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Research Article

Corecovery of Bio-Oil and Fermentable Sugars from Oil-Bearing Biomass

Godwin Severa,[1] Guneet Kumar,[2] and Michael J. Cooney[1]

[1] *Hawaii Natural Energy Institute, University of Hawaii, Honolulu, HI 96816, USA*
[2] *Suganit Systems Inc., 10903 Hunt Club Road Reston, VA 20190-3912, USA*

Correspondence should be addressed to Michael J. Cooney; mcooney@hawaii.edu

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The applicability of ionic liquid-methanol cosolvent system to both extract bio-oil and simultaneously pretreat the carbohydrate fraction of *jatropha* and safflower biomass for enzymatic hydrolysis to fermentable sugars is presented. Although pretreatment with either the cosolvent or pure ionic liquid yielded comparable hydrolysis kinetics and fermentable sugar yields on safflower whole seeds, the addition of alcohol to the ionic liquid was necessary to optimally recover both bio-oil and fermentable sugars. The ionic liquid [C2mim][Ac] was far more effective than [C2mim][$MeSO_4$] with optimum processing conditions occurring at a cosolvent concentration of 70–30 wt% of [C2mim][Ac] to methanol and a processing temperature of 120°C. Under these conditions, the majority of the bio-oil was extracted and 25.4 wt% (safflower) and 14.3 wt% (*jatropha*) of the whole seed biomass were recovered as fermentable sugars. The recovery of fermentable sugars from the carbohydrate fraction was as high as 74% and 78% for *jatropha* and safflower seeds, respectively, when using [C2mim][Ac] cosolvent. A preliminary theoretical analysis of two potential oil seed processing pathways using the cosolvent system suggested that the corecovery of bio-oil, fermentable sugars, and a protein rich meal can recover a majority of the energy contained in the original biomass—a result that improves upon the traditional approach of solely extracting bio-oil.

1. Introduction

Fuel from biomass technology has embraced the application of green chemistry to increase utilization of biomass, minimize production of waste product, and improve recovery of nontoxic solvents. Many efforts have focused on processes that recover single components, for example, the recovery of lipids from oil bearing biomass or carbohydrates from lignocellulosic biomass [1–3]. With respect to oil-seed biomass, however, few reports have focused on treatments that recover both components [4]. From this perspective, the recovery of both fermentable sugars and bio-oil from oil-seed biomass using a single application of a common "green" solvent would represent a significant contribution to the sustainable utilization of biomass resources.

The extraction of bio-oil from oil-bearing biomass using solvent or mechanical extraction techniques has been well investigated [2, 4, 12, 13]. Mechanical extraction comprises pressing the oil seed with a screw press to expel the oil and leave behind a carbohydrate- and protein-rich seed cake. Solvent extraction of bio-oil has traditionally been applied with organic solvents such as hexane under low to moderate temperatures and pressure [4]. Solvent extraction generally achieves extraction efficiencies as high as 98% whilst mechanical processes generally achieve efficiencies of less than 80% although improvements have occurred over the last decade [5]. Solvent extraction is generally considered more economical when the volume of biomass processed exceeds 250 tons per day [12].

The production of fermentable sugars from lignocellulosic biomass has also been well studied and largely focused on chemical pretreatment with dilute acids, steam explosion, or organic solvent pretreatments [1, 14–16]. These pretreatment steps are aimed at disrupting the biomass cell wall structure as a means to increase accessibility of hydrolysis enzymes [3, 17, 18]. Unfortunately, most suffer low efficiencies, require high energy use, cause loss of fermentable sugars, or form inhibitory products which affect downstream processing [3,

14, 15]. From this perspective, a more efficient and inexpensive pretreatment process with less impact on downstream processing would improve commercial viability. Recently, room temperature ionic liquids have been proposed to fill this void [3, 19, 20] due to their observed solvating power and perceived minimal impact upon the environment. Specifically, ionic liquids have been applied in the dissolution of cellulose, lignin, and lignocellulosic biomass [16, 19, 21–23].

More recently, ionic liquid based cosolvents have been shown in our lab to efficiently extract bio-oil and amphiphilic molecules from oil-seed biomass [24, 25]. In the specific case of *jatropha* biomass, the cosolvent was shown to extract and solubilize phorbol esters while partitioning the extracted bio-oil to its own separate immiscible phase. Given the reported qualities of pure solutions of ionic liquids to pretreat biomass, the question was raised as to whether the cosolvent extraction process could also pretreat the carbohydrate fraction of the biomass for subsequent enzymatic hydrolysis to recover simple sugars. To this end, this work reports on the capacity of the cosolvent to concomitantly recover both bio-oil and fermentable sugars from *jatropha* and safflower biomass. Commentary is given on the input-output efficiency of the cosolvent extraction system as well as how the cosolvent disrupts the various components of the biomass and how this relates to the efficiency of enzymatic hydrolysis.

2. Experiment

2.1. Materials and Chemicals. Methanol (Fisher scientific), 1-ethyl-3-methylimidazolium acetate ([C2mim][Ac]), 90% (Aldrich), 1-ethyl-3-methylimidazolium methyl sulfate ([C2mim][$MeSO_4$]), 98% (Aldrich), sulfuric acid (Fisher Scientific), hexane (Fisher scientific), CTec2 enzyme solution (Novozymes Inc.), HTec2 enzyme solution (Novozymes Inc.), glucose (Sigma Aldrich), and xylose (Sigma Aldrich) were used as received from manufacturer. 18 MΩ cm deionized, filtered water was used to make aqueous solutions (Barnstead E-pure water purification system). *Jatropha* seeds were obtained from University of Hawaii, Department of Tropical Plant and Soil Science and safflower seeds were obtained from Pacific Biodiesel Inc. The safflower whole seeds were ground as received with a blender. The *jatropha* whole seeds (kernel plus shell) were either ground as received with a blender or deshelled and the separated shells and kernels weighed and ground separately using a blender. The composition of safflower seeds was 14% crude protein, 39% bio-oil, 29% carbohydrates, 6% moisture, and 12% lignin. *Jatropha* whole seed had 20% crude protein, 31% bio-oil, 22% carbohydrates, 8% moisture, and 19% lignin. The protein content was determined by Kjeldahl method, bio-oil by solvent extraction method, carbohydrate by difference (100 - bio-oil (%) - proteins (%) - lignin (%) - moisture (%)) and lignin by pyrolysis method.

