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BIOSUGAR PRODUCTION FROM OIL PALM MESOCARP FIBER (OPMF) USING VISCOZYME

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ABSTRACT

In this study, the performance of HNO_3 and NaOH pretreatmentson oil palm mesocarp fiber (OPMF) was evaluated based on the residual carbohydrate. The best pretreatment condition was ascertained for reducing sugar and biosugar produced after saccharification with viscozyme. The pretreatment with 2 % (v/v) HNO_3 improved cellulose concentration from 33.14 % to 60.0 %, while hemicellulose, lignin and ash were reduced by 25.59 %, 10.0 % and 25.9 % to 10.0 %, 5.33 % and 3.0 %, respectively. Enzymatic treatment using 1g solid loading, produce highest production in terms of arabinose 10.5 g/l, glucose (1.1 g/L) and xylose (0.42 g/L) was obtained under agitation. While for 10 grams solid loading, highest level of bio-sugar was obtained with arabinose (0.8 g/L) and glucose (1.6 g/L). FESEM-EDX and FTIR images before and after saccharification successfully showed the effect of enzymatic treatment on the mesocarp fiber.

Keywords: OPMF, HNO_3, lignocellulosic, enzymatic saccharification, biosugar.

1. INTRODUCTION

The major cost in biofuel production such as ethanol, is the substrate, i.e. carbohydrate in the form of sugar; starch, cellulose, hemicellulose. Even though some sugars are easily available from agricultural by-products, straws from wheat and corn, husk from rice and bagasse [1,2].Extension of this process towards exploitation of lower-value substrates such as lignocellulose offers reduction in the production cost, make increase in the use of biofuels and in addition assuring food security. Lignocellulosic biomass is nature's most abundant raw material can be obtained from hardwood, softwood, grasses, and agricultural residues.

Biosugars, bioethanol and other kinds of biofuels are biobased products. It can be suitably established in regions with biomass availability [3]or else the market consequence will tell on the products. For example, Brazil is known for high cultivation sugar-cane [4, 5], may have the raw material of choice for bioethanol as bagasse, while USA is known for high cultivation of corn [6] may have abundance of corn straw as substrate relative to countries like Malaysia, Indonesia, and Thailand, known for high cultivation of oil palm. Since the cultivation of the crops is a gift of nature, therefore, diversification in the generation of raw materials has become necessary. A potential region for any biobased industrial products possesses surplus biomass and has no problems with food security. Understanding the fact that biobased products are region championed and Asia is the largest oil palm producer worldwide, necessitated for more exploration of its abundant oil palm biomass.

In Malaysia, the favourable weather condition encourages all year cultivation of oil palm [7], as such huge biomass generation of ~ 6.93 million tonnes in dry basis of oil palm biomass[8], a potential bioresources for the bioconversion into value-added products such as biosugars, biofuels, bioplastic, cellulose and composite. Oil palm mesocarp fiber (OPMF) is a waste material of palm oil extraction and the expectation is that oil palm mesocarp fiber biomass will grow beyond what can be burnt by the limited boiler capacity of the palm oil mills [9]. At present, the biomass is used as a boiler fuel and mulching [10] medium, consequently, alternative biotechnological procedure for overcoming the huge biomass under mild condition has become environmental mandate.

Oil palm mesocarp biomass as a lignocellulosic material is rich in, cellulose and hemicellulose therefore, can be a suitable substrate for biosugar production. Conversion of lignocellulosic can be conducted by means of enzymes or acids. Acid hydrolysis has the set back of high severity condition and production of inhibitors [11]. Combatively, enzymatic hydrolysis offers advantages of reduce energy and water utilization as they can work under unsevered conditions, offer yield improvement, in addition to the fact the enzymes are biodegradable [12].

Pretreatment is a must for these complex polysaccharides in order to expose the complex sugar to hydrolysis. Exposing the complex sugar will give access for enzymatic conversion of the complex sugar to simple sugar. Simple sugars, such as glucose and xylose are essential carbon sources for microbial fermentation to produce numerous biofuels and other value added product [13, 14, 15]. This work presents a commercial 'viscozyme' saccharification of OPMF fiber after pretreatment with HNO_3 for biosugar production, as one of the biotechnological methods to reduce biomass volume generated in palm oil industries.

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2. MATERIALS AND METHODS

2.1 Materials

OPMF (17-30 mm) was collected from Mahamurni palm oil plantation, Sedenak, Kulai, Johor Bahru, Malaysia. Viscozyme L and all sugar, namely glucose, arabinose, xylose, cellobiose, mannose and fructose were from Sigma Aldrich, USA.NaOH was purchased from RCI Labscan Thailand, while HNO_3, H_2S0_4 andn-Hexane were purchased from QRëc Malaysia.

2.2 OPMF preparation

The OPMF was washed with a commercial detergent to remove residual oil and dirt, and then rinsed till no foam was formed. The fiber was then oven dried at 70 °C for 24 hours. Impurities such as crushed kernels and shells were manually removed by hand pick in order to avoid inaccuracy in the lignocellulosic analysis. The fiber was then re-sized by grinding in a blender, and then sieved to an average fiber length of 2 mm. Then, it was stored at room temperature for subsequent experiment.

2.3 OPMF Pretreatment

Pretreatment is carried out to reduce the lignin content. The pretreatment of OPMF fiber was carried out according to Noratiqah *et al.* [16] and Iberahim *et al.* [17] with slight modification. OPMF was soaked in 1 to 6 (v/v) HNO_3 and NaOH, at a ratio of 1:10 (w/v) for 4 hours. Afterwards, autoclaved for 5 and 15 minutes at 121 °C. The pretreated fiber was filtered and washed several times with distilled water until the HNO_3 and NaOH traces was eliminated (pH value of 6 to 7). The fiber was then oven dried at 70 °C for 24 hours and then stored inside a sealed plastic container at room temperature for further use.

2.4 Analysis of OPMF lignocellulosisc

Lignocellulose composition of the mesocarpfiber was ascertained in line with the procedure described in[18]. The amount of extractives in OPMF biomass was examined by solvent extraction. Acetone, 60 ml was added to 1 g of dried biomass sample held at 90 °C for 2 hours. The sample was then dried at 105 °C until a constant weight was obtained. The weight difference before and after the extraction is the amount of the extractives[18,19]. To determine the amount of hemicellulose, 10 ml of 0.5 mol/l of NaOH solution was added to 1 g of extractive-free dried biomass held at 80 °C for 3&1/2 hours. Then, the sample was washed using deionised water (DI) until the pH value of 7, then dried to a constant weight. The difference between the sample weights (before and after treatment) is hemicellulose content [19].

Lignin content was determined by adding 30 ml of 98 wt. % H_2SO_4 for 1 g of extractive-free dried biomass. Afterwards, the sample was held at ambient temperature for 24 hours, and boiled at 100 °C for 1 hour. The mixture was filtered and the residue washed until the sulfate ion in the filtrate was untraceable (via titration with barium chloride, 10% solution); then dried to a constant weight. The residual weight was recorded as the lignin content [19]. The content of cellulose was calculated by the difference, assuming that cellulose, hemicellulose, lignin and extractives are the only constituents of the entire biomass [19, 20]. All final product yields of the biomass are expressed on a moisture-free basis [19].

