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Lignocellulosic-based Jatropha Seed Pre-Treatment Using Ultrasonic Reactive Extraction for Liquid Biofuel Production

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Pretreatment is an essential step in the production of alcohol from lignocellulosic-based materials because the biomass structural complexity severely restricts enzymatic and microbial accessibility. Due to that factor, recalcitrance to saccharification was found to be the major limitation for conversion of lignocellulosic-based material to ethanol. This study aims to reduce the recalcitrance within the biomass through delignification process and thus enhancing the enzymatic hydrolysis for bioethanol production. Jatropha seeds have been pre-treated with ultrasonic during biodiesel reactive extraction process. The cellulose, hemicellulose and lignin content of the Jatropha seed were characterized using the standard van Soest method, which employs Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) procedure. Cellulose is considered to be represented by the difference between ADF and ADL (ADF - ADL), and hemicellulose is the difference between NDF and ADF (NDF - ADF). After reactive extraction process, the seed cake still contains 26.28 % of total cellulose and hemicelluloses and the amount of lignin is significantly reduced. Non-crystalline (amorphous) structures were revealed for all samples and the surface morphology suggests that some of the lignocellulosic compound have been degraded during the pretreatment process. Crystallinity of compounds also decreased when tested by using X-Ray diffraction (XRD) method. This pretreatment introduces a potential reduction of the recalcitrance in lignocellulosic materials (lignin, cellulose and hemicelluloses) and also improves its suitability as bioethanol feedstock.

1. Introduction

In these recent years, a rapid depletion of fossil fuels has becoming a major issue in the world as a demand towards fuel consumption per number of population is explodes and it is responsible for 60 % of total world oil consumption. Moreover, the threats of imminent global warming demands entailed the urgent needs to replace fossil-based fuels with green biofuels (Toquero and Bolado, 2014). Biomass based fuels, such as bioethanol, provide a promising alternative, since their energy are already included in the global carbon cycle, implying a significant reduction in carbon dioxide release (Karagöz et al., 2012). Among biomass feedstocks (lignocellulosic biomass, starch-based and sucrose-based), lignocellulosic residues offer attractive renewable sources for sugar platform biorefinery on a large scale (Diaz et al., 2013). As they are residues and wastes, they do not compete with primary food production (Diaz et al., 2013) and they represent the most abundant carbohydrate reserve worldwide (Saha et al., 2013). About 442 billion liters of bioethanol can be produced from lignocellulosic biomass and the total crop residues as well as the crop wastes can produce $491 \cdot 10^9$ L of bioethanol per year, about 16 times higher than the current total world bioethanol production (Gupta and Verma, 2015). Currently *Jatropha curcas (J. curcas)* has shown good potential for this purpose due to high oil content in the seeds (Nunes et al., 2014) and also adapts well to climatic variations and different soil compositions (Nunes et al., 2014) allowing it to be easily cultivated in different regions.

Considering the average amount of oil extracted from *Jatropha curcas* seeds is 30 % by weight, each ton of oil extracted generates about 2.3 t of seed cake (dos Santos et al., 2014). Regarded as a crop to complement the production of biodiesel, the use of *Jatropha curcas* will produce thousands of tones of

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seed cake. In this scenario, a major challenge is to decrease the value of these residues, making the biodiesel industry more consistent and competitive. One possible strategy is to leverage the significant percentage of carbohydrates present in this residual biomass, 30 to 38 % (dos Santos et al., 2014), for the production of bioethanol through hydrolytic and fermentative processes, as has been suggested for other residues from the biodiesel industry (Macedo et al., 2011).

Currently the major strategy used for cellulosic ethanol production includes three main steps which are biomass pretreatment, enzymatic hydrolysis and ethanol fermentation (Kumar et al., 2010). However, since the lignocellulosic materials have rigid structures consisting of a carbohydrate polymer matrix, mainly cellulose and hemicelluloses which are cross-linked and strongly bounded to lignin, they severely restrict the enzymatic and microbial accessibility (Toquero and Bolado, 2014). In order to overcome this problem, efficient pretreatments are required to disrupt the heterogeneous matrix, increase surface area, increase surface porosity of the cellulosic material, and to unlock carbohydrates from their lignin association, thereby enhancing enzymatic digestibility (Toquero and Bolado, 2014).