2.2. Ionic Liquid Pretreatment prior to Carbohydrate Hydrolyses. Ionic liquid pretreatment of biomass comprised heating the biomass in sealed glass tubes filled with the ionic liquid based cosolvent at temperatures between 64 and 120°C. The ionic liquid ([C2mim][$MeSO_4$] *or* [C2mim][Ac]) was mixed with methanol at different weight ratios to a final total mass of 3.2 grams. The *jatropha* kernel was treated at 120°C for 5 hours. The safflower whole seed was treated at 64, 100, and 120°C for 5 hours. The *jatropha* whole seed (i.e., kernel and shell) and shell were treated for 5 hours at 120°C, 100, and 120°C, respectively. In all cases at least 0.8 grams of biomass was used with the 3.2 grams of cosolvent to yield a solids loading of approximately 20% (w/w). Upon completion of the pretreatment process, the mixture was centrifuged at 6260 rpm for 10 minutes producing three phases: a bottom biomass phase, a middle cosolvent phase, and a top lipid phase. The only exception to this observation was with the treatment of shell biomass which did not produce a top bio-oil (lipid) phase. The top lipid phase was recovered as reported previously [24, 26]. Approximately 10 mL of methanol was added to the combined bottom biomass and cosolvent phases in order to ensure complete precipitation of the carbohydrates and the mixture was centrifuged at 6260 rpm for 10 minutes. The bottom biomass phase was then separated from the middle cosolvent phase by decantation and washed two times with 10 mL of water and a final third time with 10 mL of 50 mM citrate buffer at pH 4.8.

2.3. Carbohydrate Hydrolyses Assay. The application of enzymatic hydrolysis across samples differed depending upon the manner in which the biomass was originally processed. Untreated biomass (i.e., controls) and biomass samples (pretreated as described in Section 2.2) were suspended in 50 mM citrate buffer at pH 4.8 at a solids loading of about 5% (w/w). To these biomass-citrate buffer mixtures dosages of Novozymes' CTec2 (for cellulose digestion) and Htec2 (for hemicellulose digestion) hydrolysis enzyme solutions were added to a final weight percent (relative to the biomass) of 12.5%. The sample mixtures were then agitated slowly between 45 and 50°C for 72 hours after which time aliquots of the mixture were syringe filtered through 0.22 um pore size filters and frozen until subsequent HPLC analysis of glucose and xylose sugar content.

2.4. HPLC Analyses of Hydrolyzed Sugars. The hydrolyzed samples from Section 2.3 were injected onto a Bio-Rad (HPX-87H) column using 5 mM H_2SO_4 as the mobile phase. The separation was achieved at 55°C using a flow rate of 0.4 mL min^{-1}. Calibration curves were made with aqueous standard solutions of glucose and xylose. The retention times of glucose, and xylose were 13.3 and 14.3 min, respectively. Unless otherwise stated, carbohydrate yields are reported as weight percent fermentable sugars, glucose and xylose, relative to the initial mass of the biomass (that is whole seed, shell, or kernel) which includes the bio-oil, protein, and carbohydrate fractions. As such that carbohydrate yields may appear low relative to the literature-reported values for biomass that were almost entirely cellulosic in nature (i.e., woods).

2.5. Scanning Electron Microscopy. Dried specimens of hydrolyzed biomass were mounted on aluminum stubs using silver paste and sputtered with gold/palladium in a Hummer 6.2 sputter coater. Images were viewed using a Hitachi

Table 1: Weight percent fermentable sugars recovered from safflower whole seeds pretreated with [C2mim][Ac]-MeOH at 64, 100, and 120°C. Yields are reported relative to weight of whole seed.

wt% [C2mim][Ac]	Safflower whole seed (wt% glucose, xylose)[a]					
	64°C		100°C		120°C	
	glucose	xylose	glucose	xylose	glucose	xylose
45%	11.8 (0.1)	3.1 (0.0)	13.7 (0.2)	5.4 (0.5)	15.4 (0.4)	7.3 (0.2)
60%	—	—	—	—	16.4 (0.5)	7.3 (0.4)
70%	11.2 (0.3)	3.5 (0.0)	15.4 (0.3)	7.0 (0.1)	17.4 (0.2)	8.0 (0.2)
80%	—	—	—	—	17.3 (0.3)	7.6 (0.1)
100%	12.1 (0.0)	3.5 (0.2)	14.5 (0.2)	5.9 (0.2)	16.7 (0.2)	7.5 (0.1)

[a]The reported values are averages of at least two samples. The values in brackets are the deviations of the measured values from the mean.

S-4800 field emission scanning electron microscopy at an acceleration voltage of 10 kV.

3. Results and Discussion

3.1. Effect of Pretreatment on Enzymatic Hydrolysis. The safflower whole seeds possessed a total of 29 wt% carbohydrates relative to total biomass available for hydrolysis to fermentable sugars. The efficiency of pretreating safflower whole seeds with [C2mim][Ac]-methanol cosolvent correlated better with process temperature than with the relative proportion of [C2mim][Ac] to methanol (Table 1). Glucose and xylose yields at 64°C were similar to those obtained on untreated safflower whole seed (i.e., 13.1 and 4 wt%, resp., relative to weight of whole seed, data not presented in Table 1) indicating that the pretreatment process was inefficient at this temperature. Pretreatment at 120°C, however, increased glucose and xylose yields by approximately 56% and 112%, respectively (e.g., from 11.2% to 17.4% between 64°C and 120°C for glucose at a [C2mim][Ac] to methanol ratio of 70 : 30). The inefficient digestion of safflower whole seed at low temperature (i.e., 64°C) in either cosolvent or pure ionic liquid likely resulted from the fact that the safflower whole seeds have a high lignocellulose composition that requires higher temperatures to initiate the breakage of the intermolecular hydrogen bonding.

With respect to the relative ratio of [C2mim][Ac] to methanol, the fermentable sugar yields slightly increased with increasing concentration of [C2mim][Ac], with a maximum of yield 25.4 wt% (relative to weight of whole seed) achieved at a [C2mim][Ac] to methanol weight ratio of 70–30 after which only a slight drop off in yield was observed as the [C2mim][Ac] concentration increased from 70 wt% to purity (Figure 1). The maximum yield achieved at a [C2mim][Ac] to methanol of 70 wt% is attributed to the impact of methanol dilution and the more efficient mixing that occurs at lower viscosity [24, 27].