2.5 Enzymatic Saccharification

2.5.1 Enzymes

Viscozyme L, is an endo-1, 3 (4)-beta-glucanase, a commercial cell wall degrading enzyme complex from *Aspergillusaculeatus*, with activity of 100 Fungal Beta-Glucanase Units (FBGU) /ml (declared activity from the product data sheet). The enzyme is also a commercial enzyme preparation containing various enzymatic activities, mainly cellulase, hemicellulose and pectinase.

2.5.2 Saccharification

Saccharification process was performed in a 250 ml Erlenmeyer flask using 3.80 U/ml viscozyme with substrate loading of 1 % (w/v) and 10% (w/v) OPMF. The process was conducted at 50 °C, 150 rev/min using incubator shaker (LABWIT, ZHWY-100D). A 5 ml sample was drawn at 24 hour interval and was centrifuged at 3500 rpm, 4 °C, for 20 mins. Sampling was done from day 1 to 10. The supernatants were collected and analysed using 3, 5-dinitro salicylic acid (DNS), Gas chromatography and mass spectrophotometer (GCMS) and High performance liquid chromatography (HPLC). Non-enzymatic experiment with the same substrate loading and physicochemical conditions was simultaneously conducted as a control experiment.

2.6 Reducing sugars determination

The reducing sugar concentration was determined using spectrophotometer UV-vis (Thermo Fisher SCIENTIFIC), Model G10S UV-Vis, in line with the 3, 5-dinitro salicylic acid (DNS) method [21]. The enzymatic saccharification was expressed as the yield of sugars by the quantification of the reducing sugars concentration in the filtrate, as resulted from the DNS method.

2.7 Field Emission Scanning Electron Microscopy and Energy-dispersive X-ray spectroscopy (FE-SEM & EDX)

The surface morphology together with elemental analysis of OPMF and HNO_3-treated OPMF samples was observed both under a FE-SEM-EDX (FESEM; SU8020, Hitachi, Japan) with an Oxford Inca EDX (Oxford Instruments, Oxfordshire, UK). Prior to the FE-SEM-EDX examination, oven-dried OPMF samples were mounted on the stub and gold-coated in a sputter coater Quorum, Q150R (United Kingdom). The FE-SEM-EDXmicrographs were obtained with an acceleration voltage of 2.0 kV.

2.8 Fourier Transform Infra Red (FTIR)

FTIR analysis was recorded using a Nicolet IS5 device (Thermo scientific, USA) at wave number range, 4000 to 500 cm^{-1}. The instrument was operated at a resolution of 4 cm^{-1}, data spacing 0.482 cm^{-1} with a scan

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cycle/s of 32, accessories with iD5 Attenuated total reflectance (ATR) to obtain reflection spectra of OPMF samples.

2.9 Gas Chromatography Mass Spectrometry (GC-MS) sample preparation

Sample preparation for the GCMS analysis was carried out in the ratio of 1:10 sample to n-Hexane(extraction solvent) for 4 hour at 200 rev/min, after which were centrifuged at 3500 rpm, 4 °C, for 20 min. The upper layer was discarded and the lower layer was subjected to vacuum rotary evaporator to remove any residual solvents [22, 23]. The sample was filtered through a 0.45 syringe filter to 1 ml vials.

2.10 Gas Chromatography Mass Spectrometry (GC-MS)

Biosugars and all the phytochemicals in the samples were detected by GC-MS carried out on a GC (Gas chromatography) CLARUS 680 Perkin Elmer system attached to a Mass spectrometer (MS) perkilin Elmer, employing the following instruments settings: GC, performed using a30 cm x 0.25 mm id x 0.25 µm film thickness, HP- 5MS column, Agilent, Malaysia. Mass scanning range of 50 to 650 m/z revealed a mass spectra record of 2.48 scans s^{-1}. Sample injection was at 280°C, interface 280°C with separations achieved by using the following temperature settings: isothermal heating of 3 mins at 80°C, followed by a 5°C /min to 315°C (oven ramp). The final isothermal heating was at 315°C, 14 and mins for polar and lipophilic samples respectively. Compounds identifications were carried out by comparing the GC-MS spectral database with the National Institute of Standards and Technology library (NIST05) [23].

2.11 High Performance Liquid Chromatography (HPLC)

Polyoses production in the sample was detected by using HPLC Agilent 1100 series, Refractive index detector (RID), and a Shodex SP0810, Carbohydrate Analysis Column (8.0 mm ID x 300 mL). Modified methods by Noratiqah *et al.* [16]was adopted, however, better peaks were obtained at 0.6 ml/min flow rate of 100% filtered (x7 via a filter paper) nanopure as mobile phase. The combination of glucose, arabinose, xylose, cellobiose, mannose and fructose were used as the standards sugars and all were of analytical grades with 99% purity. A concentration 1 % (w/v) of each of the standards was diluted into a nano pure and filtered through a syringe filter 0.2 µm into a 1 ml vials.

3. RESULT AND DISCUSSIONS

3.1 Lignocellulose Composition of OPMF

The lignocellulose composition of the mesocarp fiber used in this experiment is shown in Table-1. The cellulose, hemicellulose, lignin and ash content prior to pretreatment were analyzed to be 33.14 %, 25.59 %, 25.38 % and 5.33 % respectively. Previous studies reported almost a similar OPMF composition with this study with a higher hemicellulose content; cellulose (34.5 %), hemicellulose (31.8 %), lignin (25.7 %) and ash (3.5 %) [23].Lim *et al.* [9] reported lower cellulose content with a higher hemicellulose and lignin composition; cellulose (21.3 %), hemicellulose (31.9 %) and lignin (26.9 %).

Table-1. Lignocellulose composition of the OPMF.

Lignocellulosic content	OPMF Before treatment	OPMF after enzymatic saccharification	OPMF after Pretreatment								
			1 %		2 %		4 %		6 %		Time (min)
			NaOH	HNO_3	NaOH	HNO_3	NaOH	HNO_3	NaOH	HNO_3	
Cellulose	33.14	49.58	39.0	38.2	45.2	60.0	58.00	53.2	54.5	30.4	5
			43.8	39.4	46.5	55.43	60	38.5	53.0	28.0	15
Hemicellulose	25.59	0.0	20.4	17.6	16.0	10.0	12.0	3.0	4.5	0.0	5
			19.3	13.3	14.4	8.3	11.0	0.0	3.0	0.0	15
Lignin	25.38	14.4	23.34	22.5	18.6	14.6	16.3	10.0	10.4	7.6	5
			22.34	20.0	16.6	12.2	16.0	7.3	9.0	5.0	15
Extractives	5.33	3.1	4.7	5.0	3.4	3.0	3.0	2.8	2.4	1.7	5
			4.0	4.6	2.5	3.0	2.2	2.8	2	1.2	15

3.2 OPMF Pretreatment

Pretreatment is basically carried out to increase the cellulose and reduce the lignin content to allow for efficient enzymatic conversion of biomass. At 1 % concentration of HNO_3 and NaOH, no much improvement in the lignocellulose composition was observed (Table 1). Optimum improvement in cellulose composition was obtained with HNO_3 at 2 % and 5 min of autoclaving. This condition increased the composition of cellulose from 33.14 to 60.0 % (Table 1), with a percentage increased by 81.0 %. Hemicellulose and lignin content decreased from 25.59 % and 25.38 % to 10.0 % and 14.6 %, with percentage reduction of 60.9 %, 42.47 % respectively.

