each flask was taken regularly and analysed by gas chromatography. The process will be stopped after 50 days digestion.

F. Analysis

The constituent of raw materials and pre-treated materials was determined according to NREL Laboratory Analytical Procedure. The sugar content was determined based on monomer content measured after two-step acid hydrolysis procedure. In the first step, samples were treated with 72% (w/w) H_2SO_4 at 30°C for 2 h. The reaction mixture was then diluted to 4% (w/w) H_2SO_4 and autoclaved at 121°C for 1hour.The hydrolysis solution was filtered and analysed for sugar content by HPLC on an ion exchange column (Aminex, HPX-87P, Bio-Rad, Hercules, CA). Glucose from enzymatic hydrolysis was analysed by HPLC using another ion exchange column (Aminex, HPX-87H, Bio-Rad, Hercules, CA) at 60°C with 5mM sulphuric acid as the eluent at a flow rate of 0.6 mL/min.

Gas chromatography (Auto System Perkin Elmer, Waltham, MA) was used to determine the methane produced in anaerobic digestion. This equipment was equipped with a packed column and a thermal conductivity detector (Perkin Elmer). The cellulose crystallinity was examined using Fourier Transform Infra Red (FTIR) spectrometer (Nicolet iS10, Thermo Fisher Scientific), with an average of 32 scans and resolution of 4 cm^{-1} in the range of 500 cm^{-1} and 4000 cm^{-1}. Pre-treatment was performed in duplicate, while enzymatic and anaerobic digestions were performed in triplicate. The analysis results were delivered as an average of the data.

III. RESULT AND DISCUSSION

A. Raw Material Contents

The composition of untreated and pre-treated OPEFB is summarized in Table 1. The lignin content of the untreated material was 29.60% of dry-weight. The xylan content that represents the composition of hemicelluloses in the material was 21.97% of dry-weight, and glucose as a cellulose content was 38.74% of dry-weight material. Compare to other lignocellulosic biomass, OPEFB is one of the lignin-rich plants. This indicates that OPEFB can be classified as grass type (herbaceous), regarding similarity constituent with grass type of biomass (Table 2).

The lignin value was decreased to around 23% after the pre-treatment (Table 1). However, there is no significant difference in delignification carried out in different treatment condition. The varying concentration of ammonia reagent and additional treatment, following with acid treatment were not resulting in significant different of lignin removal. In addition, the xylan contents of pre-treated OPEFB samples were lower than that of the untreated sample. 5% ammonia as a reagent was decreased xylan higher than 7.5% and 10% ammonia concentration. It proves that xylan, as a main part of hemicelluloses, was easy to hydrolyse by low base concentration. The degradation resulted as a consequence of cleavage of the linkages between lignin and hemicelluloses (Lignin-Carbohydrate Complexes, LCC). A two-step treatment with 5% ammonia and 0.1M H_2SO_4 resulted in a removal of xylan by 81.75%, compared to 43.74% removal of xylan during one-step pre-treatment using 5% ammonia. This indicates that greater amount of xylan was released during acid pre-treatment. It proves that base and acid solution, even in a low concentration, could be easier dissolving hemicellulose in biomass content. On the other hand, the acid pre-treatment was reduced the lignin content of lignocellulosic biomass in small number also.

The pre-treatment with a lower concentration of ammonia (5%) also resulted in a reduction of existing glucan content in the lignocellulosic biomass, as a result of the dissolution of sugars contained in the material. But this phenomenon did not appear in a higher concentration of ammonia. Table 1 also shown that synergetic effect of two-step pre-treatment (ammonia and acid) not so significantly to remove lignin and reduce of glucan. The result of two steps pre-treatment showed a similar value with single ammonia pre-treatment. Furthermore, the acid pre-treatment was working well to reduce the amount of xylan in lignocellulosic biomass.