Given the small difference in yields between 70 and 100 wt% [C2mim][Ac] to methanol, the rate of the hydrolysis was studied at 120°C (Figure 2) in order to determine if pretreatment with 70 wt% [C2mim][Ac] would produce much shorter hydrolysis times. The results indicate that pretreatment of safflower whole seed with [C2mim][Ac] at both 70 and 100 wt%, however, yielded similar hydrolysis rates for cellulose and hemicellulose (Table 2), suggesting

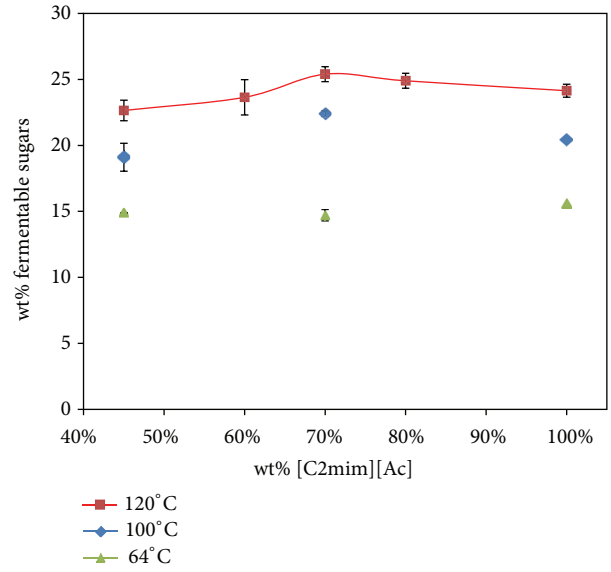


Figure 1: Total fermentable sugar yields relative to weight of whole seed from enzyme hydrolysis of safflower whole seed pretreated at various temperatures.

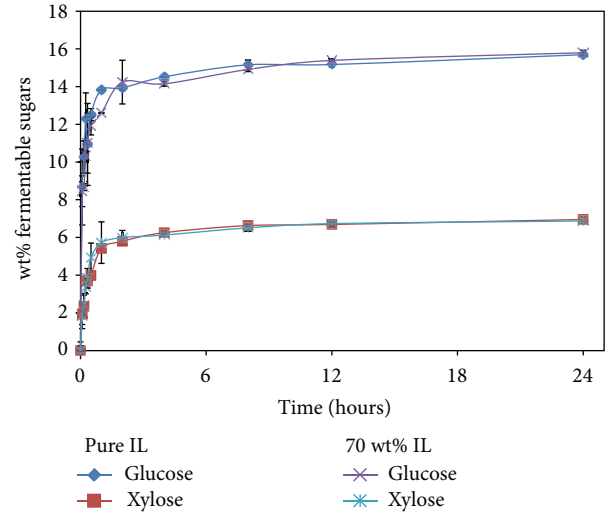


Figure 2: Effect of ionic liquid concentration on hydrolysis kinetics of safflower whole seed, using pure and 70 wt% [C2mim][Ac] at 120°C.

Table 2: Initial rates of fermentable sugar formed during hydrolysis of safflower whole seeds. Initial hydrolysis rates were calculated from the data obtained during the first 10 minutes of hydrolysis.

wt% [C2mim][Ac]	Initial rate of formation (mg/mL/min)[a]	
	70%	100%
glucose	0.43 (0.007)	0.44 (0.002)
xylose	0.12 (0.022)	0.12 (0.019)

[a]The reported values are averages of two samples. The values in brackets are the deviations of the measured values from the mean.

that the dilution of [C2mim][Ac] with methanol did not significantly reduce the rate of hydrolysis.

Pretreatment of safflower whole seed with methyl sulfate as the anion, however, was ineffective. Pretreatment with [C2mim][$MeSO_4$] at temperatures ranging between 64 and 120°C resulted in yields similar to those of untreated safflower whole seed at all temperatures, Table 3, confirming the literature-reported efficacy of acetate anion to pretreat biomass prior to enzymatic hydrolysis. The acetate anion is better able to hydrogen bond with the cellulose and to disrupt its crystalline structure, making the bonds more susceptible to enzymatic hydrolysis [20]. As such, the lower yields associated with the methyl sulfate anion are attributed to the inability of the [$MeSO_4$] anion to effectively interact with the cellulosic component of the biomass despite the fact that the [C2mim][$MeSO_4$] based cosolvent is reported to support excellent bio-oil extraction from oil-bearing biomass [24, 25].

Following the pretreatment of safflower whole seed biomass, the effect on *jatropha* whole seed was similarly evaluated at 120°C but using only acetate as the anion (Figure 3). The efficiency of fermentable sugar production increased slightly as the relative concentration of [C2mim][Ac] was increased from 40 to 70 wt%, with a step jump observed between 70 and 80 wt% followed by a gradual linear increase until pure [C2mim][Ac] was achieved. This result contrasted against the slight decrease in yield found for safflower whole seed biomass as the relative concentration of [C2mim][Ac] was increased above 70 weight percent. It was hypothesized that the variation in yield response was a result of the *jatropha* whole seeds possessing a much harder and thicker shell as compared to safflower whole seeds which possess a thin shell that is difficult to separate from the kernel.

To confirm this hypothesis, the *jatropha* shell and kernel were independently pretreated and their hydrolysis yields investigated. Yields from the kernel biomass (data not shown) pretreated at 120°C in 70 and 100 wt% [C2mim][Ac] were equivalent at 9 wt% total fermentable carbohydrates (relative to weight of kernel), inferring that [C2mim][Ac] concentration had negligible effect on the efficiency of subsequent enzymatic hydrolysis. By contrast, pretreatment of the *jatropha* shell with [C2mim][Ac] cosolvent (Figure 4) led to fermentable sugar profile similar to that of the *jatropha* whole seed (i.e., shell plus kernel, Figure 3) confirming that the presence of the *jatropha* shell is the source of the observed variation in fermentable sugar yields.