In this study, *Jatropha curcas* seed cake is obtained from the residual after biodiesel extraction in which ultrasonic-assisted reactive extraction is implemented for simultaneous oil extraction and transesterification process (Zahari et al., 2014). Recently, biodiesel production technique using reactive extraction or in-situ process provides almost complete extraction with up to 95 % efficiency (Siatis et al., 2006). Even though the process has successfully improved the oil production, the seed residue still being left out as solid waste (Mohit et al., 2011) and has to be soaked in order to remove the excess oil from Jatropha pressed cake prior for bioethanol conversion process (Zahari et al., 2014). However, the residual seeds retrieved from reactive extraction process coupled by ultrasonication were consisting of completely de-oiled residues which make the preparation step unnecessary (Zahari et al., 2014) and also provides a better decomposition of biomass residues having easier accessible and better degradable fibre for bioethanol production process. Moreover, ultrasonication is also capable in disrupting the biological structure and increasing the seed surface area (Bychkov et al., 2012) which would serve as the physical pre-treatment and indicates that the biodiesel residual does not required further pretreatment for bioethanol conversion. Hence, this study will determine the total lignocellulosic composition (cellulose, hemicelluloses and lignin) of the seed cake after biodiesel extraction in comparison to the composition in raw Jatropha seed by using van Soest fibre characterization method to ensure that there is an adequate amount of remaining cellulose and hemicelluloses for further bioconversion process. This study is also aiming to prove that delignification process occur during ultrasonication extraction process hence showing that further pretreatment is unnecessary for bioethanol conversion process. Scanning Electron Microscopic (SEM) and X-Ray Diffraction (XRD) analysis were also used in this study to observe the morphological and crystallinity changes after ultrasonic reactive extraction process.

2. Material and Methods

2.1 Sample preparations

The fresh stalk of *Jatropha curcas* sample was purchased and supplied from BIONAS Company, Malaysia. Commercial grinder (Mill A11, IKA, German) was used to grind the raw seeds into average sizes for about 0.85 – 1.00 mm in the mechanical sieve. The seeds were oven-dried for 2 h to minimize the moisture in the seeds. After ultrasonic extraction, the Jatropha seed cakes are being stored in fume chamber to avoid contamination of the samples setup.

2.2 Van Soest fiber determination methods

The fibre content characterization was applied to both raw seed Jatropha and extracted Jatropha seed cake. The cellulose, hemicelluloses and lignin contents of both samples were characterized using standard van Soest methods (Wang, 2008), which employs NDF, ADF and ADL procedures. NDF represents the total lignocellulosic components exist in biomass (hemicelluloses, cellulose, lignin and water soluble compounds) while ADF represents the amount of total cellulose, lignin and water soluble compounds in the biomass.

2.2.1 Neutral Detergent Fiber (NDF)

NDF fiber are the residues remaining after digestion in a detergent solution which predominantly hemicelluloses, cellulose and lignin. Approximately 1.00 ± 0.01 g of prepared sample was put in the conical flasks. Reagents were added in the following order: 100 mL of room temperature neutral detergent solution (30.00 ± 0.01 g sodium lauryl sulphate, USP; 18.61 ± 0.01 g disodium dihydrogen ethylene-diamine tetra acetic dehydrate, reagent grade; 4.56 ± 0.01 g disodium hydrogen phosphate, anhydrous, reagent grade; and 10 ± 0.01 ml 2-ethoxy-ethanol (ethylene glycol, monoethyl ether), purified grade, were added into distilled water to make up a 1 L ± 0.3 mL solution. The mixture was agitated to dissolve the

chemicals and a pH range of 6.7-7.1 was obtained.), 2 ± 0.015 mL decahydronaphthalene, and 0.50 ± 0.01 g sodium sulphite. After that, the mixture was heated to boiling and refluxed by using condenser, round bottle neck and heating mantle for 60 ± 1 min, timed from the onset of boiling. After boiling, slight vacuum suction was applied and the mixture were washed thrice with 50 ± 1 mL of hot water (80-90 ºC) and then the liquid was filtered and twice with cold acetone in the same manner. Finally, the crucible was dried at 100 ± 1 ºC for 8 h (±1 min) in the drying oven and cooled in a desiccator and then weighed. The yield of recovered neutral-detergent fibre was reported as the total lignocellulosic content.