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Further increased in the concentration of HNO3 to 6 % could not improve the cellulose content, however, lignin and hemicellulose was consistently removed. NaOH pretreatment results in equal cellulose improvement at 4 % NaOH 15 mins autoclaving. Cellulose increased from 33.14 to 60 % with a percentage increase of 81.0 %. Hemicellulose and lignin content decreased from 25.59 % and 25.38 % to 11.0 % and 16.0 %, with a percentage decrease of 57.01 and 58.72, respectively. Since one of the major requirements of a good pretreatment is that it must improve cellulose production before and after saccharification as well as remove lignin shield of biomass (Sun and Cheng, 2002). Considering the two methods, both have improved the cellulose content, but percentage reduction of lignin with HNO_3 is higher 42.47% as compared to the 16.0 %, NaOH. Thus pretreatment with HNO3 may likely give higher saccharification yield. Besides that less acid is consumed as 2 % as compared to the NaOH of 4 %.

Ioelovich and Morag [24] studied the enzymatic conversion of switch grass after acidic pretreatment; they reported an increase in cellulose content by 55 %, lignin content by 28 % and reductions in hemicellulose content by 7 %. Treatment of fibers with HNO_3 may react with OPMF by oxidizing carbonyl groups and nitrating aromatic components to subunits. A similar condition was reported with wood aspen fiber [25]. This aids in hydrolysing the fiber to low molecular units. Digestibility of lignocellulose structure is stuck by the presence of lignin and the goal of pretreatment is to disrupt the lignin structure and unsettle the crystalline structure of cellulose so that enzymes can have an easy access [26].

In acid pretreatment there is a direct relationship between cellulose increment and hemicellulose reduction as much of the hemicellulose components are released into the pretreatment hydroxylates, it has been reviewed by Mosier *et al*. [26]that glucose yields from cellulose rise with hemicellulose removal to almost 100 %. Dilute acid treatment increases substrate porosity which in turn increased enzymatic hydrolysis [27].

The use of nitric acid as a pretreatment agent has been effective in increasing the cellulose composition of oil palm biomass with reduction of lignin content [28]. Therefore, the use of HNO_3 for the pretreatment of OPMF is recognized as effective in increasing cellulose and reducing the unwanted component of the OPMF.

3.2.1 Field Emission Scanning Electron Microscope (FE-SEM-EDX)

The FE-SEM-EDX analysis revealed the surface morphology of OPMF. The fiber structure before and after pretreatment is shown in Figure-1. The surface of the untreated OPMF looked smoother than the pretreated mesocarp fiber which is somewhat rougher and textured. The smoothness is as a result of the presence of lignin that cemented the cellulose and the hemicellulose[17] while the roughness is as a result of the effect of HNO_3 pretreatment on the lignocellulose structure. Study on disruption of oil palm empty fruit bunch (OPEFB) revealed HNO_3 as a highly potent solvent that brought about disruption of lignocellulose[16].The unpretreated fiber has on its surface globular bodies attaching themselves into aggregates, presumably silica. Analogous observation of silica spreading on the entire mesocarp fiber was reported in literatures [29, 30, 31].

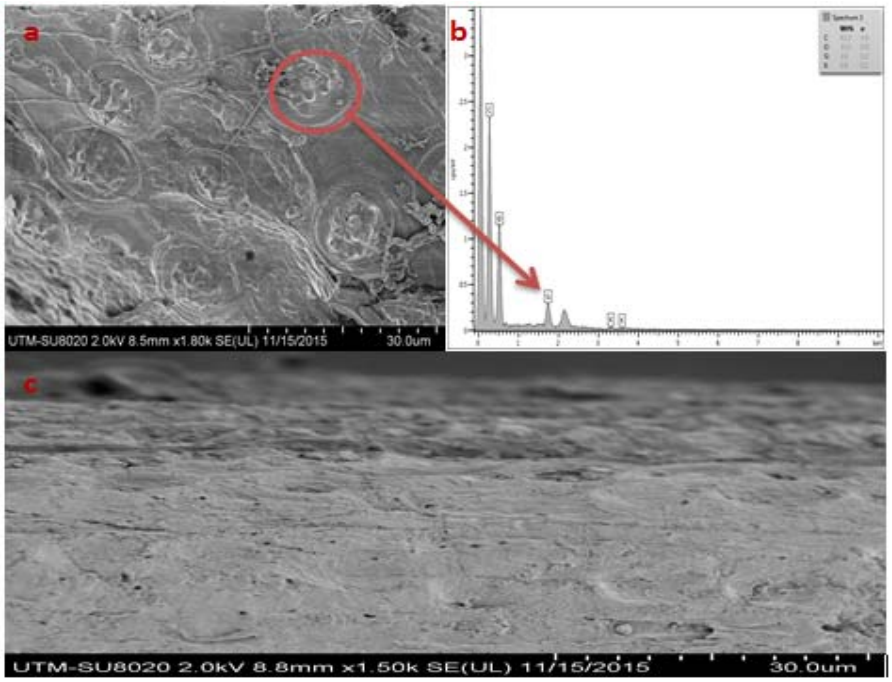

Figure-1. (a) Untreated OPMF (b), Microanalysis spectrum of untreated OPMF indicate the particle is silica (c) OPMF after pretreatment with 4% HNO3 4 min).

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To buttress the above assertion, elemental analysis by EDX also showed 3.9 % (w/t) silica in OPMF as in Figure-2(b). Other elements in EDX analysis revealed the presence of carbon (C), oxygen (O) and potassium (K).Nordin *et al.* [32] also employed the EDX tool to verify silver particles bodies as silica [32]. Their findings also revealed the same elemental content as in this study, with the additional element, K. A shaft looks at the surface of the pretreated fiber unveiled tiny pores spaces as in Figure-1 (c). The presence of these spaces indicates the further effect of the acid in removing the lignin, hemicellulose and presumably silica. The removal of the silica bodies on the lignocellulose surface will ease the access of enzymes to the cellulose structure[33]. After the enzymatic saccharification of the fiber, severe disruption occurred to the lignocellulose structure and completely damaged the wall of the fibers, as seen in Figure-1 (d). An analogous observation was described as in[17, 34]. With the images differences observed, it can be concluded that HNO_3 has effectively modified the lignocellulose structure of OPMF.

3.2.2 Spectral analysis by FTIR

FTIR spectroscopy was used in identifying the functional groups and chemical constituents of the OPMF before and after HNO_3 pretreatment. The samples were referred to as OPMF (un-treated) and HNO_3OPMF (treated with HNO_3), respectively (Figure-3). The key differences between these two spectra are seen conspicuously at peaks 2162.23 cm^{-1}, 1979.60 cm^{-1}, 1733.01 cm^{-1}, 1550-1506.57 cm^{-1} and 1420 cm^{-1}. Table-2 contains the description of wavenumbers in terms of the components based on the functional groups in the OPMF and HNO_3OPMF.