As anticipated, the yield of fermentable sugars from *jatropha* shell at 100°C were lower than that observed at 120°C

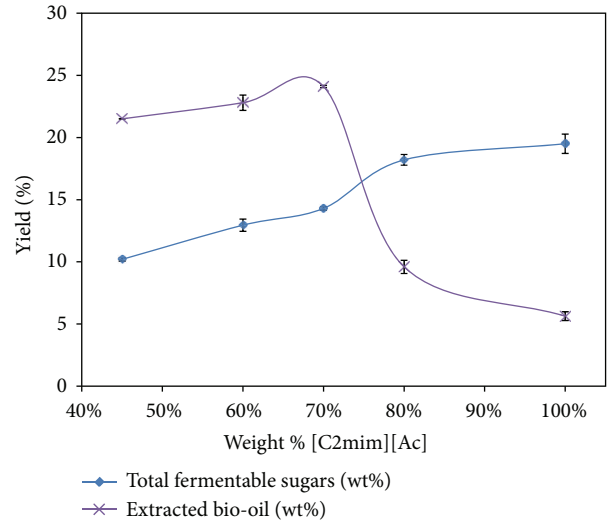


Figure 3: Total fermentable sugars and bio-oil yields relative to weight of whole seed obtained from pretreated *jatropha* whole seed (kernel plus shell) at different concentrations of [C2mim][Ac].

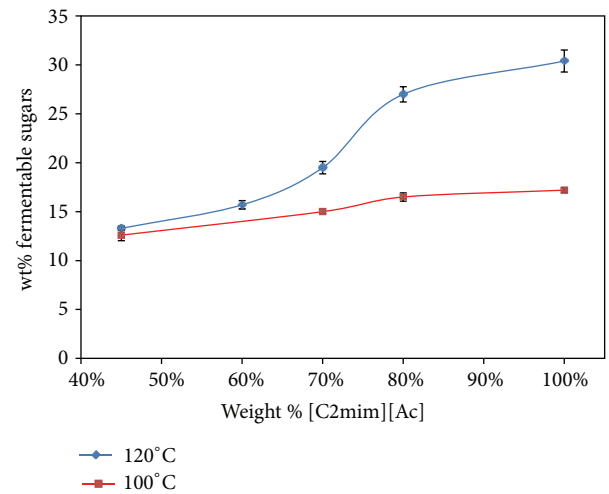


Figure 4: Total fermentable sugar yields relative to weight of *jatropha* shell released from *jatropha* shell at different temperatures after pretreatment.

with the biggest changes in yields occurring after 70 wt% [C2mim][Ac]. Unlike the safflower biomass (which does not contain a hard outer shell like *jatropha*), it appears that [C2mim][Ac] concentrations above 70 wt% are necessary to effectively disrupt the intermolecular bonds in the hard and thick *jatropha* shell [28]. The acetate anion enhances the separation of carbohydrates from the lignin protective matrix and also disrupts the hydrogen bonding between the cellulose microfibrils [14]. This can cause partial dissolution of the biomass into the solvent and, in expansion of the biomass matrix, all of which enhance the penetrability of the hydrolysis enzymes.

The xylose content from the *jatropha* shell for pretreatments in both pure and 80 wt% [C2mim][Ac] was quite similar (Table 4), indicating that hemicellulose was also

Table 3: Weight percent fermentable sugars (relative to weight of whole seed) released from safflower whole seed pretreated with the co-solvent [C2mim][$MeSO_4$]-MeOH.

Wt% [C2mim][$MeSO_4$]	Safflower whole seeds (wt% glucose, xylose)[a]					
	64°C		100°C		120°C	
	glucose	xylose	glucose	xylose	glucose	xylose
45%	10.5	2.3	9.9	2.4	—	—
70%	10.8	2.3	11	2.2	10.6	2.5
100%	—	—	—	—	11.5	2.2

[a]The reported values represent repeated HPLC measurements that have been verified to vary by no more than four percent of the reported values.

Table 4: Weight percent fermentable sugars (relative to weight of *jatropha* whole seed or *jatropha* shell) recovered from *jatropha* whole seed and *jatropha* shell pretreated with the cosolvent [C2mim][Ac]-MeOH at 100 and 120°C.

wt% ionic liquid	*Jatropha* whole seed[a,c]		*Jatropha* shell[b,d]			
	120°C		100°C		120°C	
	glucose	xylose	glucose	xylose	glucose	xylose
45%	9.1 (0.1)	1.1 (0.0)	10.8 (0.3)	1.8 (0.1)	11.2 (0.1)	2.1 (0.0)
60%	10.8 (0.3)	2.1 (0.0)	—	—	12.6 (0.3)	3.1 (0.0)
70%	11.7 (0.0)	2.6 (0.1)	12.2 (0.1)	2.8 (0.1)	14.2 (0.6)	5.3 (0.2)
80%	14.1 (0.3)	4.1 (0.0)	13.3 (0.2)	3.2 (0.2)	17.7 (0.4)	9.3 (0.0)
100%	15.5 (0.3)	4.0 (0.3)	14.1 (0.1)	3.1 (0.1)	21.7 (0.7)	8.7 (0.2)

[a,b]The reported data are averages of at least two samples. The values in brackets are the deviations of the measured values from the mean.
[c]*Jatropha* whole seed fermentable sugar values are relative to weight of *jatropha* whole seed.
[d]*Jatropha* shell fermentable sugar values are relative to weight of *jatropha* shell.

digested to the same extent. Thus, the slight difference in total fermentable sugars (3.4 wt%) recovered from the *jatropha* shell using pretreatments of pure or 80 wt% [C2mim][Ac] at 120°C is predominately due to the incomplete utilization of cellulose during the enzyme hydrolysis of the shell in 80 wt% [C2mim][Ac]. That said, the small difference in yield (i.e., 3.4 wt%) does suggest that the sole pretreatment of *jatropha* shell can effectively be executed using only 80 wt% [C2mim][Ac] at 120°C if one desire to use less ionic liquid.

To estimate the efficiency relative to the carbohydrate fraction of the oil seed biomass (as opposed to the amount of whole seed biomass as reported previously and in the tables) the amounts of recovered fermentable sugars were converted to hydrolyzed carbohydrates (cellulose and hemicellulose) using 0.9 and 0.88 as conversion factors for glucose and xylose concentrations to cellulose and hemicellulose, respectively. These values were then used to estimate the carbohydrate fraction of the whole seeds (i.e., 29 wt% and 22 wt% for safflower and *jatropha*, resp.). The calculations indicate that the [C2mim][Ac] cosolvent yields carbohydrate recovery efficiencies (relative to the baseline carbohydrate content of the whole seeds) as high as 74% achieved at 80 wt% [C2mim][Ac] on *jatropha* and 78% with 70 wt% [C2mim][Ac] on safflower seeds.