2.3 2.2.2 Acid Detergent Fiber (ADF)

Approximately 1.00 ± 0.01 g of prepared sample was put in the conical flasks , to which about 100 mL of room temperature neutral detergent solution (20.00 ± 0.01 g cetyltrimethylammonium bromide (CTAB), technical grade was added to 1L ± 0.3 mL of 1 ± 0.01 N H_2SO_4 previously standardized and mixed.) was added. 2 mL of decalin was added in the mixture and the mixture was heated to boiling and refluxed for 60 ± 1 min, timed from the onset of boiling. After boiling, slight vacuum suction was applied and the mixture were washed thrice with 50 ± 1 mL of hot water (80-90 ºC) and then the liquid was filtered twice with cold acetone in the same manner. Finally, the crucible was dried at 100 ± 1 ºC for 8 h (±1 min) in the drying oven and cooled in a desiccator and then weighed.

2.4 2.2.3 Acid Detergent Lignin (ADL)

In ADL procedure, the ADF procedure is used as a preparatory step. The detergent removes the acid soluble materials that would interfere with the lignin determination. Firstly, the filter crucible containing the acid-detergent fibre was placed in a ceramic dish for support. About 50 ± 1 mL cooled (15 ± 1 ºC) 72 % H_2SO_4 was added to the residue and stirred with a glass rod to a smooth paste, breaking all lumps, and allowing the excess acid to gravity drain. Another 50 mL of 72 % H_2SO_4 was added and stirred at hourly intervals and the excess acid was allowed to drain away by gravity. After the 3 h (±1 min) treatment with 72 % H_2SO_4, it was filtered under vacuum to remove as much acid as possible, and washed with hot water (80 - 90 ± 1 ºC) until it was free from acid (the pH of 7 from the filter crucible outflow). The filter crucible was then placed in a drying oven at 100 ± 1 ºC for 2 h (±1 min) and weighed after cooling in a desiccator overnight. The remaining residue was ashed in a muffle furnace at 500 ± 5 ºC for 2 h (±1 min). Then the filter crucible was cooled in a desiccator and weighed once completely cool.

2.5 Lignocellulosic composition determination

Hemicellulose is represented by the difference between NDF and ADF (NDF - ADF), and cellulose is represented by the difference between ADF and ADL (ADF - ADL). Meanwhile, ADL percentage obtained in the study is representing the lignin composition in the samples.

2.6 Scanning Electron Microscopic (SEM)

The physical properties of raw Jatropha seed sample and ultrasonic extracted sample were characterized by Scanning Electron Microscopy, SEM (JEOL JSM-6360 LA) employed in Institute of Oceanography (INOS), University Malaysia Terengganu. Single layer of samples was put carefully on the clean disk with having double sided tapes for the SEM imaging of about 10 min with x 5,000 resolution for selected samples.

2.7 X-Ray Diffraction (XRD) analysis

X-Ray Diffraction (XRD) patterns were recorded with a Rigaku XRD system employed in Physics Laboratory, School of Ocean Engineering, University Malaysia Terengganu.

3. Results and Discussion

3.1 Fiber characterization analyses

Based on the results obtained, the average amount of Neutral Detergent Fiber (NDF) or total lignocellulosic composition is 50.92 % for raw seed Jatropha and 38.2 % for ultrasonic extracted Jatropha seed cake. For Acid Detergent Fiber (ADF), 42.38 % and 23.75 % residue is left in raw Jatropha seed and Jatropha seed cake. Whereas for Acid Detergent Lignin (ADL), raw seed Jatropha contributes 26.84 % of total lignin and 11.62 % lignin contained in extracted Jatropha seed. The amount of lignin showed a reduction from raw seed sample towards the extracted one because delignification process is occurred to remove the recalcitrance within the Jatropha carbohydrate structure.