The presence of acetylenic compounds is not very common in the FTIR spectra of lignocellulolytic structure. The spectra allied with the C≡C structure can be its characteristic and are assigned with wavenumber 2140-2100 cm^{-1} while aromatic combinations includes 2000-1660 cm^{-1}[35]. The presence of carbonyl bond (C=O) in OPMF was located at 1733.01 cm^{-1} and 1746 01 cm^{-1}. These bonds represent ketone, aldehyde or carboxylic acid, characteristics of hemicellulose [31]. The bond is seen weaker as shown in the HNO_3OPMF spectrum. The band at 1505 cm^{-1} according to [31] represents aromatic ring of lignin is more marked on the OPMF spectrum than HNO_3OPMF spectrum, this band was elucidated with C=C-C characteristics of wavenumber 1510-1450 cm^{-1} aromatic ring stretch[35]. According to Pandey [36], wave number in the range of 1590-1600cm^{-1} matched to typical band of aromatic skeleton, a future best qualified for lignin. The OPMF among its component has lignin as the only compound with aromatic monomers.

Bahrin *et al.* [37]described the presence of cellulose with 1420 and 1430 cm^{-1}bands, correspondingly amorphous and crystalline structures respectively. Mahmud *et al.* [29], further labelled amorphous and crystalline cellulose I to be associated with band 1420 cm^{-1} and cellulose II to 1430 cm^{-1}, both as a result of C-H bending. Moreover, adsorption at 1246 cm^{-1} aligned to polysaccharides like cellulose which is seen in both spectra, by and large hemicellulose 1254 cm^{-1}, as a result of C–O stretching of acetyl group in hemicellulose. The increase in the intensity indicated the improvement in cellulose content after HNO_3OPMF. The intensity of the peak at 1420 reduced after HNO_3pretreatment, this shows reduction in the crystalline cellulose, a condition required for successful enzymatic conversion [29]. Intra and inter molecular stretching band of O-H is observed between a range of 3200-3400 cm^{-1}, which appeared broader in the OPMF than the HNO_3 fiber, implying the longevity in the chain polymers, similar results was reported in [38, 39].

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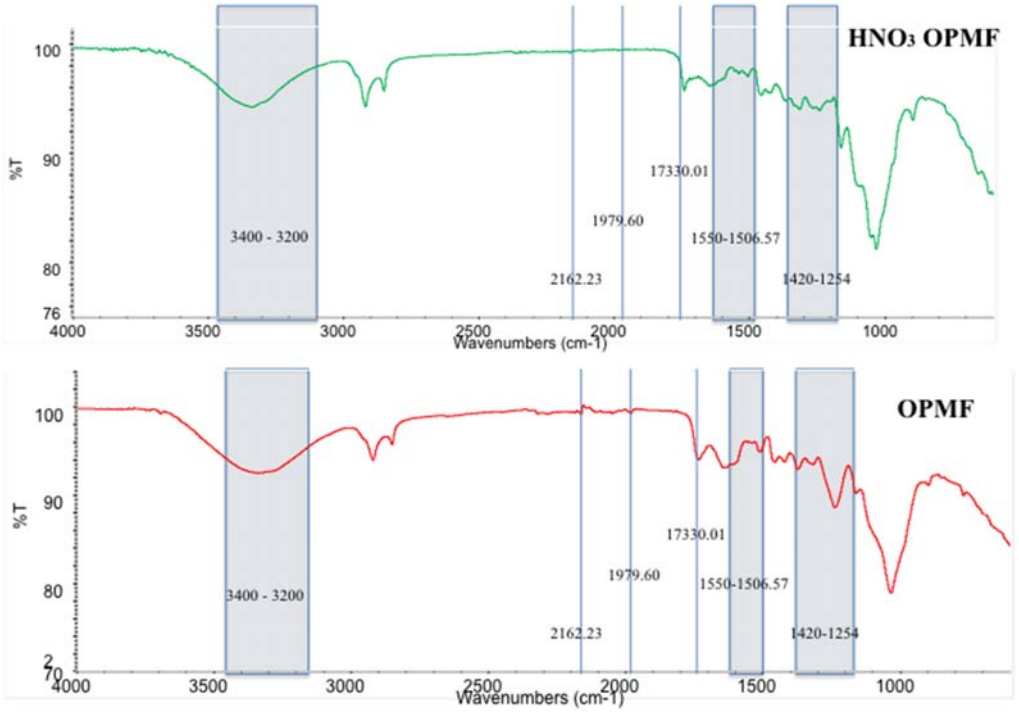

Figure-2. FTIR spectra of OPMF and pretreated HNO_3OPMF.

The FTIR results have revealed the chemical changes associated with the fiber after HNO_3 pretreatment. Bands disappearance associated with hemicellulose removal shows that the pretreatment applied was effective in reducing the unwanted chemical content that normally slows down enzymatic biomass conversion to biosugar.

Table-2. Description of components based on the functional groups in the OPMFs.

Wave number (cm^{-1})	Vibration/ Functional groups	Component	Reference
2140-2100 2000-1660	C≡C/ Aromatic combination	Lignin	[35]
17330.01 17400.01	C=O/ ketone, aldehyde or carboxylic acid	Hemicellulose	[31]
3200-3400	OH/intra and inter molecular stretch	Elongation in polysaccharide	[38,39]
1505, 1510–1450	C=C-C / aromatic ring stretch	Lignin	[40][31,35]
1590-1600	Aromatic skeleton	Lignin	[41]
1420, 430	C-H/ C-H bending	Cellulose, amorphous and crystalline, crystalline cellulose I & II	[29,37]
1254	C-O/ acetyl group stertch	hemicellulose	[29]

3.3 Enzymatic treatment of OPMF

Series of sampling was carried out every day for 10 days as seen in Figure-3. From Figure-4, for 1 g solid loading, the highest reducing sugar was obtained on days 5 and 6 with production equivalent of 3.3 g/L all with agitation, stationary experiment gave a corresponding result of 3.1 and 3.2 respectively. Highest reducing sugar of 10 g solid loading was on day 2, 1.2 g/L, under agitation, a corresponding stationary experiment gave 1 g/L. Further, increase in the solid loading up to 50 g could not increase the amount of reducing sugar produced. Analysis of each category shows that agitation plays a role in enzymatic saccharification of mesocarp fiber. Probably the solid loading is high for the enzymatic degradation.

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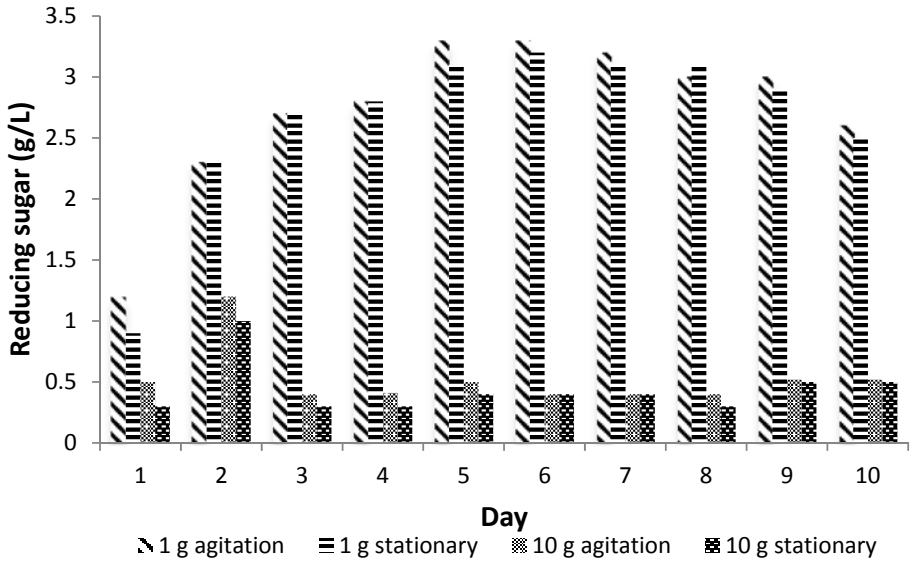

Figure-3. Reducing sugar produced at 1g and 10 g solid loading.