3.2. Coextraction of Bio-Oil. Both safflower and *jatropha* seeds contain significant amounts of bio-oil and its co-extraction during the pretreatment step is desired. Previous reports on the extraction of bio-oil from *jatropha* kernel [24] using the cosolvent system at 64°C have shown a nonlinear response in yield to ionic liquid weight percent. Thus, we characterized the extraction of bio-oil from *jatropha* whole seed biomass at a pretreatment temperature of 120°C. As seen in Figure 3, maximum oil yields are observed at [C2mim][Ac] concentrations approaching 70 wt% after which a significant drop off in yield was observed. For the purpose of multi-component extraction of bio-oil and recovery of fermentable sugars, Figure 3 suggests an optimal [C2mim][Ac]-methanol ratio of 70 : 30 wt%.

Table 5: Bio-oil yields relative to weight of whole seed from safflower whole seed treated with the cosolvent [C2mim][Ac]-MeOH at 120°C.

wt% ionic liquid	Safflower[a] (wt% bio-oil)
45%	37.5 (0.4)
70%	35.8 (1.5)
100%	30.5 (0.5)

[a]The reported data are averages of two samples. The values in brackets are the deviations of the measured values from the mean.

Similar to that found previously for *jatropha* oil seeds [24], pretreatments of safflower whole seed using [C2mim][Ac] concentrations between 45 and 70 wt% produced the highest extracted bio-oil yields (Table 5). Pure [C2mim][Ac] provided the least efficient bio-oil yield at 30.5 wt% (relative to whole seed) providing further confirmation that 70 wt% [C2mim][Ac] is more appropriate for the effective multicomponent extraction of biooil and fermentable sugars from both safflower and *jatropha* whole seeds.

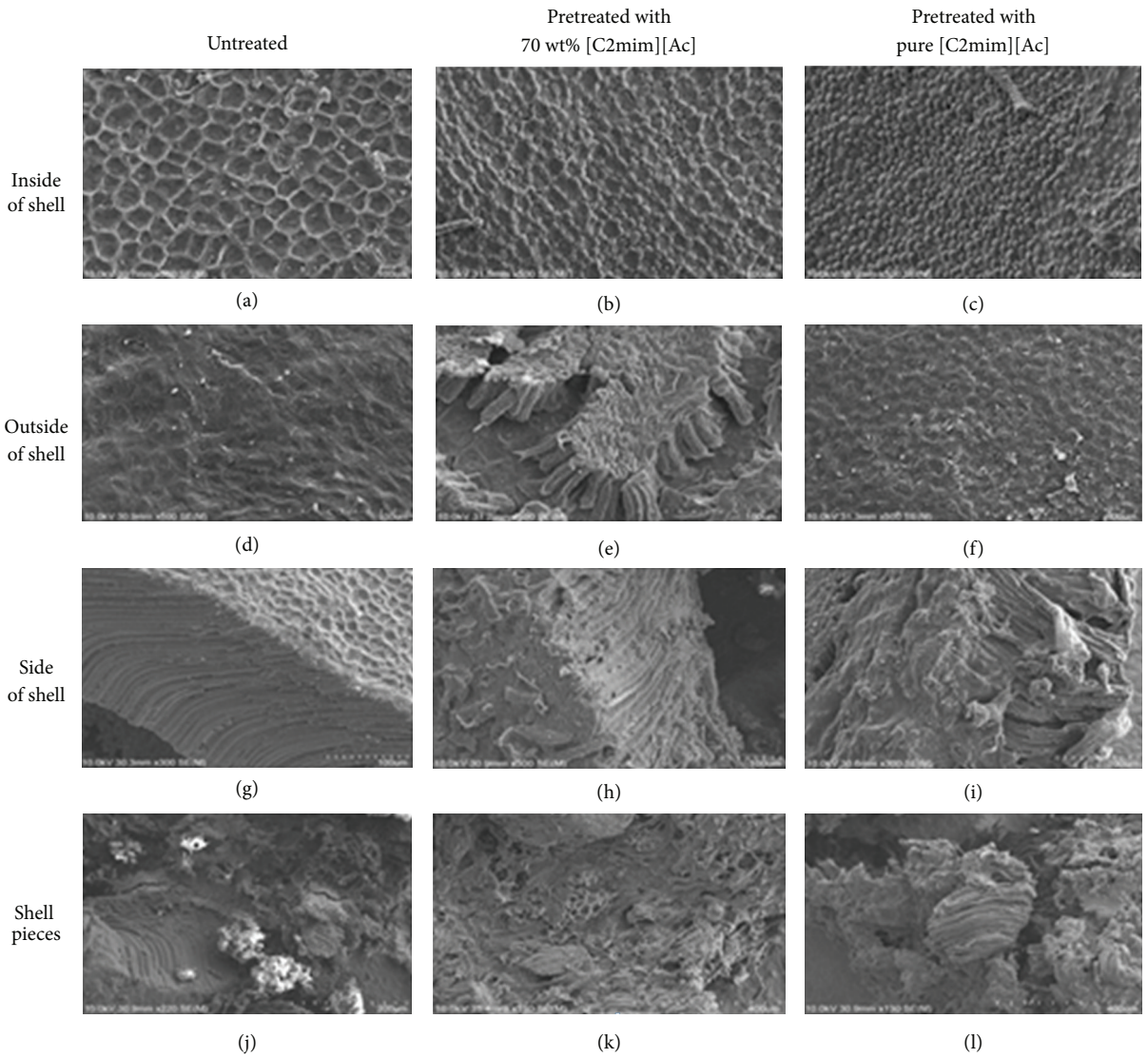
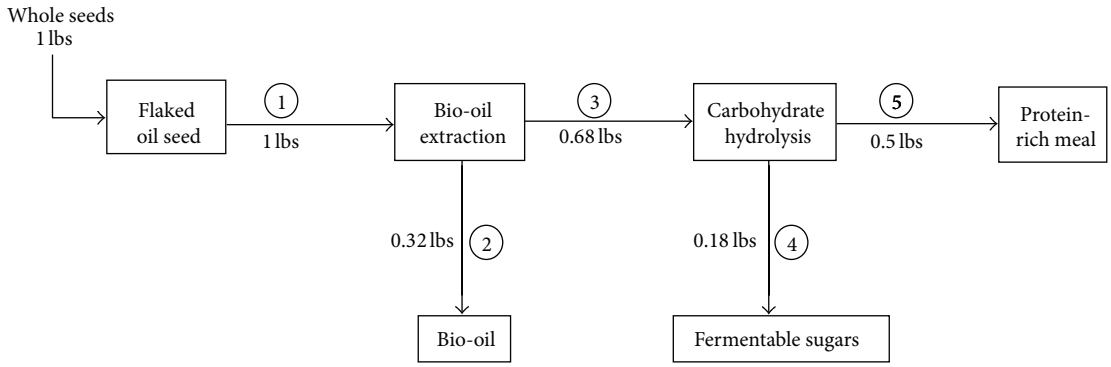