The results also indicate that the ultrasonic reactive extraction had prepared the seed cake for further bioconversion process without any other pretreatment needed. The pre-treatment effects also can be

proven by comparing the amount of hemicelluloses obtained from the deduction between NDF and ADF (NDF-ADF) which is 8.53 % raw Jatropha seed and the percentage is increasing in extracted Jatropha seed which is 14.45 % in value. The value is increasing because some of the lignin cellulose in the raw seeds has been converted into easier degradable portions (decreasing value in cellulose). Moreover, small portion of the biomass cellulose component might be considered as hemicelluloses since the pre-treatment had broken the cellulose crystalline structure into slightly amorphous, making it more susceptible to be dissolved during the fibre characterization. The comparison of cellulose, hemicelluloses and lignin composition before and after ultrasonic reactive extraction is shown in Figure 1.

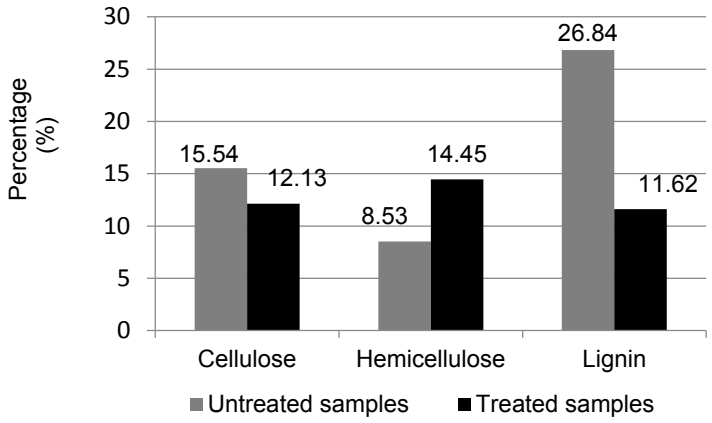

Figure 1: Figure of comparison in total lignocellulosic composition between raw seed Jatropha (Untreated samples) and ultrasonic reactive extracted Jatropha seed cake (Treated samples)

The value of cellulose and hemicelluloses resulted from this study also shows that the seed cake still having a high amount of total fibre content in about 27 % and can be used for further bioconversion. The other studies were also obtained 27 – 50 % of total cellulose and hemicelluloses content in the various pre treated Jatropha seed cakes for bioethanol conversion as shown in Table 1. The results obtained showed the total lignocellulosic components are not 100 % in value because in the biomass there are some of water soluble components dissolved during the van Soest fiber characterization method (Wang, 2008).

Table 1: Table of lignocellulosic composition of pre-treated Jatropha seed cake

References	Liang et al., (2010)	Demissie et al., (2013)	dos Santos et al., (2014)	Experimental Result of Study
Cellulose (%)	13.5	13.6	16.88	12.13
Hemicellulose (%)	26.8	22.57	10.41	14.45
Lignin (%)	12.4	10.47	33.29	11.62

3.2 Surface morphology quantification

Since a large fraction (± 15 %) of lignin is removed during ultrasonic reactive biodiesel extraction, it is interesting to examine the physical morphology of both raw seed Jatropha sample and extracted Jatropha seed. SEM of both samples was obtained to verify the biomass structural change and the SEM micrographs of both samples were shown in Figure 2.

It was clearly observed that significant morphological changes indeed occurred after extraction process by using ultrasonic reactive extraction method. The raw seed sample morphology had a relatively smooth and intact surface structure and strongly bounded to each other which might be recalcitrant to enzymatic attack and its adsorption onto the biomass, resulting in lower enzymatic conversion from this surface morphology (Subhedar et al., 2014).

Significantly different from the raw seed sample, the extracted sample showed uneven and disrupted surface structure mainly due to the breaking up of lignin carbohydrate matrix during extraction process. It leads to an accumulation of some lignin complexes that condensed on the surface of extracted sample. The goal of pretreatment in order to delignify the biomass and increase the available surface area of

cellulose and hemicelluloses to increase enzymatic hydrolysis has achieved and prove that there is unnecessary to treat the extracted samples with another pretreatment for bioethanol conversion process.