TABLE I
THE COMPOSITION OF UNTREATED AND TREATED OPEFB

Treated	**Lignin (%)**	**Xylan (%)**	**Glucan (%)**
Untreated	29.60	21.97	38.74
5% (w/w) Ammonia	24.10	12.36	23.01
5% (w/w) Ammonia + H_2SO_4	23.64	4.01	27.94
7.5% (w/w) Ammonia	24.59	18.61	32.73
7.5% (w/w) Ammonia + H_2SO_4	23.02	12.40	31.94
10% (w/w) Ammonia	23.62	19.85	36.93
10% (w/w) Ammonia + H_2SO_4	22.44	10.31	37.16

TABLE II
COMPARISON OF COMPOSITION OF LIGNOCELULLOSIC BIOMASS [17]

Treated	**Lignin (%)**	**Xylan (%)**	**Glucan (%)**
Hardwood	18 – 25	24 - 40	40 - 55
Softwood	25 - 35	25 - 35	45 - 50
Corn cob	15	35	45
Paper	0 - 15	0	85 - 99
Rice straw	18	24	32
Baggase	18 - 20	30 - 32	33 - 35
Grass	10 - 30	25 - 50	25 - 40

B. Enzymatic Hydrolysis

The effects of various treatment conditions on lignin and hemicellulose removal, crystallinity index, and enzymatic digestibility are shown in Table 3. One step pre-treatment offered higher insoluble solid recovery yield (around 88%) comparing with that of the two-step treatment. The solid lost under the two-step treatment was resulted by the removal of all the constituent of lignocelluloses, mainly hemicelluloses, as a result of the additional dilute-acid treatment step, and by the dissolution of sugars in the raw material. However, the removal of lignin was almost similar in both one-step and two-step pre-treatment methods due to the characteristic of lignin that can only be dissolved in alkaline (Table 1 and Table 3).

TABLE III

THE EFFECT OF VARIOUS TREATMENT CONDITIONS ON THE REMOVAL OF LIGNIN AND HEMICELLULOSE, THE CRYSTALLINITY INDEX, AND ON THE ENZYMATIC DIGESTIBILITY*

	NH_3 conc. (%)	Insoluble solid recovery yield (%)	Lignin removal (%)	Xylan removal (%)	Glucan recovery yield (%)	Crystallinity index (TCI)	Enzymatic digestibility**
Untreated	-	-	-	-	-	2.11	13.00
One-step	5	87.83	18.58	43.74	59.40	1.65	18.48
	7.5	88.48	16.93	15.29	84.49	1.60	18.76
	10	88.36	20.20	9.65	95.33	0.80	23.23
Two-step	5	82.46	20.14	81.75	72.12	1.30	18.68
	7.5	77.70	22.23	43.56	82.45	1.28	21.82
	10	76.83	24.19	53.07	95.92	1.24	21.58

*based on untreated OPEFB

**%theoritical maximum glucose after 96 hrs saccharification (g/g glucan added)

Moreover, a small amount of xylan can also be dissolved during the alkaline pre-treatment. This is typical of alkaline pre-treatment, which generally has a stronger effect on lignin than hemicellulose and cellulose.The retaining of hemicelluloses and cellulose into solid fraction was highest in 10% ammonia solution. It indicates that lower concentration of ammonia means a higher concentration of water in the solution, and it has big opportunity to break the chain of hemicelluloses and dilute the monomeric sugar from hemicellulose into solution.

The highest lignin removal was obtained when 10% (w/w) alkaline pre-treatment was combined with 0.1M dilute sulphuric acid pre-treatment. However, this sample did not show the best enzymatic digestibility after following enzymatic hydrolysis. Fig.1 showed the variation of glucose yield from enzymatic hydrolysis of untreated and treated OPEFB samples. The yield of glucose was increased from 13 (g/g glucan added) to 23.23 (g/g glucan added) after the pre-treatments. The highest increase of 78,7%, in glucose yield, was obtained after treatment by 10% (w/w) ammonia without a following additional dilute-sulphuric acid treatment step. This indicates that in addition to the presence of lignin there are other factors that influence the action of the enzymes on the biomass. According to Table 1 and Table 3, the presence of glucan after treatment on treated OPEFB gave a significantly impact to the enzymatic digestibility.

