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ULTRASONICATED *JATROPHA CURCAS* SEED RESIDUAL AS POTENTIAL BIOFUEL FEEDSTOCK

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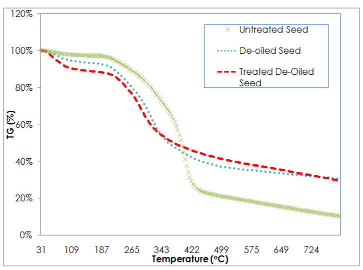
Graphical abstract



Abstract

This study focuses on the prospect of *Jatropha Curcas* seed residual from the ultrasonic in-situ process which is used as a biofuel raw material especially for producing bioethanol. Reactive extraction process coupled with ultrasonic system were used for simultaneous oil extraction and transesterification of *Jatropha Curcas* seed. Using ethanol as the solvent, alkaline catalyst (sodium hydroxide) and with the aid of ultrasonic device, about 50% oil from the initial seeds was extracted, which is equivalent to Soxhlet extraction performance. The seeds were being chemically and physically characterized with ultimate analyses, with SEM and XRD as potential bioethanol raw material. SEM and XRD profile exhibited loosen compounds in the ultrasonicated residues and provided a better accessible and easier degradable fiber for assisting bioethanol production process compared to the initial seeds. The positive effects of the ultrasonic reactive extraction for *Jatropha Curcas* seed pre-treatment is beneficial towards bioethanol production and could further be used as a solvent in the latter process.

Keywords: Biodiesel, bioethanol, cavitation, reactive extraction, ultrasonic

Abstrak

Kajian ini menumpukan kepada prospek penggunaan sisa biji *Jatropha Curcas* daripada proses ultrasonik *in-situ* sebagai bahan mentah bahan api bio terutamanya bagi penghasilan bioethanol. Proses pengekstrakan aktif yang digabungkan dengan sistem ultrasonik berfungsi secara serentak bagi pengekstrakan minyak dan juga melakukan proses transesterifikasi kepada biji *Jatropha Curcas*. Menggunakan ethanol sebagai pelarut, pemangkin alkali (natrium hidroksida) dan bantuan peralatan ultrasonic, sehingga 50% minyak boleh diekstrak, iaitu setara dengan kemampuan pengekstrakan Soxhlet. Sisa biji tersebut diklasifikasikan secara kimia dan secara fizikal menggunakan analisis ultima, menggunakan SEM dan XRD sebagai bahan mentah yang berpotensi untuk bioethanol. Profil SEM dan XRD menunjukkan ikatan yang lebih rapuh bagi sisa biji yang melalui proses ultrasonik dan memberikan ruang serta memudahkan capaian kepada fiber untuk memudahkan proses penghasilan bioethanol berbanding biji yang asal. Kesan positif proses pengekstrakan aktif digabungkan dengan ultrasonik sebagai pra-rawatan kepada biji *Jatropha Curcas* boleh membantu proses penghasilan bioethanol dan berpotensi untuk digunakan semula sebagai bahan pelarut di peringkat awal.

Kata kunci: Biodiesel, bioethanol, kavitasi, pengesktrakan aktif, ultrasonik

1.0 INTRODUCTION

Currently, inedible oil crop such as Jatropha is vastly being used for fuel source primarily as biodiesel feedstock. Its seed contains about 300-350g/kg oil; which can be extracted and used as a fuel directly or in its transesterified form, further, as the substitute for diesel or so-called biodiesel [1]. However, substantial amount of toxic residue or seed cake which contains about 65% of the seed [2] are being discarded after extraction and transesterification of the oil portion. Therefore, approximately 3 tons of waste in the form of seed cake would be produced for every ton of processed Jatropha oil [1]. Apart from the oil content, the seeds of *Jatropha Curcas* plant are also rich in nutrient with significant content of degradable biomass content [1] in the form of fiber (14 w/w%), carbohydrates (50 w/w%) and lignin (19 w/w%) [3], as such, it becomes a suitable feedstock for further biofuel production especially bioethanol. The biological conversion from *Jatropha Curcas* seed cake includes pre-treatment, hydrolysis, and fermentation because the recalcitrance of lignocelluloses is the major barrier for production of bioethanol. Hence, pre-treatment is identified as a suitable approach to overcome those compounds recalcitrance to unwind cellulose from hemicelluloses and lignin chains [4], thus making cellulose more susceptible to hydrolysis towards its conversion into glucose [5]. It would be highly beneficial in terms of the processing time and chemical usage if the pre-treatment could be achieved within the production steps because it represents one of the major economic costs [6].

