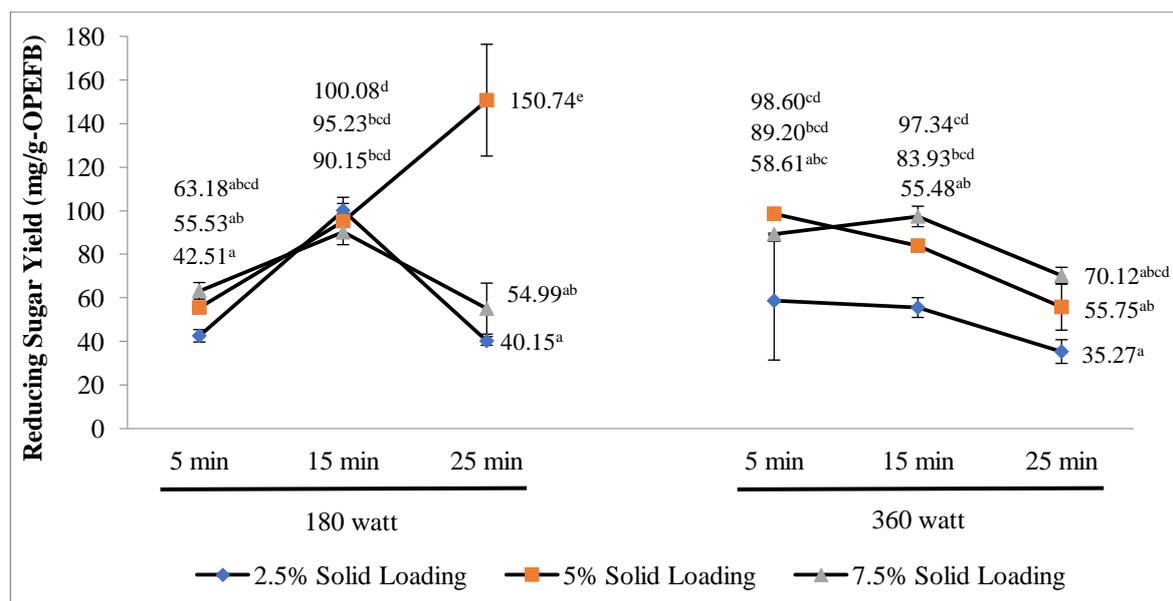
Figure 1. The yield of reducing sugar in hydrolyzate at different pretreatment conditions. The different superscript letters showed that there was a significant difference in the mean value ($p < 0.05$).

Meanwhile, at the highest solid loading (7.5%) and 180 watts, the rise of reducing sugar took place just when the pretreatment was conducted at the range between 5 and 15 min from 63.18 mg/g to 90.15 mg/g. This indicated that more structure of OPEFB was destructed during pretreatment. However, when the pretreatment was conducted at a longer duration, going to 25 min, there was the decrease of reducing sugar from 90.15 to 54.99 mg/g. The MW pretreatment was not fairly able to decompose the rest of the material structure. It might be too bulky of OPEFB size.

The yield of reducing-sugar was prior estimated that it could be improved by increasing the power level from 180 to 360 watts. However, more severe pretreatment conditions declined the obtained reducing sugar as shown in Fig. 1. The extended pretreatment time also showed the unexpected result in which the longer pretreatment time was conducted, the lower reducing sugar was obtained at 360 watts for all solid loading. This could lead to further degradation of reducing sugar into furan such as furfural and 5-hydroxymethylfurfural (5-HMF) from xylose and glucose dehydration, respectively. Both by-products inhibited the process of enzymatic hydrolysis. The reducing sugar yield eventually leveled off or no significant change occurred ($P > 0.05$).

According to the results above, The pretreatment using 5% solid loading conducted at 180 watts for 25 min gave high reducing sugar. To ensure this is the best condition, the irradiation time was extended up to 30 min as shown in Fig. 2.