As it is shown in Table 3, pre-treatment with 10% ammonia conditions resulted in the lowest xylan removal, highest glucan recovery, as well as lowest crystallinity index, and as a consequence, the enzymatic digestibility was the highest for that sample. The presence of xylan does not affect the action of the enzymes used in this study because xylanase for the degradation of xylan to xylose was not included. As a result of the high concentration of solvent, the loss of sugars during the pre-treatment was slight. Therefore, a high enzymatic digestibility, as well as a high glucan recovery, could be achieved after this pre-treatment.

In addition, a two-step process with additional dilute-acid treatment step is not necessary, since comparing the result for to 10% ammonia treatment with or without an additional dilute-sulphuric acid treatment. The glucan recoveries in both cases were almost the same, 95,33 and 95,92%, respectively, in Table 3. On the other hand, the following process after ammonia treatment, the acid pre-treatment, was increasing the xylan removal. The percentage of xylan removal in the lignocellulosic residue after ammonia treatment was increased two-fold from the one step process. Therefore, the selectivity of ammonia treatment was proven by the result. The acid pre-treatment will be very effective to separate hemicelluloses from lignocellulosic biomass. Two-step pre-treatment could be the best technology to isolate the fraction of lignocelluloses, in the form of cellulose, hemicelluloses, and lignin.

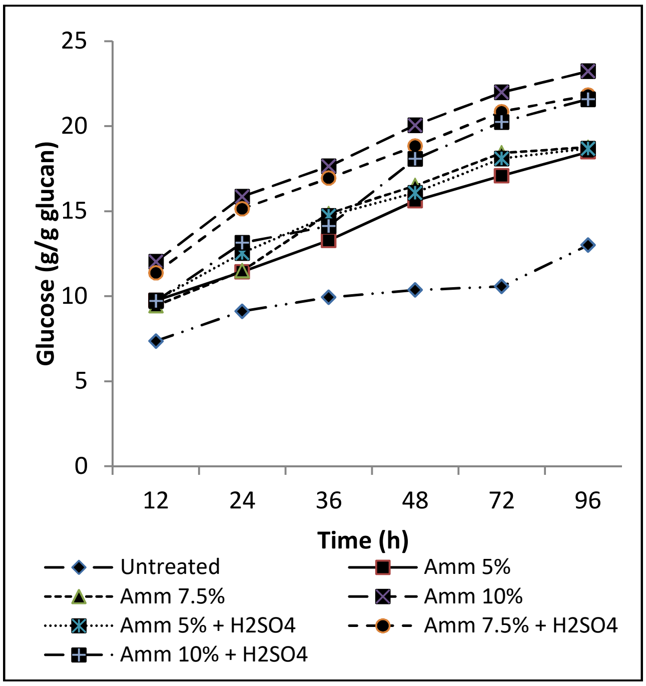

Fig. 1 Enzymatic digestibility of untreated and treated OPEFB

TABLE IV

COMPARISON OF SAA PROCESS AT ANY CONDITION

Material	Delignification (%)	Glucose Yield (%)
Corn cob [18]	80 - 90	92
Corn stover [11]	55 - 74	85
Barley hulls [16]	50 - 60	83
Rice straw [19]	35 - 60	82
Sorghum [20]	44	84