Figure 5: SEM images of *jatropha* shell taken at 100 μm, ((a)–(i)), 200 μm (j), and 400 μm ((k)-(l)).

3.3. SEM Analysis of Pretreated Biomass. The *jatropha* shell was found to be the most difficult component to enzymatically hydrolyze as evidenced by the large variation of fermentable sugar yields with ionic liquid concentration. To confirm this finding, the pretreated *jatropha* shell was visualized using high-resolution imaging (Figure 5). The micrographs of untreated shell (control) showed intact rod-like fibril structure and smooth outside surface. A surface layer covering the fibril cell structure can be seen from the inside view of the shell. No singular or loose fibers were observed in the untreated shell. SEM images of treated shells showed significant difference between untreated and pretreated (70 wt% [C2mim][Ac] and pure [C2mim][Ac]) *jatropha* shell biomass. For example, the pure [C2mim][Ac] pretreated shell possessed a loose distorted fiber structure and pores (Figures 5(i) and 5(l)) likely due to the delignification of the biomass. The inside surface layer of the *jatropha* shell was entirely removed during pretreatment exposing the top surface of the fibril structure (Figure 5(c)). The 70% (w/w) [C2mim][Ac] pretreated *jatropha* shell showed similar disruption of the biomass though to a slightly less extent than pure [C2mim][Ac]. The SEM analysis supports the enzyme hydrolysis data which showed high fermentable sugar yields with 70 wt% [C2mim][Ac] and pure [C2mim][Ac]. Improved contact between the enzyme and biomass occurs due to the disruption of the outer surface layers of the *jatropha* shell, distortion and loosening of the fiber structure, and creation of pore structure aids in enzyme hydrolysis of the *jatropha* shell.

4. Systems Analysis

The commercialization of a fuel from oil seed industry will be enhanced by the utilization of multiple components such as proteins, carbohydrates, and lignin to compensate for the marginal market value of the bio-oil product. As the ionic liquid cosolvent system reported in this work appears to support



Stage number	wt% lb lb[1]				
	1	2	3	4	5
	Flaked oil seed	Bio-oil	Seed cake	Fermentable sugars	Protein-rich meal
Moisture	6	0	—	0	—
Bio-oil	32	100	2	0	0
Total fermentable sugars[a]	18	0	34	100	0
Protein	19	0	28	0	48
Lignin	17	0	24	0	41
Ash	6	0	8	0	10
Total	98	100	96	100	99

[a]Taken as a two to one ratio of glucose to xylose

Figure 6: Mass balances for solvent extraction of *jatropha* whole seed and composition of the intermediate stages. Whole *jatropha* seed taken to comprise moisture (6%), bio-oil (32%), total fermentable sugars (18%), protein (19%), lignin (17%), and ash (6%). The values in the tables are averages taken from the literature and consequently do not always sum to precisely 100 [5–8].

pathways for additional byproducts, we have attempted a first pass systems' analysis that evaluates two specific pathways to process *jatropha* oil seed into the following products: (1) fermentable sugars, (2) bio-oil, and (3) protein-rich meal.

The first pathway begins with the flaking of the *jatropha* whole seed (Figure 6). The flaked *jatropha* whole seed is assumed treated with the ionic liquid based cosolvent as discussed previously. The top bio-oil phase is assumed fully recovered leaving behind the cosolvent and digested biomass. The cosolvent phase is also recovered and recycled whilst the cosolvent pretreated biomass (seed cake) is hydrolyzed with enzymes to convert the structural carbohydrates into fermentable sugars (e.g., glucose and xylose). The protein-rich biomass remaining after carbohydrate extraction is assumed desolvatized for use as a supplement to animal feed. A mass balance (assuming complete recovery of the bio-oil, carbohydrates, and protein-rich meal from the whole seeds) applied to this pathway suggests that 1 pound of *jatropha* whole seed yields approximately 0.18 pounds of fermentable sugars, 0.32 pounds of bio-oil, and 0.50 pounds of protein-rich meal. The analysis also assumes effective bio-oil and carbohydrate recovery which is expected to occur as a result of multiple cosolvent extraction cycles performed in a multistage countercurrent extractor or by accelerated solvent extraction techniques. A preliminary energy analysis based on inputs (*jatropha* whole seeds) and outputs (bio-oil, carbohydrates, and protein-rich meal) was performed to ascertain the feasibility of *jatropha* processing. The results, shown in Table 6, approximate a significant energy recovery of 10,954 Btu and a positive cost analysis of $0.77 recovered per pound of whole *jatropha* seed processed. The cost analysis was estimated from the market prices of inputs and outputs of soybean processing and is presented as an approximate indicator of profitability (see Table 6).

Based upon the heats of combustion of the products from the first pathway, roughly 99% of energy available in the *jatropha* whole seed is recovered compared to only 49% if only the bio-oil is recovered. The energy analysis does not include the processing energy inputs for the pretreatment and hydrolysis steps; thus, inclusion of this input energy is anticipated to lower the overall system efficiency. More, we propose the conversion of the carbohydrate fraction to fermentable sugars (can be used for fermentation of bio-oils or alcohols) as preferable to its use as an animal feed (particularly given that the presence of any level of phorbol ester toxins complicates its use in this manner) [29, 30].

The second pathway considered likewise begins with the whole seed but processes only the kernel after it has been

Table 6: Energy and price balances from *jatropha* whole seed processing using first and second pathways.