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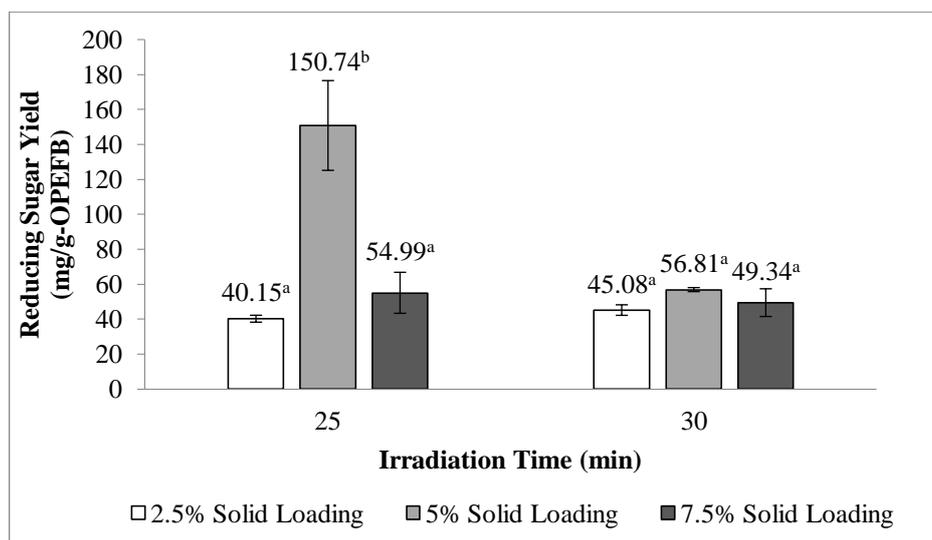
Figure 2. The reducing sugar obtained after enzymatic hydrolysis at two pretreatment times (25 and 30 min). MW pretreatment was performed at 180 watts power level using 2.5-7.5% solid loading. The different superscript letters showed that there was a significant difference in the mean value ($p < 0.05$).

The result showed that the reducing sugar yield reduced from 150.74 mg/g to 56.81 mg/g when the MW pretreatment time was conducted longer by 5 min using 5% solid loading at 180 watts. Meanwhile, for the other solid loading, no significant change took place when the time was extended ($p > 0.05$). This was perhaps because the amount of cellulose and hemicellulose in solid phase of pretreated OPEFB had completely degraded. Thus, the extension of time gave no significant change of reducing sugar yield.

Several previous researches had been reported with different biomass. For instance, Lu et al. [20] reported that the MW pretreatment could increase the yield of reducing sugar, approximately 2.9 times higher than the yield obtained with untreated rice straw. Lai et al. [21] also mentioned that the MW pretreatment oil palm trunk and fibers could increase the cellulose released and remove hemicellulose and lignin. Singh et al. [22] stated that the effect of hemicellulose and lignin removal in large quantities was able to increase the reducing sugar obtained. Binod et al. [17] investigated the production of bioethanol using this pretreatment type, and it is successful to produce a high amount of bioethanol.