The compound generated from gas chromatography and mass spectrophotometer analyses are contained in Figure-4. The first five compounds in the Figure-4 are sugars, namely; DL-arabinose, xylose, fructose, glucose and mannose. The presence of the phenols, flavone, 5-hyroxy-4-methoxy-7 methyl, and glycerol in the saccharification hydrosylate is not surprising as the palm pressed fiber oil which is extracted from the oil palm fruit is rich in phenols and phenolics, lignocellulosics and fatty acids of which the glycerol is the back bone.

Moreover, the sterilization of fruits (palm) deactivates polyphenoloxidases which are able to preserve the phenolic phytochemicals in the wastes streams from the oil mills. Phenols are biologically active compound and the detection of this compound indicates that phenols in oil palm mesocarp can withstand the physicochemical pretreatment applied. Research showed that oil palm phenolics possesses anti-proliferative actions on tumour cells both in vitro and in vivo and can boost the growth of unaffected cells [42]. Other important biologically active components of the mesocarp fiber such as carotenoids, tocopherols, tocotrienol, are not detected probably due to the effect of the pretreatment that may have caused their denaturation.

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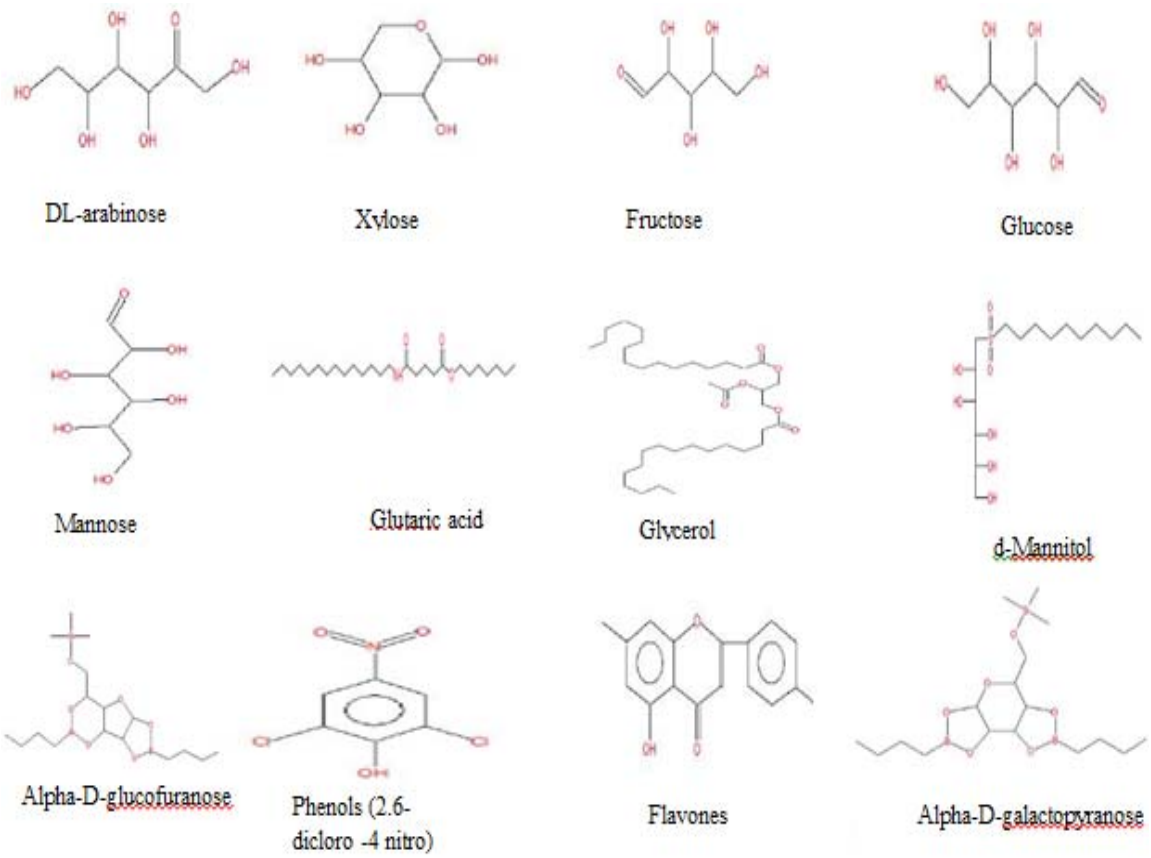

Figure-4. Structures of compounds generated from saccharification of OPMF.

3.4 Biosugar generated from saccharification of OPMF

A preliminary analysis was conducted to detect the entire components of the OPMF after pretreatment and enzymatic saccharification. HPLC analysis quantified the polyoses from the enzymatic saccharification of OPMF which showed 3 major biosugars, namely; glucose, xylose and arabinose as a result of the conversion of cellulose, xylan and arabinan respectively.

The highest biosugar yield obtained is arabinose on day 6, as shown in Figure-5, where 1 g solid loading OPMF results to 10.5 g/l (agitation) and 1.0 g/l (stationary). Solid loading of 10 g gave the highest yield of arabinose on day 2, with 0.8 g/l and 0.7 g/l, under agitation and stationary experiments, respectively. Glucose yield was also the highest on day 6, with 1.1 g/l in both experiments, while at 10 g solid loading 1.0 g/l and 0.9 g/l was attained under agitation and stationary experiment respectively, the result is presented in Figure-6. In the case of xylose, 1g solid loading yielded 0.2 g/l and 0.1 g/l, while higher loading of 10 g yielded 0.35 g/l and 0.31 g/l corresponding to agitation and stationary experiment. Xylose conversion was not detected during the first two days and the last three days of 10 day saccharification as seen in Figure-8. A careful observation of the Figures (5, 6 and 7), in the entire different substrate loading, agitation played a role in improving the enzymatic degradation of the OPMF. A reason ascribed to that is due to the heterogeneous nature of the systems, and therefore, the enzymes and products need a transfer from the solid substrate to the solvent bulk.