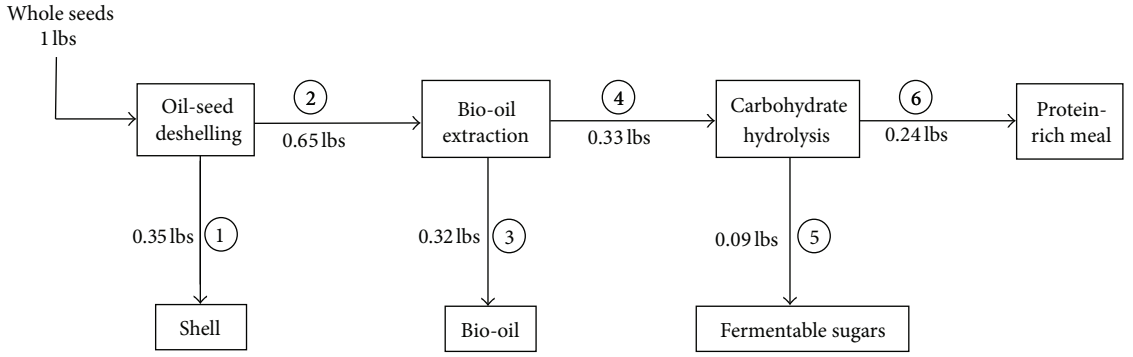
	First pathway	Second pathway
Heat of combustion inputs (Btu/lb whole seed)[a]	10963	10963
Heat of combustion outputs (Btu/lb whole seed)[b]	10954	7586
% recovery	99	69
costs of feedstock/lb[c]	0.22	0.22
market value of products/lb of whole seed[d]	0.99	0.55
% profit	350	150

[a]Heat of combustion of inputs stated as that of *jatropha* whole seed [9].

[b]Heats of combustion of outputs obtained from *jatropha* oil (17498 Btu/lb), fermentable sugars (glucose (6700 Btu/lb) and xylose (6710 Btu/lb) in 2 : 1 ratio) and protein-rich meal (calculated from kernel cake (7825 Btu/lb) and seed cake (10791 Btu/lb) by substracting the contribution from carbohydrate components) [5, 9, 10].

[c]Costs of inputs estimated from commodity price of soybean whole seed [11].

[d]Price of protein-rich meal and bio-oil estimated from soybean and fermentable sugars from 25 kg glucose at Sigma Aldrich [11].



Stage number	wt% lb lb^{-1}					
	1	2	3	4	5	6
	Shell	Kernel	Bio-oil	Kernel cake	Fermentable sugars	Protein meal
Moisture	9	4.5	0	0	0	0
Bio-oil	1	48	100	2	0	0
Total fermentable sugars[a]	32	9	0	18	100	0
Protein	4	32	0	69	0	86
Lignin	49	1	0	2	0	2.5
Ash	5	4	0	10	0	13
Totals	100	98.5	100	101	100	101.5

[a]Taken as a two to one ratio of glucose to xylose

Figure 7: Mass balances for solvent extraction of *jatropha* kernel and the composition of intermediate stages. Whole *jatropha* seed taken to comprise moisture (6%), bio-oil (32%), total fermentable sugars (18%), protein (19%), lignin (17%), and ash (6%). The values in table are averages taken from the literature and consequently do not always sum to precisely 100 [5–8].

separated from the shells (Figure 7). The separation of the shell from the kernel theoretically improves the yield of bio-oil recovered per unit volume of biomass processed but at the cost of lowering the yield of fermentable sugars. In our example, 1 pound of *jatropha* whole seed yields 0.65 pounds of kernel which produces approximately 0.09 pounds of fermentable sugars, 0.32 pounds of bio-oil, and 0.24 pounds of protein-rich meal. As seen in Table 6, the second pathway recovers a lower amount of energy (69% of caloric value of whole seed) and our cost analysis suggests a profit of $0.33 per pound processed. However, the energy balance and cost analysis improve if only the bio-oil is extracted.

5. Conclusions

In this study, an ionic liquid, methanol cosolvent was shown for the first time to effectively extract bio-oil and recover fermentable sugars from oil-seed biomass. This represents an improvement over current approaches that have focused solely on the recovery of bio-oil or fermentable sugars [3]. Effective co-recovery of both bio-oil and fermentable sugars was shown to require both cosolvent components with an optimal concentration of 70–30 wt% [C2mim][Ac] to methanol ratio and a processing temperature of 120°C. Under these conditions, nearly all the bio-oil (35.8 and 24.1 wt%

relative to weight of whole seed) was extracted and autopartitioned to a separate immiscible phase and approximately 25.4 and 14.3 wt% (relative to weight of whole seed) of fermentable sugars were recovered from the safflower and *jatropha* whole seeds, respectively. This constitutes a combined carbohydrate and bio-oil co-recovery of 61.2 and 38.4 wt% of the safflower and *jatropha* whole seeds, demonstrating a new pathway for processing increased products from oil-seed biomass. A first pass model analysis suggested that the most optimal processing pathway would be to pretreat the *jatropha* whole seed with the cosolvent and recover bio-oil, fermentable sugars, and a protein-rich meal.

Conflict of Interests

The authors confirm that there is no conflict of interests with regard to financial gain for any of the companies or entities referred to in the Methods section of the paper from which reagents, chemicals, and other materials were purchased. Funding from this work was solely as stated in the acknowledgement and no interaction occurred with the mentioned commercial entities commercial identities (i.e., Fisher scientific, Aldrich, Novozymes Inc., and Sigma Aldrich) other than to purchase chemicals, reagents, or materials from them through typical catalogue purchase.

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References

[1] A. T. W. M. Hendriks and G. Zeeman, "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresource Technology*, vol. 100, no. 1, pp. 10–18, 2009.

[2] M. Cooney, G. Young, and N. Nagle, "Extraction of bio-oils from microalgae," *Separation and Purification Reviews*, vol. 38, no. 4, pp. 291–325, 2009.

[3] M. Mora-Pale, L. Meli, T. V. Doherty, R. J. Linhardt, and J. S. Dordick, "Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass," *Biotechnology and Bioengineering*, vol. 108, no. 6, pp. 1229–1245, 2011.

[4] H. E. Synder and T. W. Kwon, *Soybean Utilization*, Van Nostrand Reinhold, New York, NY, USA, 1987.

