ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

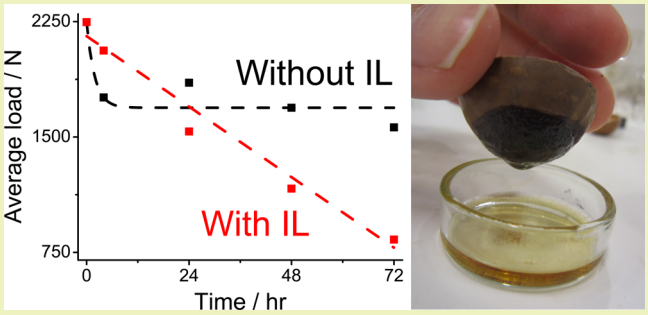
Pretreatment of Macadamia Nut Shells with Ionic Liquids Facilitates Both Mechanical Cracking and Enzymatic Hydrolysis

Wuan Xin Teh, Md. Mokarrom Hossain, Trang Quynh To, and Leigh Aldous*

School of Chemistry, UNSW Australia, Sydney, New South Wales 2052, Australia

Supporting Information

ABSTRACT: The effect of ionic liquids upon the mechanical and (bio)chemical integrity of macadamia nut shells (from *Macadamia integrifolia*) has been investigated. Whole macadamia nuts-in-shell are notoriously difficult to crack, and the Australian macadamia nut shells used in this study required 2240 ± 430 N of force to crack. Ionic liquids were screened for their solubility values, with 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) able to dissolve 5.5 ± 0.5 wt % macadamia nut shell. Treatment with small quantities of [Emim][OAc] resulted in weakened whole nut-in-shells that could be cracked with only ca. 46% of the displacement (0.67 ± 0.16 mm), ca. 34% of the force (760 ± 240 N) and ca. 15% of the energy (0.25 ± 0.10 J per shell) relative to no treatment. Further treatment by dissolution and precipitation of macadamia nut shell, followed by enzymatic hydrolysis with cellulase, resulted in the release of 80 ± 15% of the expected glucose content, relative to 1.3 ± 1.0% before any pretreatment.



KEYWORDS: *Macadamia, Nuts-in-shell, Facilitated biomass processing, Weakening biomass, Ionic liquids*

INTRODUCTION

Ionic liquids (ILs) are increasingly difficult to define, due to their increasing diversity in chemistry, structure and function. A general definition of ILs is that they are liquid below 100 °C, and at this point, it should be an ionic compound. A staggering number of IL structures are possible.[1] Of these, a relatively small number of ILs possess the ability to act as nonderivatizing (to a degree[2]) near-universal solvents for a wide range of lignocellulosic biomasses, spanning from soft and hard woods to rice husks, bagasse, straws and grasses, as examples.[3] With weight percentage solubility values exceeding 50% for lignin[4] and cellulose[5] in ILs, and with complicated biomass samples dissolving relatively intact either at room temperature[6] or within minutes at elevated temperatures,[7] comparable solvents for biomass processing do not exist. The negligible volatility of most ILs implies theoretically zero volatile organic components (VOCs) pollution during application and ease of recyclability. When combined with tunable toxicity and functionality,[8] tentative "green" credentials are apparent in the potential replacement of other solvents with ILs.

Nuts (encased in nut shells) require processing to isolate the edible components from the waste lignocellulosic components. Biorefinery processing of nonedible lignocellulosic biomasses typically aims to significantly disrupt the holocellulosic components (e.g., to facilitate enzymatic accessibility) and to remove and isolate the lignin.[9] ILs have been highlighted as significant pretreatment media that can facilitate grinding of wood,[10] the subsequent enzymatic hydrolysis of the wood,[11] the selective dissolution of individual fractions of wood[12] as well as the complete dissolution and subsequent fractionation of wood into cellulosic,[13] lignin[13] and hemicellulosic[14] fractions. As noted above, ILs have since been demonstrated to be near universal solvents for lignocellulosic biomass, although to the best of our knowledge they have not been investigated with respect to nut shells in general, and macadamia nut shells in particular.