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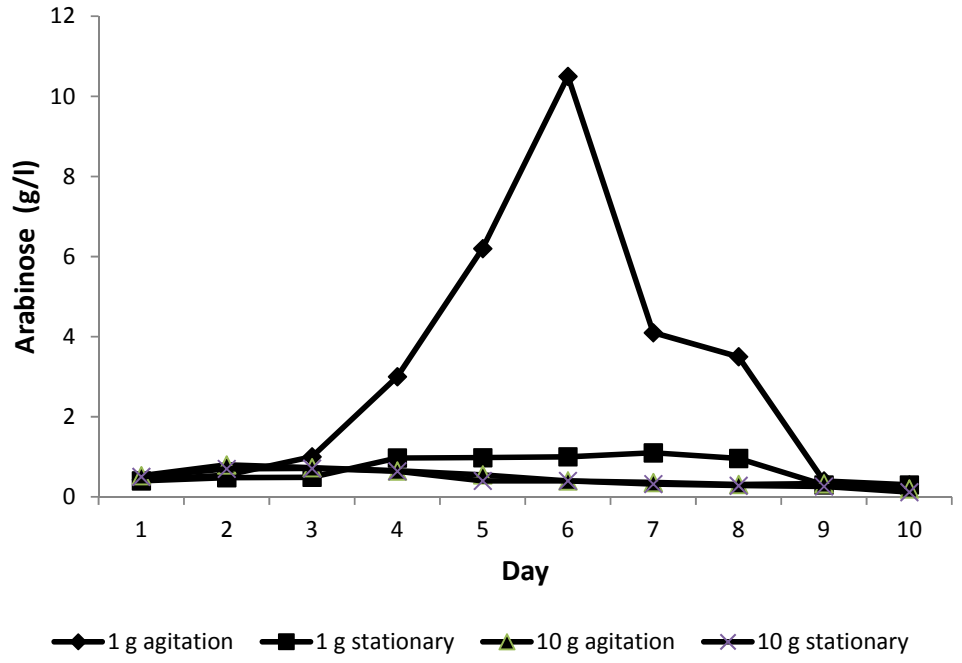

Figure-5. Arabinose production from 1 g and 10 g solid loading.

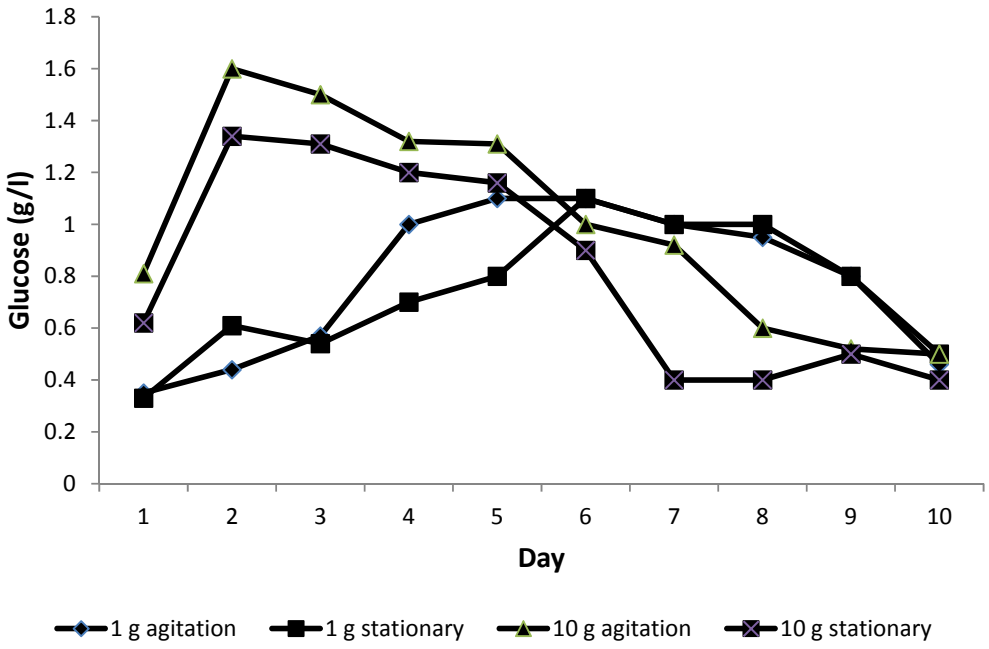

Figure-6. Glucose production from 1 g and 10 g solid loading.

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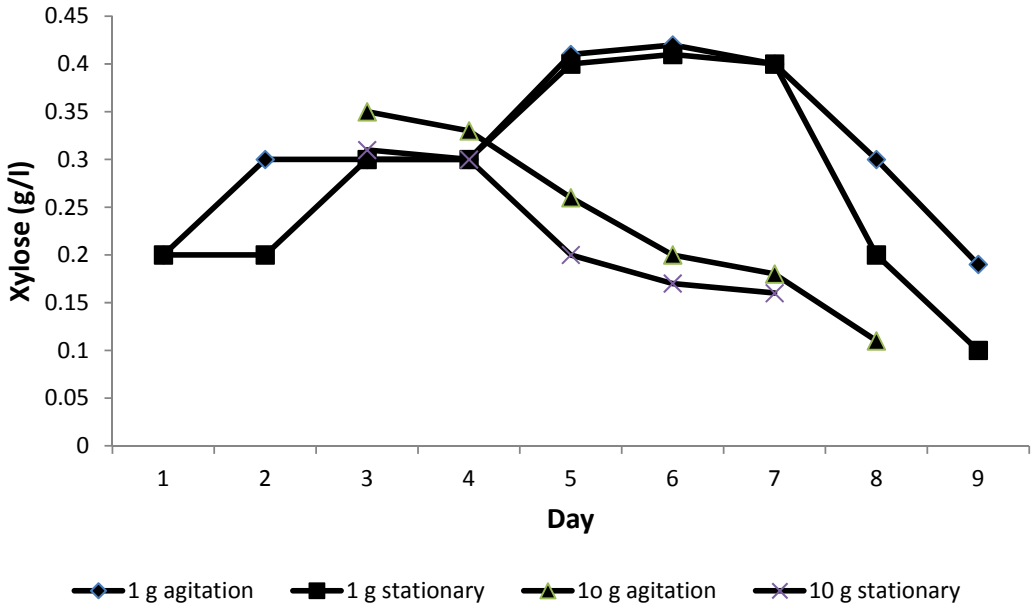

Figure-7. Arabinose production from 1 g and 10 g solid loading.

Analysis of the lignocellulose composition of OPMF after the enzyme treatment showed a decrease in content of cellulose and hemicellulose from 60 % and 10 % to 49 % and 0 %, respectively. Therefore, there was a complete conversion of the hemicellulose as as seen in table 1, a reason that led to the highest production of arabinose. Figure-8, below shows the OPMF after enzymatic treatment. The figure displays some perforation as a result of the enzymatic attack.

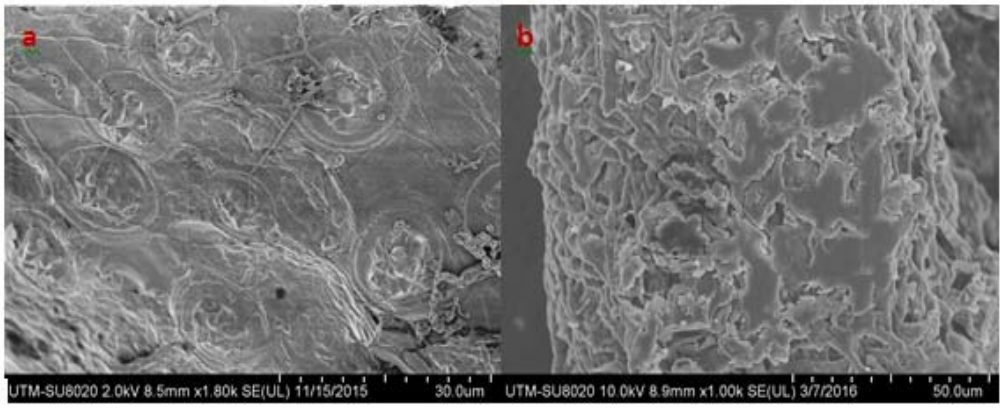

Figure-8. (a) Untreated OPMF (b) OPMF after enzymatic treatment.