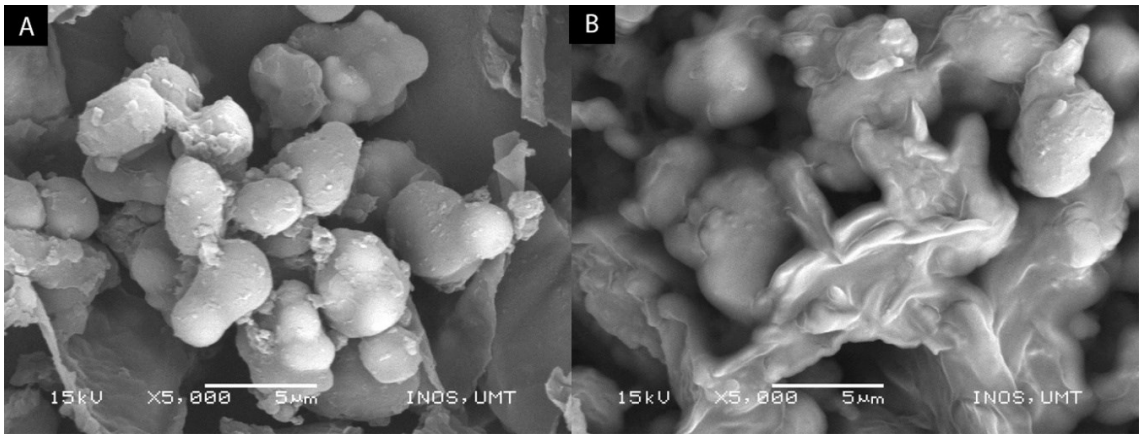

Figure 2: SEM Image of ultrasonic reactive Jatropha seed cake (A); SEM Image of raw Jatropha seed (B)

3.3 Physical crystallinity analysis

The XRD patterns for both samples are shown in Figure 3. The intensity distribution in the region of interest (diffraction angle, 2θ = 18 – 24°) usually displays two peaks. The peak at 20° is due to (0 2 1) line in cellulose I and (1 0 1) line in cellulose II (Kumar et al., 2010). From the result, we can clearly observe that the peak intensity in raw Jatropha seed is about 400 - 450 cps and the value becoming lower (300 - 350 cps) in the extracted Jatropha seed cake sample. The intensity of extracted samples showing decreasing pattern probably due to crystallinity of the samples breaking down (Supamanthanon et al., 2011) during biodiesel ultrasonic reactive extraction.

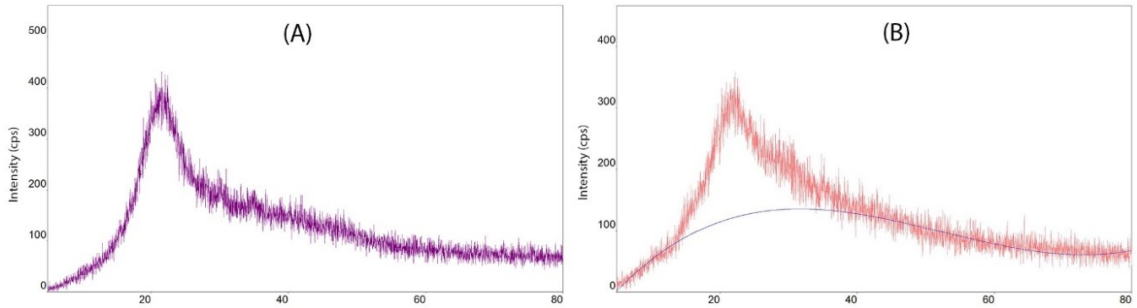

Figure 3: XRD images for raw Jatropha seed cake (A) and ultrasonic reactive extracted Jatropha seed cake (B)

4. Conclusions

The extracted *Jatropha curcas* seed cake contains high amount of lignocellulosic components (cellulose and hemicelluloses) and is comparable to results reported by other studies. Surface morphological shows a significance changes under Scanning Electron Microscopic (SEM) analysis. Meanwhile X-ray Diffraction (XRD) analysis also reveals the declining pattern in peak intensity distribution mainly due to the breaking down of sample during pretreatment process. A significance reduction amount of lignin percentage was observed in van Soest fiber determination analysis and proves that delignification process has occurred during ultrasonic extraction of biodiesel. Referring to all the results obtained, this study concludes that there is unnecessary pretreatment needed towards the ultrasonic extracted *Jatropha curcas* seed cakes after biodiesel extraction for another bioconversion process as the ultrasonic itself acts as a pretreatment step during biodiesel extraction.

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