Macadamia is the generic term referring to a number of species of trees that are native to Australia. *Macadamia integrifolia* trees are now extensively cultivated for their edible, round seeds that are encased in a tough, smooth casing. These seeds (and tough seed coat) are popularly mistaken as nuts (and nut shell).[15] Due to the widespread adoption of this misconception, they will be referred to as nuts, nut-in-shell and nut shell throughout this paper.

Macadamia cultivation has now expanded to much of Australia, as well as the United States of America (Hawaii and California), South Africa, Brazil and several other countries. From 2006 to 2011, Australia accounted for roughly 38% of the worldwide production of madacamia nuts (ca. 40 000 metric tonnes of shelled nuts per annum from Australia alone), with South Africa accounting for 23% and the USA for 16%.[16]

Macadamia nut shells have been described as an "isotropic wood"[17] and possesses many remarkable physical properties. Compared to annealed aluminum, the shells possess half the

Received: February 17, 2015
Revised: March 18, 2015
Published: March 18, 2015

DOI: 10.1021/acssuschemeng.5b00126
ACS Sustainable Chem. Eng. 2015, 3, 992−999

density of the metal but only fracture at twice the yield strength of the metal.[18] The strength in tension and specific strength of the shells exceeds that of concrete.[18] Their work of fracture is about 1 order of magnitude higher than modern, high quality ceramics, while their elastic modulus is nearly 2 orders of magnitude lower.[18] The microstructure consists of "vascular bundles" of "rods" of lignified cells in close-packed format.[6,18] The shell contains extensive fracturing which act to relieve strain, and the entire structure actively diverts or even terminates cracks.[19] The first ca. 160 μm of the exterior shell is dense and tough, while the remaining ca. 1.5 to 5 mm shell underneath is significantly more open and porous.[20]

Macadamia nut shells are notoriously difficult to mechanically crack, and represent a significant technical challenge, specifically when the objective is to isolate the considerably more fragile nut intact. Extensive and sophisticated commercial cracking facilities are therefore required. As nuts are purchased from farmers whole-in-shell, and the sell-on price for bulk whole macadamia nuts (currently ca. \$17 kg^{-1} in Australia) slightly exceeds that for nut halves and vastly exceeds that of smaller nut fragments (ca. \$0.50 kg^{-1}), a tremendous incentive drives innovation in the macadamia nut shell cracking industry. Numerous patents are available spanning from sophisticated mechanical impacting[21] and cutting[22] devices through to high-powered dual laser beams for disintegrating the shell.[23]

The water content of macadamia nuts-in-shell is reduced prior to cracking,[24] due to the associated shrinking of the nut and minor embrittlement of the shell, thus partially facilitating shell cracking. Often this process involves the combustion of materials such as liquefied petroleum gas in order to provide a continuous stream of hot air to massive silos or batch column driers over a period of several days.[25] Therefore, although in theory macadamia nut plantations are a form of carbon sequestration,[26] the actual cracking process is significantly polluting. The shells and other waste (ca. 70% of the nut-in-shell weight) are underutilized, whereas considerable combustion of primarily fossil fuels is driven by the final high value of the nut.

In this work, we have investigated the chemical pretreatment of macadamia nuts-in-shell with ILs. This represents a novel route to (chemically) facilitate the mechanical cracking of macadamia nuts-in-shell. Under unoptimized conditions, IL-treated shells were cracked with only ca. 34% of the force and ca. 15% of the energy relative to untreated nut-in-shells. The IL pretreated nut shell flour could be further dissolved in a larger volume of IL (up to 5.5 ± 0.5 wt % macadamia nut shell in [Emim][OAc]). This IL pretreatment significantly facilitated downstream treatment of the waste lignocellulosic shells. A cellulose-rich fraction could be easily recovered from the IL, from which 80 ± 15% of their total glucose content[27] could be released after 48 h enzymatic hydrolysis treatment, compared to 1.3 ± 1.0% from macadamia nut shell flour with no prior pretreatment.

MATERIAL AND METHODS

Materials and Instruments. All biomass samples and ILs were used as received, except where specified in the main text.