As presented in this study 1 g enzyme loading resulted in higher sugar yield, which, according to Arapoglou *et al.* [43] the yield of enzymatic hydrolysis depends on factors such as enzyme type and its concentration, substrate concentration, and the process conditions such as temperature and pH.

The choice of viscozyme in this case is not questionable as literatures have reported the enzymes to be successful in hydrolyzing different substrate including micronized biomass, soybean hulls, agave bagasse, sugarcane bagasse as well as oil recovery from shea seeds[44,45,46,47]. Viscozyme preparation contains various enzymatic activities mainly cellulase and hemicellulose. Cellulase formulations contain a broad range of enzymes that work synergistically to convert cellulose to simpler sugars, namely endo-and exo-glucanases, and β-glucosidase. Endoglucanases randomly hydrolyzes available intra molecular β- 1,4-glycosidic bonds of the cellulose chains, creating new chain ends; exoglucanases further cleave cellulose chains at the ends which release soluble cellobiose or glucose, and β-glucosidases hydrolyze cellobiose to glucose. The degradation of hemicellulose is accompanied by endo-1,4-β-xylanase (xylanase) enzymes cleaving the β-1,4-

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glycosidiclinkage between xylose residues in the back bone of xylans (a major predominant polymer of hemicellulose). Next to the xylan include L-Arabinan that require α-L-arabinanases and a-L-Arabinofuranosidases to hydrolyze mannan L -arabinan and arabinofuranosyl-content of Hemicelluloses. Therefore, in this case, it can be presumed that 10 grams above of OPMF is higher for the enzymatic conversion.

The highest total reducing sugars produced by means of commercial enzymes were achieved with the overall conversions of OPMF, glucan and xylan of 87% and 60.73% respectively. Most researchers that produce higher values than this study use enzyme consortia probably to arrive at higher yields or the nature of the substrate necessitated that, as some substrate require multiple enzymes such as liquozyme and ternamyl for starch liquefaction. In this study, interest was in finding out the biosugar type that can be obtained from the OPMF using viscozyme as a sole enzyme.This will enable the study of the performance of individual enzyme in consortia.

4. CONCLUSIONS

OPMF was treated with HNO_3which improved cellulose concentration from 33.14 to 60.0 %, while hemicellulose, lignin and ash were reduced by 25.59 %, 10.0 %, 25.9 % to 10.0 % and 5.33 % to 3.0 % respectively. The enzymatic treatment with 3.80 U/ml enzymes hydrolyzed OPMF to biosugars in terms of glucose, arabinose and xylose. For 1g solid loading, highest production in terms of arabinose 10.5 g/l, glucose (1.1 g/L) and xylose (0.42 g/L) was obtained under agitation. While for 10 grams solid loading, highest level of bio sugar was obtained with glucose (1.6 g/L) and arabinose (0.8 g/L).

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REFERENCES

[1] C.S. Goh, K.T. Tan, K.T. Lee, S. Bhatia. 2010. Bio-ethanol from lignocellulose: status, perspectives and challenges in Malaysia, Bioresource Technology. 101: 4834-4841.

[2] V. Menon, M. Rao. 2012. Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept, Progress in Energy and Combustion Science. 38: 522-550.

[3] S. Kim, B.E. Dale. 2004. Global potential bioethanol production from wasted crops and crop residues, Biomass and Bioenergy. 26: 361-375.

[4] J.H. Jung, W. Vermerris, M. Gallo, J.R. Fedenko, J.E. Erickson, F. Altpeter. 2013. RNA interference suppression of lignin biosynthesis increases fermentable sugar yields for biofuel production from field-grown sugarcane, Plant biotechnology journal. 11: 709-716.

[5] J. Martines-Filho, H.L. Burnquist, C.E. Vian. 2006. Bioenergy and the rise of sugarcane-based ethanol in Brazil.

[6] M.-H. Chen, P. Kaur, B. Dien, F. Below, M.L. Vincent, V. Singh. 2013. Use of tropical maize for bioethanol production, World Journal of Microbiology and Biotechnology. 29: 1509-1515.

[7] S. Yusoff. 2006. Renewable energy from palm oil–innovation on effective utilization of waste, Journal of cleaner production. 14: 87-93.

[8] M.S. Nazir, B.A. Wahjoedi, A.W. Yussof, M.A. Abdullah. 2013. Eco-friendly extraction and characterization of cellulose from oil palm empty fruit bunches, BioResources. 8: 2161-2172.

[9] S.H. Lim, A. Samsu Baharuddin, M.N. Ahmad, M. Shah, U. Kalsom, A. Rahman, S. Abd Aziz, M.A. Hassan, Y. Shirai. 2009. Physicochemical changes in windrow co-composting process of oil palm mesocarp fiber and palm oil mill effluent anaerobic sludge, Australian Journal of Basic and Applied Sciences. 3: 2809-2816.

[10] S.-H. Kong, S.-K. Loh, R.T. Bachmann, S.A. Rahim, J. Salimon. 2014. Biochar from oil palm biomass: A review of its potential and challenges, Renewable and Sustainable Energy Reviews. 39: 729-739.

[11] G.E. Anthon, D.M. Barrett. 2008. Combined enzymatic and colorimetric method for determining the uronic acid and methylester content of pectin: application to tomato products, Food chemistry. 110: 239-247.

[12] C. Israilides, A. Vlyssides, D. Arapoglou, T. Varzakas, R. Marchant, A. Vlysides. 2008. Integrated management of potato starch wastes, Proceedings of the Waste and Resource Management–A Shared Responsibility (Waste, 2008).

©2006-2017 Asian Research Publishing Network (ARPN). All rights reserved.

[13] O. Hassan, T.P. Ling, M.Y. Maskat, R.M. Illias, K. Badri, J. Jahim, N.M. Mahadi. 2013. Optimization of pretreatments for the hydrolysis of oil palm empty fruit bunch fiber (EFBF) using enzyme mixtures, Biomass and Bioenergy. 56: 137-146.

[14] M.J. Lau, M.W. Lau, C. Gunawan, B.E. Dale. 2010. Ammonia fiber expansion (AFEX) pretreatment, enzymatic hydrolysis, and fermentation on empty palm fruit bunch fiber (EPFBF) for cellulosic ethanol production. Applied biochemistry and biotechnology. 162: 1847-1857.

[15] S.I. Mussatto, C.J. Silva, I.C. Roberto. 2006. Fermentation performance of Candida guilliermondii for xylitol production on single and mixed substrate media, Applied microbiology and biotechnology. 72: 681-686.

[16] K. Noratiqah, M. Madihah, B.S. Aisyah, M.S. Eva, A. Suraini, K. Kamarulzaman. 2013. Statistical optimization of enzymatic degradation process for oil palm empty fruit bunch (OPEFB) in rotary drum bioreactor using crude cellulase produced from Aspergillus niger EFB1. Biochemical Engineering Journal. 75: 8-20.