Recent biodiesel advancement enables extraction and transesterification conducted at the same time through reactive extraction method for optimal oil extraction and biodiesel yield. This in-situ process eliminates the requirement of pre-extraction process by directly using the oil-bearing seed. This process has been studied for different oil crop seeds including Jatropha [7-13], castor [14-16], sunflower seed [17], cottonseed [18], soybean [19, 20] and rapeseed [21]. It is a technological advancement to reduce biodiesel production cost by integrating multiple biodiesel processing stages which constitute over 70% of the overall cost, even when using refined oil as feedstock [22]. The seed is added into a mixture consists of acid/base catalyst, a small portion of hexane and a large amount of alcohol (methanol, ethanol or propanol), agitated at near-boiling point temperature of the selected alcohol and stirred using a mechanical stirrer or using low frequency sound wave. The operation of the process provides the suitable pre-treatment condition to change the physical and chemical structure of the lignocelluloses seed especially for ultrasonic-assisted process [23]. Ultrasonic used in the process could induce physical pre-treatment through the formation of cavitation bubbles in the liquid phase that grow and violently collapsed. Furthermore, addition of ultrasonication could induce mechanical treatment for disrupting biological structure and increase the surface area [24] while enhancing the saccharification of cellulose for maximum cavitation effect at 50°C [25]. Moreover, the thermal temperature employed in the process could deactivate the toxic phorbol ester while producing crude biodiesel, glycerol by-product and complete de-oiled seed residues [13].

Generally speaking, studies are conducted towards optimization on catalyst loadings, alcohol ratios, reaction temperatures and reaction times in the in-situ process, with a primary focus towards achieving high biodiesel yield and purity. The intention of this study, however, is to evaluate the effects of those parameters to react as ultrasonic-assisted alkaline pre-treatment on the Jatropha seed. It is known that alkaline pre-treatment is the most suitable method for many feedstocks in bioethanol production compared to other available pre-treatment choices [26], especially for agricultural residues through delignification effect [6]. By doing so, a single in-situ process could be utilized to produce biodiesel and provides a suitable seed residues such as the bioethanol feedstock. Thus, it is hypothesized that the in-situ process could be used for a whole utilization of Jatropha seed (oil portion and seed residue) in generating both biodiesel and bioethanol.

In this context, the research aims at the fundamental insight of ultrasonic-assisted reactive extraction as a physical, chemical and alkaline pre-treatment on *Jatropha Curcas* seed. The result of the study has focus on the characterization of Jatropha seed residues for its suitability as bioethanol raw material.

2.0 EXPERIMENTAL

2.1 Materials

The Jatropha seeds were bought fresh from Bionas (M) Sdn. Bhd. Extraction solvents consist of ethanol and n-hexane together with acetic acid and sodium hydroxide (NaOH) were purchased from Merck Malaysia. All chemicals and solvents used in this study were of analytical grade.

2.2 Seed Preparations

Commercial grinder was used to grind the seeds and grouped into average sizes below 0.75 mm using the mechanical sieve. For analytical purpose, prior to Soxhlet and reactive extraction experiments, the seed were dried in a convection drying oven at the temperature of 105°C for a minimum of 4 hours until the difference in weight is below 0.1%. De-oiled Jatropha seed samples were obtained by extracting the oil content using conventional Soxhlet extraction (EPA Method 3045C) using n-hexane as the solvent. Triplicate seed samples were made for all experiments.