[5] W. M. J. Achten, L. Verchot, Y. J. Franken et al., "*Jatropha* biodiesel production and use," *Biomass and Bioenergy*, vol. 32, no. 12, pp. 1063–1084, 2008.

[6] R. Staubmann, "Biogas production from *Jatropha curcas* press-cake," *Applied Biochemistry and Biotechnology A*, vol. 63-65, no. 1–3, pp. 457–467, 1997.

[7] Y. Liang, T. Siddaramu, J. Yesuf, and N. Sarkany, "Fermentable sugar release from *Jatropha* seed cakes following lime pretreatment and enzymatic hydrolysis," *Bioresource Technology*, vol. 101, no. 16, pp. 6417–6424, 2010.

[8] A. M. J. Kootstra, H. H. Beeftink, and J. P. M. Sanders, "Valorisation of *Jatropha curcas*: solubilisation of proteins and sugars from the NaOH extracted de-oiled press cake," *Industrial Crops and Products*, vol. 34, no. 1, pp. 972–978, 2011.

[9] K. Openshaw, "A review of *Jatropha curcas*: an oil plant of unfulfilled promise," *Biomass and Bioenergy*, vol. 19, no. 1, pp. 1–15, 2000.

[10] M. A. V. R. da Silvaa, M. D. M. C. R. da Silvaa, A. I. M. C. L. Ferreiraa, Q. Shib, B. F. Woodfieldb, and R. N. Goldbergc, "Thermochemistry of alpha-D-xylose(cr)," *Journal of Chemical Thermodynamics*, vol. 58, pp. 20–28, 2012.

[11] USDA, *Oilseeds: World Markets and Trade*, United States Department of Agriculture, 2013.

[12] T. Adriaans, "Suitability of solvent extraction for *Jatropha curcas*," Ingenia Consultants and Engineers, for FACT Foundation, 2006.

[13] M. J. Cooney and C. K. H. Guay, "Heterotrophic algal-biodiesel production: challenges and opportunities," in *Biofuel and Bioenergy from Biowastes and Lignocellulosic Biomass*, S. K. Khanal, Ed., pp. 346–370, American Society of Civil Engineers, 2010.

[14] G. Brodeur, E. Yau, K. Badal, J. Collier, K. B. Ramachandran, and S. Ramakrishnan, "Chemical and physicochemical pretreatment of lignocellulosic biomass: a review," *Enzyme Research*, vol. 2011, Article ID 787532, 17 pages, 2011.

[15] L. C. Sousa, S. P. Chundawat, V. Balan, and B. E. Dale, "'Cradle-to-grave' assessment of existing lignocellulose pretreatment technologies," *Current Opinion in Biotechnology*, vol. 20, no. 3, pp. 339–347, 2009.

[16] H. Zhao, G. A. Baker, and J. V. Cowins, "Fast enzymatic saccharification of switchgrass after pretreatment with ionic liquids," *Biotechnology Progress*, vol. 26, no. 1, pp. 127–133, 2010.

[17] C. Li, B. Knierim, C. Manisseri et al., "Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification," *Bioresource Technology*, vol. 101, no. 13, pp. 4900–4906, 2010.

[18] I. P. Samayam, B. L. Hanson, P. Langan, and C. A. Schall, "Ionic-liquid induced changes in cellulose structure associated with enhanced biomass hydrolysis," *Biomacromolecules*, vol. 12, no. 8, pp. 3091–3098, 2011.

[19] R. P. Swatloski, S. K. Spear, J. D. Holbrey, and R. D. Rogers, "Dissolution of cellose with ionic liquids," *Journal of the American Chemical Society*, vol. 124, no. 18, pp. 4974–4975, 2002.

[20] *Ionic Liquids*, B. Kirchner, Ed., vol. 290 of *Topics in Current Chemistry*, Springer, New York, NY, USA, 2009.

[21] X. Zhao, K. Cheng, and D. Liu, "Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis," *Applied Microbiology and Biotechnology*, vol. 82, no. 5, pp. 815–827, 2009.

[22] M. Zavrel, D. Bross, M. Funke, J. Büchs, and A. C. Spiess, "High-throughput screening for ionic liquids dissolving (ligno-)cellulose," *Bioresource Technology*, vol. 100, no. 9, pp. 2580–2587, 2009.

[23] Y. Pu, N. Jiang, and A. J. Ragauskas, "Ionic liquid as a green solvent for lignin," *Journal of Wood Chemistry and Technology*, vol. 27, no. 1, pp. 23–33, 2007.

[24] G. Severa, G. Kumar, M. Troung, G. Young, and M. J. Cooney, "Simultaneous extraction and separation of phorbol esters and bio-oil from *Jatropha* biomass using ionic liquid-methanol co-solvents," *Separation and Purification Technology*, vol. 116, pp. 265–270, 2013.

[25] G. Young, F. Nippgen, S. Titterbrandt, and M. J. Cooney, "Lipid extraction from biomass using co-solvent mixtures of ionic liquids and polar covalent molecules," *Separation and Purification Technology*, vol. 72, no. 1, pp. 118–121, 2010.

[26] G. Young, F. Nippgen, S. Titterbrandt, and M. J. Cooney, "Lipid extraction from biomass using co-solvent mixtures of ionic liquids and polar covalent molecules," *Separation and Purification Technology*, vol. 72, no. 1, pp. 118–121, 2010.

[27] Z. Wei, Y. Zu, Y. Fu et al., "Ionic liquids-based microwave-assisted extraction of active components from pigeon pea leaves for quantitative analysis," *Separation and Purification Technology*, vol. 102, pp. 75–81, 2013.

[28] D.-A. Z. Wever, H. J. Heeres, and A. A. Broekhuis, "Characterization of physic nut (*Jatropha curcas* L.) shells," *Biomass and Bioenergy*, vol. 37, pp. 177–187, 2012.

[29] H. P. S. Makkar, K. Becker, F. Sporer, and M. Wink, "Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*," *Journal of Agricultural and Food Chemistry*, vol. 45, no. 8, pp. 3152–3157, 1997.

[30] J. Martínez-Herrera, P. Siddhuraju, G. Francis, G. Dávila-Ortíz, and K. Becker, "Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico," *Food Chemistry*, vol. 96, no. 1, pp. 80–89, 2006.