[17] N.I. Iberahim, J.M. Jahim, S. Harun, M.T.M. Nor, O. Hassan. 2013. Sodium hydroxide pretreatment and enzymatic hydrolysis of oil palm mesocarp fiber. International Journal of Chemical Engineering and Applications. 4: 101.

[18] L. Lin, R. Yan, Y. Liu, W. Jiang. 2010 In-depth investigation of enzymatic hydrolysis of biomass wastes based on three major components: cellulose, hemicellulose and lignin. Bioresource Technology. 101: 8217-8223.

[19] C. Di Blasi, G. Signorelli, C. Di Russo, G. Rea. 1999. Product distribution from pyrolysis of wood and agricultural residues. Industrial & engineering chemistry research. 38: 2216-2224.

[20] S. Li, S. Xu, S. Liu, C. Yang, Q. Lu. 2004. Fast pyrolysis of biomass in free-fall reactor for hydrogen-rich gas. Fuel Processing Technology. 85: 1201-1211.

[21] G.L. Miller. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry. 31: 426-428.

[22] Y.-M. Choo, S.-C. Yap, C.-K. Ooi, A.-N. Ma, S.-H. Goh, A.S.-H. Ong. 1996. Recovered oil from palm-pressed fiber: a good source of natural carotenoids, vitamin E, and sterols. Journal of the American Oil Chemists' Society. 73: 599-602.

[23] B. Neoh, Y. Thang, M. Zain, A. Junaidi. 2011. Palm pressed fibre oil: A new opportunity for premium hardstock? Int. Food Res. J. 18: 769-773.

[24] M. Ioelovich, E. Morag. 2012. Study of enzymatic hydrolysis of pretreated biomass at increased solids loading, Bioresources. 7: 4672-4682.

[25] R.A. Young. 1992. Activation and characterization of fiber surfaces for composites. ACS symposium series (USA).

[26] [26] N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Lee, M. Holtzapple, M. Ladisch. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource technology. 96: 673-686.

[27] C. Cara, E. Ruiz, J.M. Oliva, F. Sáez, E. Castro. 2008. Conversion of olive tree biomass into fermentable sugars by dilute acid pretreatment and enzymatic saccharification. Bioresource Technology. 99: 1869-1876.

[28] M. Umikalsom, A. Ariff, H. Zulkifli, C. Tong, M. Hassan, M. Karim.1997. The treatment of oil palm empty fruit bunch fibre for subsequent use as substrate for cellulase production by Chaetomium globosum Kunze. Bioresource Technology. 62: 1-9.

[29] N.A.N. Mahmud, A.S. Baharuddin, E.K. Bahrin, A. Sulaiman, M.N. Naim, R. Zakaria, M.A. Hassan, H. Nishida, Y. Shirai. 2013. Enzymatic saccharification of oil palm mesocarp fiber (OPMF) treated with superheated steam, Bioresources. 8: 1320-1331.

[30] Y.Y. Then, N.A. Ibrahim, N. Zainuddin, H. Ariffin, W.M.Z.W. Yunus, B.W. Chieng. 2014. The influence of green surface modification of oil palm mesocarp fiber by superheated steam on the mechanical properties and dimensional stability of oil palm mesocarp fiber/poly (butylene succinate) biocomposite. International journal of molecular sciences. 15: 15344-15357.

[31] N.S.H.M. Yunos, A.S. Baharuddin, K.F.M. Yunos, M.N. Naim, H. Nishida. 2012. Physicochemical property changes of oil palm mesocarp fibers treated with high-pressure steam. BioResources. 7: 5983-5994.

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[32] N.I.A.A. Nordin, H. Ariffin, Y. Andou, M.A. Hassan, Y. Shirai, H. Nishida, W.M.Z.W. Yunus, S. Karuppuchamy, N.A. Ibrahim. 2013. Modification of oil palm mesocarp fiber characteristics using superheated steam treatment. Molecules. 18: 9132-9146.

[33] D. Dietrich, S. Hinke, W. Baumann, R. Fehlhaber, E. Bäucker, G. Rühle, O. Wienhaus, G. Marx. 2003. Silica accumulation in Triticum aestivum L. and Dactylis glomerata L. Analytical and bioanalytical chemistry. 376: 399-404.

[34] T.Y. Ding, S.L. Hii, L. Ong. 2012. Comparison of pretreatment strategies for conversion of coconut husk fiber to fermentable sugars. BioResources. 7: 1540-1547.

[35] J. Coates. 2000. Interpretation of infrared spectra, a practical approach. Encyclopedia of analytical chemistry.

[36] K. Pandey. 1999. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. Journal of Applied Polymer Science. 71: 1969-1975.

[37] E.K. Bahrin, A.S. Baharuddin, M.F. Ibrahim, M.N.A. Razak, A. Sulaiman, S.A. Aziz, M.A. Hassan, Y. Shirai, H. Nishida. 2012. Physicochemical property changes and enzymatic hydrolysis enhancement of oil palm empty fruit bunches treated with superheated steam. Bio Resources. 7: 1784-1801.

[38] R.M. Soom, A.A. Aziz, W. Wan Hasamudin. 2009. Solid-state characteristics of microcrystalline cellulose from oil palm empty fruit bunch fibre. Journal of oil palm research. 21: 613-620.

[39] K.O. Reddy, C.U. Maheswari, M. Shukla, J. Song, A.V. Rajulu. 2013. Tensile and structural characterization of alkali treated Borassus fruit fine fibers. Composites Part B: Engineering. 44: 433-438.

[40] L.-P. Xiao, Z.-J. Sun, Z.-J. Shi, F. Xu, R.-C. Sun. 2011. Impact of hot compressed water pretreatment on the structural changes of woody biomass for bioethanol production, Bioresources. 6: 1576-1598.

[41] A. Pandey, P. Selvakumar, C.R. Soccol, P. Nigam. 1999. Solid state fermentation for the production of industrial enzymes. Current science. 77: 149-162.

[42] S.D. Sekaran, S.-S. Leow, N. Abobaker, K.K. Tee, K. Sundram, R. Sambanthamurthi, M.B. Wahid. 2010. Effects of oil palm phenolics on tumor cells in vitro and in vivo. African Journal of Food Science. 4: 495-502.

[43] D. Arapoglou, T. Varzakas, A. Vlyssides, C. Israilides. 2010. Ethanol production from potato peel waste (PPW). Waste Management. 30: 1898-1902.

[44] S. Otu, V. Dzogbefia, E. Kpikpi, E. Essuman. 2015. Comparative Effect of Crude and Commercial Enzyme in Shea Fat Extraction.

[45] J. Yoo, S. Alavi, P. Vadlani, V. Amanor-Boadu. 2011. Thermo-mechanical extrusion pretreatment for conversion of soybean hulls to fermentable sugars. Bioresource Technology. 102: 7583-7590.

[46] D. Navarro, M. Couturier, G.G.D. Silva, J.-G. Berrin, X. Rouau, M. Asther, C. Bignon. 2010. Automated assay for screening the enzymatic release of reducing sugars from micronized biomass. Microbial cell factories. 9: 1.

[47] J. Hernández-Salas, M. Villa-Ramírez, J. Veloz-Rendón, K. Rivera-Hernández, R. González-César, M. Plascencia-Espinosa, S. Trejo-Estrada. 2009. Comparative hydrolysis and fermentation of sugarcane and agave bagass1e. Bioresource Technology. 100: 1238-1245.