2.3 Ultrasonic-Assisted Reactive Extraction

A reactive extraction experiment was conducted using a probe sonicator consists of S.S. Velocity Horn fitted with PZT Crystals operating at a frequency of 20 KHz and 120 Watts. Irradiation of mixed reactants occurs in a 500 mL round bottom flasks for 10 minutes because it is already sufficient for biodiesel production [13, 27] and already within the proposed ultra-sonication pre-treatment time in the range of 0.5 to 150 minutes [28, 29]. Dried Jatropha seeds were weighted into the flasks filled with pre-heated reactant mixture which consists of catalyst within the solvent of ethanol plus n-hexane for completed polar and non-polar lipid contents. Alkaline catalyst using NaOH in the concentration of 2M was employed during the process. Losses for mixture of solvents (ethanol + hexane) during the in-situ process were minimized by having a condenser attached to the system with running tap water. The reaction was neutralized at the end of each experiment using acetic acid solutions before the residual seed is separated through Whatman GF/C filter paper in a vacuum filtration. For comparison among de-oiled seeds, Jatropha seed was also extracted using conventional Soxhlet extraction method (EPA Method 3540 C).

2.4 Characterization of Seeds

2.4.1 Ultimate Analysis

Ultimate analysis of seeds were done using Therm FlashEA 1112 Elemental Analyzer equipped with two combustion columns made of quartz glass for the analysis of carbon, hydrogen, nitrogen, and sulfur in high oxygen condition. The samples were weighted into tin containers on Mettle Toledo balance (for microgram measurement). The combustion products were separated using gas chromatography and measurement given in percentages by a thermal conductivity detector (TCD) and data acquisition board.

2.4.2 Elemental Analysis

The seeds were solubilized using acid digestion method (EPA Method 3050B); followed by trace metals analyses using Varian SpectrAA 220FS for the measurement of potassium (K), copper (Cu), manganese (Mn), iron (Fe), nickel (Ni), zinc (Zn) and magnesium (Mg). The system consists of four lamp positions for multiple elements analysis using a rotating mirror to select operating lamp and high density deuterium background correction as standard. Four mixtures of standard solutions range from 0.2 – 5 mg/L were used for calibration of the system.

2.4.3 TGA Profiles

The temperature profile of the seeds was recorded using Mettler Toledo TGA/SDTA851 in both nitrogen and oxygen atmosphere with a flow rate of 20 mL/min and the heating rate of 10°C/min. About 5 mg of the seed material was loaded onto the alumina pan and heated from about 30 – 800°C.

2.4.4 XRD Patterns

XRD patterns were recorded with a Rigaku XRD system using CuKa radiation measured in the 2θ range between 3° to 120° with 0.020 resolutions and speed of 2.00.

2.4.5 SEM Analysis

The SEM system from JEOL was employed in the Institute of Oceanography (INOS), Universiti Malaysia Terengganu (UMT), for imaging purpose of the morphology of seeds surface. Samples were put carefully to obtain a single layer on the cleaned disk having double-sided tape for the SEM imaging. The disks with single layer sample were fine coated in an auto fine coater-gold for about 10 minutes. SEM images with x1000 resolution were taken for selected sample.

3.0 RESULTS AND DISCUSSION

3.1 Characteristic and Composition

An average moisture and oil content of 7.85 and 49.54 wt%, respectively were found in the Jatropha seed used in the study. The literature reveal an agreement with the result, having moisture in the range of 4.75 – 19.57% [30] depending on the seed dimensions (sizes and shapes). The moisture within the seed was removed through drying before the ultrasonic-assisted reactive extraction process to avoid formation of soap that could inflict the analysis and the result of the residual seed compositions. Chemical and physical characteristics were conducted for three different seed samples: 1) Untreated seeds (ground) and dried raw seed); 2) De-oiled seeds (conventional Soxhlet extracted seed); and 3) Treated de-oiled seeds (residual seeds after ultrasonic-assisted reactive extraction process). Table 1 below shows the result of ultimate analysis, which displayed the major compounds in the three categorized seeds.