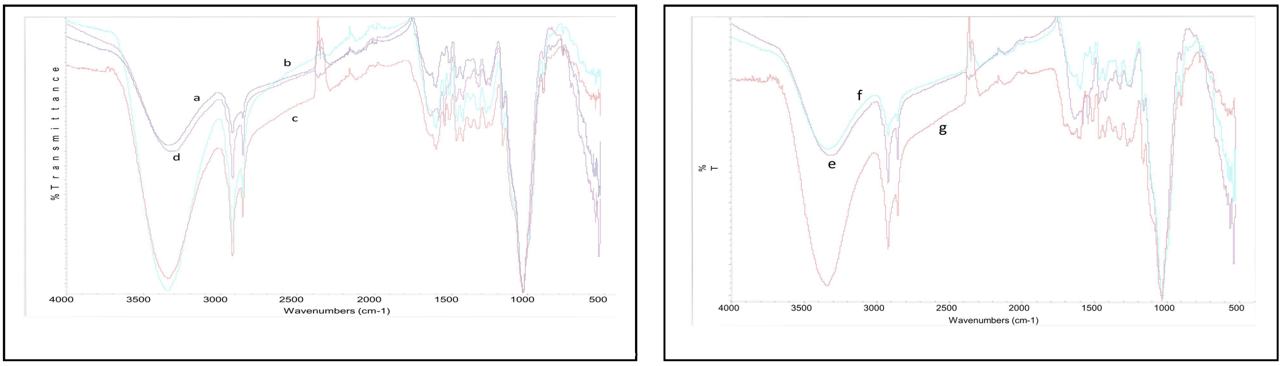

Fig. 2 Fourier transform infra red (FTIR) spectra of the untreated and treated OPEFB, (a) untreated; (b) 5%(w/w) NH_3; (c) 7.5%(w/w) NH_3; (d) 10%(w/w) NH_3; (e) 5%(w/w) NH_3 + 0.1M H_2SO_4; (f) 7.55%(w/w) NH_3 + 0.1M H_2SO_4; (g) 10%(w/w) NH_3 + 0.1M H_2SO_4

Comparing to the result of other researchers in SAA process at any condition (Table 4), these result has a similarity with other. In other studies, using SAA method with different lignocellulosic feedstock, the enzymatic digestibility of rice straw pre-treated at 69°C, 10 hours, 21% ammonia was 82% after 48 hours of saccharification time [18], corn stover treated at 60°C, 12 hours, 15% ammonia was 85% after 72 hours of saccharification time [20], and barley hull treated at 75°C, 48 hours, 15% ammonia was 83% after 96 hours enzymatic hydrolysis [15]. These results thus show that the enzymatic digestibility and hydrolyzability depend on feedstock, pre-treatment temperature, and ammonia concentration. It proves that SAA process is a promised technology in bioconversion process to produce monosaccharide (glucose) from lignocellulosic biomass.

The crystallinity index was obtained from the data of FTIR spectra. FTIR spectroscopy is a successful technique for the characterization the structure of cellulose [21]. Fig. 2 shows the IR spectra of the untreated vs. treated material. The 1,425 and 898 cm^{-1} absorption bands, which are assigned to the crystalline cellulose I and cellulose II, respectively, were used to study the type of crystalline cellulose and the changes in the crystallinity. Table 3 shows the total crystallinity indexes (TCI), which can be calculated as the absorbance ratio A1,425/A898 from the spectra.

Comparing the FTIR spectra in Fig. 2 shows that pre-treatments with ammonia in various concentrations reduced the absorbance band corresponding to cellulose I (crystalline cellulose) and on the other hand increased the absorbance band corresponding to cellulose II (amorphous part of cellulose), resulting in a decrease in total crystallinity indexes after the treatments. As Table 3 shows, one-step pre-treatment with 10% ammonia have the lowest index of crystallinity and also gives the highest enzymatic digestibility. The correlation between two effects of SAA process indicates that highest digestibility of enzymatic will obtained by decreasing of cellulose crystallinity.

The acid pre-treatment was not given the real effect to TCI. Table 3 shows that after soaking in acid solution, the TCI of the various sample has a similar value (around 1.28). The result shown that additional step with the acid solution was not given any advantages in reduced lignin content, decreased the crystallinity of cellulose and enhanced the enzymatic digestibility.

C. *Anaerobic Digestion*

Biogas (methane) production by anaerobic digestion was the effective method to calculate the successful of pre-treatment beside enzymatic digestion. Fig. 3 had shown the methane production of untreated and treated OPEFB per day (for 50 days treatment). At normal condition, based on theoretical, 1 gram cellulose will produce 415mL methane. The result shows that methane production was increased after ammonia pre-treatment for almost two-fold. The 10% ammonia concentration gave the highest result for this process after 50 days fermentation. Regarding Table 1 and Table 3, it proves that lignin removal, the crystallinity of cellulose and solid retained has significant effect to produce methane.