3.3. Comparison of MW Pretreatment to Autoclave Pretreatment

In this study, the performance of MW pretreatment was also compared to autoclave pretreatment. The yield obtained in both pretreatments was shown in Fig. 3. Overall, the autoclave pretreatment gave the higher yield both during pretreatment and enzymatic hydrolysis as compared to the MW pretreatment.
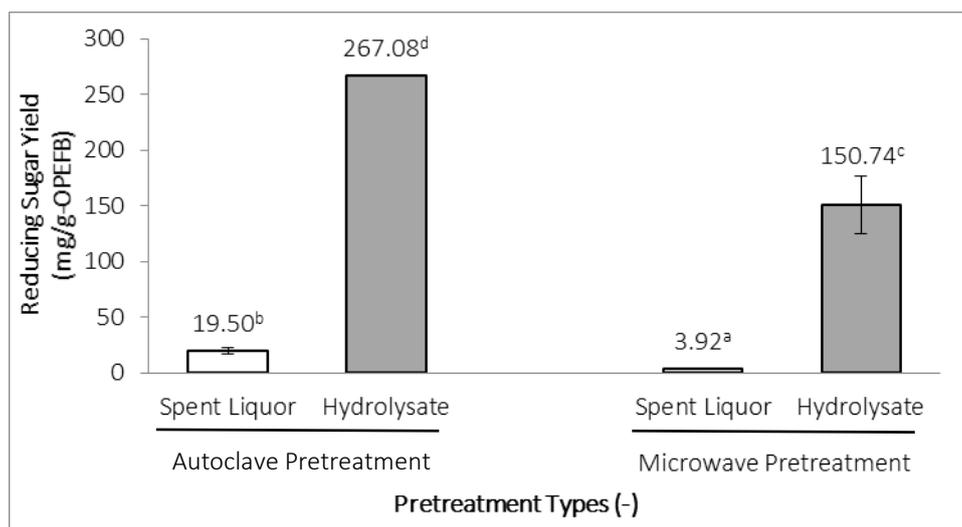
Figure 3. The reducing sugar obtained in the spent liquor after pretreatment and hydrolyzate. Autoclave pretreatment was performed at 115°C for 60 min using 10% solid loading whereas the MW pretreatment was performed at 180 watts power lever for 25 min using 10% solid loading. The different superscript letters showed that there was a significant difference in the mean value ($p < 0.05$).

The reducing sugar yield in spent liquor after autoclave pretreatment was approximately 5 times higher than the yield after MW pretreatment. This showed that autoclave pretreatment could release more reducing sugar and retain this amount from sugar decomposition due to harsh conditions. Besides, when pretreated solid was further hydrolyzed enzymatically, more sugars of autoclave pretreatment were recovered. This indicated that autoclave pretreatment was more successful in deconstructing the OPEFB structure. The less performance of MW pretreatment might be caused by more structure of OPEFB that was not degraded. The MW pretreatment might require the other solution apart from water for the acceleration of reaction rate.

Several studies on the MW pretreatment assisted with alkaline, acid, and an ionic solution had been reported. A comparison of the reducing sugar yielded was illustrated in Table 2. As compared to other studies, this study gave the lowest reducing sugar. This showed that the use of other solutions, apart from water, could increase reducing sugar yield.

Table 2. Reducing sugar in spent liquor after microwave pretreatment

Raw Materials	Parameters					Reducing Sugar		Ref.
	Power Level (watts)	Microwave Intensity (°C)	Irradiation Time (min)	Solid Loading (%)	Solution (-)	Xylose (mg/g)	Glucose (mg/g)	
Switchgrass	250	-	10	10	2% NaOH	127	289	[30]
Catalpa sawdust	400	-	6	5	2.25% Ca(OH)$_2$	-	402.73	[34]
Oil palm empty fruit bunch fiber	180	-	12	10	3% NaOH	-	411	[35]
Sugarcane bagasse	600	-	4	10	1% NaOH	-	665	[17]
Cotton Plant Residue	300	-	6	17.5	1% NaOH	-	495	[36]
Sorgum bagasse	-	130	60	10.5	28% NaOH	-	420	[37]
Mission grass	-	120	10	6.7	3% NaOH	-	409	[38]
Miscantus Sinensis	300	-	30	6.7	1.78% H$_3$PO$_4$	-	622	[39]
OPEFB	180	-	25	5	Water	151 ± 26		This Study

4. CONCLUSION

Power level, irradiation time, and solid loading significantly affected the yield of reducing sugar. The increase of the reducing sugar yield with the extended MW pretreatment time just took place at the lowest power level (180 watts) and solid loading 10%. Even though, sugar obtained in spent liquor was too little, maximally around 3.92 mg/g-OPEFB, after the pretreated solid was enzymatically hydrolyzed, the yield dramatically elevated up to 150.74 mg/g-OPEFB at 180 watts for 25 min and using 10% solid loading. This indicated that the MW pretreatment could improve the production of reducing sugar. However, the use of water as a solution did not give higher yield as compared to autoclave pretreatment (liquid water hot pretreatment or autohydrolysis).

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