Table 1 Jatropha Seeds Compositions

PARAMETER		SAMPLES		
		Untreated Seeds	De-oiled Seeds	Treated De-oiled Seeds
Moisture		5.33%	13.86%	10.70%
Proximate Analysis	Ash Yield	5.49%	10.77%	11.88%
Ultimate Analysis	Carbon	55.52%	40.63%	37.17%
	Hydrogen	8.66%	5.69%	5.15%
	Nitrogen	3.46%	6.09%	4.36%
	Sulphur	ND	ND	ND
	Oxygen (by difference)	26.87%	36.82%	41.44%
Carbohydrate Profile	Lignin Content	8%	20%	15%
	Crude Protein	26.62%	42.31%	31.96%
Elemental / Macro & Micro Nutrients	Potassium	1.833%	3.835%	3.242%
	Copper	0.002%	0.003%	0.003%
	Manganese	0.003%	0.005%	0.005%
	Ferum	0.007%	0.011%	0.012%
	Nickel	0.373%	0.393%	0.350%
	Zinc	0.011%	0.011%	0.017%
	Magnesium	5.042%	7.534%	6.346%

High carbon and oxygen contents suggest a positive comparison of the seed with other potential biomass feedstocks for bioconversion. Smaller content of carbon in the de-oiled seed and treated de-oiled seed is caused by the extraction of fat and lipid (long chain carbon molecules) [23]. Nitrogen would mostly represent the form of proteins and amino acid. There will be an increase of crude protein content in the de-oiled and treated de-oiled seed, compared to untreated seed. It indicated that the treatment process does not significantly remove those nutrients that could probably serve as valuable nitrogen source during the bioethanol fermentation process [31, 32].

However, protein content was shown slightly below the value of 59.1% [1] for the de-oiled seeds and even lower for the treated de-oiled seed. This could result from solubilisation of organic matter by the ultrasonic pre-treatment [33] especially with the cavitation bubble which opens the surface of the seed substrates. However, this provide a good extractive aspect of sonication during the biodiesel in-situ process and also preferable for further bioethanol conversion process by allowing better reaction of the seed substrate surface that could cut down the overall process time. With the presence of macro & micro nutrients exhibited by the elemental analysis, it could provide a better insight upon supplement required to be added into the bioethanol feedstock for optimum yield.

3.2 Thermal Stability

TGA analysis was conducted to compare thermal stability of Jatropha seeds, before and after the extraction process. It is also to determine whether the presence of different catalysts (acid/alkaline) would have any effect on the accessibility of cellulose, hemicelluloses, lignin, and other extractives. Figure 1 shows the TG curves that measures the seed weight loss as a function of temperature.

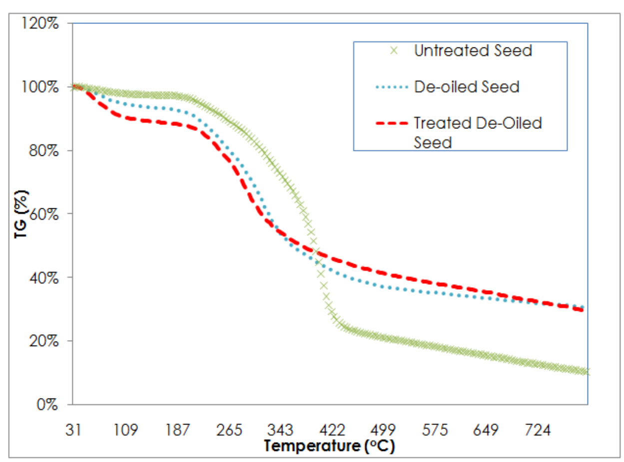


Figure 1 TGA Profiles of Jatropha Seeds

There are two significant weight loss which can be observed by thermal degradation attributed to hemicelluloses decomposition (200 – 300^{o}C) and cellulose decomposition (300 – 450^{o}C) [34]. Almost similar pattern is being exhibited by both de-oiled and treated de-oiled seed samples with better decomposition at a lower temperature for those chemically extracted seeds. This result would suggest that the biomass matrix of the seed had been broken down and hence making it easier for the biomass compounds to be thermally degraded, especially for the ultrasonicated residual seed.