Methane production in two-step pre-treatment was not studied in this paper. Based on the result of enzymatic digestion, acid-solution pre-treatment did not have significant effect to enhance the digestibility of anaerobic fermentation, due to the characteristic of acid pre-treatment does not improve the glucan recovery. However, the composition of glucan in treated biomass is the main factor in increasing the digestibility.

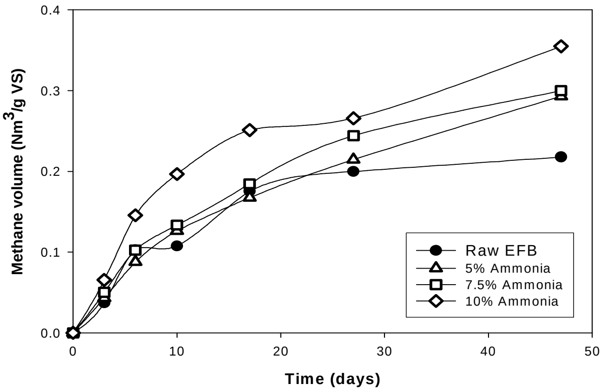

Fig. 3 Methane production of Untreated and Treated OPEFB per day

The result from two analytical methods, enzymatic and anaerobic digestions, has shown a similar trend for untreated and treated material. The digestibility of treated OPEFB was enhancing due to increasing of ammonia concentration as a per-treatment reagent. Furthermore, the correlation of two digestibilities was illustrated in Fig. 4. Fig. 4 had shown the correlation of digestibility of anaerobic versus enzymatic digestion. There is a linear correlation between anaerobic and enzymatic digestion for untreated and treated OPEFB. The anaerobic digestion will increase while the enzymatic digestion enhanced. It means that pre-treatment had an impact not only to enzymatic digestibility in producing bio-ethanol but also improve the digestion of anaerobic to produce methane as biogas.

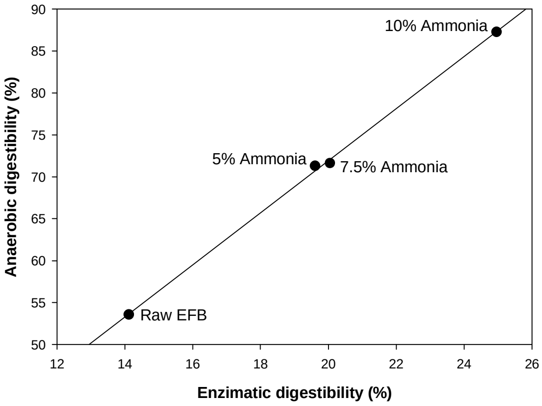

Fig. 4 Correlation of digestibility of anaerobic versus enzymatic digestion

IV. CONCLUSIONS

In spite of the expectations that the presence of lignin and hemicellulose in biomass can inhibit the enzymatic hydrolysis, our results showed that the main factor in increasing the enzymatic digestibility after aqueous-ammonia treatment of OPEFB is the reduced the crystallinity in the structure of cellulose and enhanced the glucan recovery. Even the higher removal of lignin or hemicelluloses had an impact to the digestibility, but the effectiveness is not significant. However, the selectivity of ammonia solution as a delignification agent in removing lignin, retaining hemicelluloses and cellulose into a solid fraction, and reducing the crystallinity of cellulose are the advantages of SAA process for bio-conversion by enhancing the enzymatic digestibility and anaerobic digestion. The two-step of pre-treatment process, soaking with ammonia solution and following with acid solution, was not significantly effected to both of digestibility methods. This process was necessary to isolate the component of lignocellulosic biomass into three main fractions, i.e. cellulose, hemicelluloses, and lignin.

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