3.3 Seed Surface Morphology

Seed surface morphology was being looked into by using SEM and XRD for determination of seed product suitability as bio product, mainly looking into its cellulose structure. The SEM image and XRD pattern taken from the Untreated Seeds, De-oiled Seeds, and Treated De-oiled Seeds is shown in Figures 2a, 2b, and 2c, respectively.

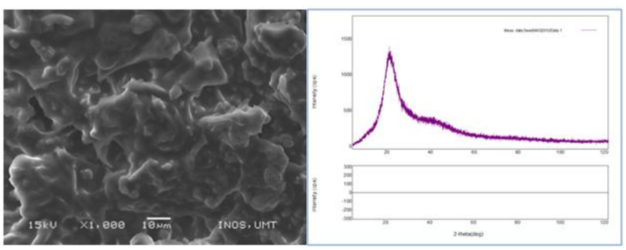


Figure 2a SEM Image and XRD Pattern for Untreated Seed

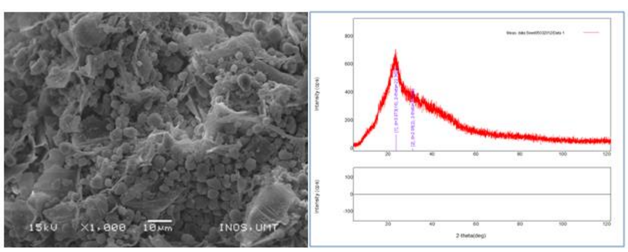


Figure 2b SEM Image and XRD Pattern for De-oiled Seed

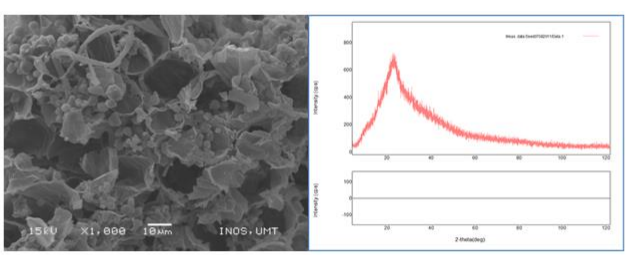


Figure 2c SEM Image and XRD Pattern for Treated De-oiled Seed

All XRD patterns revealed amorphous structure which is mixture of crystalline structure (primarily cellulose) and non-crystalline structure (starch, hemicelluloses, lignin etc.). It is a fact that the alkaline catalyst usage does not dissolve the seed material but the SEM images of treated de-oiled seed seem to suggest that some of the seed physical compounds could have been dissolved during the process, as suggested by the ultimate analysis data. The images have shown lesser presence of starch granules on the surface and quite loosen fiber of the seed rather than the extracted seed. However, not all starch or easily dissolved substances in the seed are extracted along by the process as the residual seed remains amorphous. Even though it is not a pure cellulose substance, the fibrous material, shown by the SEM image, might hold a good possibility for the loosen fiber of the seed lignocelluloses structure as potential bioethanol raw material.

4.0 CONCLUSION

The experimental results show that the ultrasonic-assisted reactive extraction using alkaline catalyst can function simultaneously for biodiesel production and as a pre-treatment step of the Jatropha seed. The data exhibit slight solubilisation of the organic matter shown by the lower value of carbon and protein content but also contribute towards delignification effect as expected by alkaline pre-treatment for the seeds. Moreover, the seed residues being retrieved consist of completely de-oiled seed and visually become almost powdered-form compared to its original sieved sizes. These findings reveal a positive insight towards combining both biodiesel and second-generation bioethanol feedstock, especially with the ultrasonication aid in-situ process towards full utilization of Jatropha seed as an alternative fuel source.

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