Macadamia nut shell flour, average diameter quoted by supplier as 212 μm, was kindly donated by Micro Milling Pty Ltd. (NSW, Australia). Whole macadamia nuts-in-shell and shell fragments were kindly donated by Macadamias Direct (NSW, Australia). Macadamia nuts were initially screened by briefly placing them in water. Breached shells were apparent by bubble formation and discarded. The nuts-in-shell that sank were classed as ripe (representing full shell development). These nuts-in-shell were then air-dried on the bench overnight before further experiments.

The ILs 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF_6]), 1-ethyl-3-methylimidazolium tetrafluoroborate ([Emim][BF_4]), 1-ethyl-3-methylimidazolium dicyanimide ([Emim][$N(CN)_2$]), 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([Emim][CF_3SO_3], 1-ethyl-3-methylimidazolium thiocyanate ([Emim][SCN]), 1-ethyl-3-methylimidazolium diethylphosphate ([Emim][DEP]), 1-ethyl-3-methylimidazolium ethylsulfate ([Emim][$EtSO_4$]), 1-ethyl-3-methylimidazolium hydrogen sulfate ([Emim][HSO_4]) and 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) were all purchased (IoLiTec, Germany). All were specified as "high purity grade", which indicates >98% purity by NMR, except [Emim][OAc] ("technical grade", >95% purity by NMR). All associated certificates of analysis indicated that purity >99% for both anion and cation ion chromatography assays.

All thermal gravimetric analysis (TGA) experiments were performed using a TGA/DSC 1 STARe system (Mettler-Toledo, Switzerland). Approximately 10 mg of sample was placed in a platinum crucible and heated at a rate of 10 °C min^{-1} under a continuous flow of 30 mL min^{-1} Argon. All IR spectra were recorded using a computer controlled (OMNIC software) Avatar 370 FT-IR (Thermo Nicolet, USA) infrared spectrophotometer. Water content was measured by Karl Fischer titration using an 831 KF Coulometer (Metrohm, Switzerland). UV−vis spectra were acquired using a Cary 100 Bio (Varian, Australia) UV−vis spectrophotometer. Microscope experiments were performed using an Olympus CX31-P polarizing microscope (Olympus, Australia) using an Instec STC200 heating platform and QImaging Micropublisher 3.3 RTV digital camera. X-ray diffraction (XRD) experiments were performed using Phillips X'pert Multipurpose X-ray diffraction system (MPD) equipped with graphite monochromatized Cu Kα radiation (λ = 1.54 Å) in the 2θ range of 5−95° with a step size of 0.026° and a scanning rate of 0.15° s^{-1}.

Solubility and Fractionation Tests. Solubility was screened in a range of ILs under quiescent conditions in a heater block, adding 1 wt % of macadamia nut shell flour to the heated IL every 24 h and occasionally manually shaking the vessel. The IL was considered saturated when the flour visually remained undissolved.

Subsequent solubility tests in [Emim][OAc] were performed in a round-bottom flask, to which 1 g of IL and a known quantity of macadamia nut shell flour was added. The mixture was placed into an oil bath and heated at 110 °C for 18 h, maintaining continuous stirring using a Teflon-coated stirrer bar at 700 rpm. The neck of the flask remained open to the atmosphere.

As a general fractionation process, 10 mL (ca. 10 times the volume of the IL) of an acetone−water mixture (1:1) was added to solutions of macadamia in the IL in the flask and stirred for 20 min.[13] Cellulose-rich solid residue was recovered via simple filtration using Millipore nylon filter (0.22 μm) and dried overnight at 70 °C under vacuum. All other fractionation tests followed the same general volumes and processes, with the different solvents employed detailed in the main text.

Cracking Experiments. The required load to crack the Macadamia nut-in-shell was measured using a computer controlled Universal Testing Machine, Instron 5565 (5kN capacity, software version Bluehill 3) on compression loading mode. The load was applied through the hilum axis of the nut between two parallel plates (Figure S1 of the Supporting Information) maintaining a compression speed of 1 mm min^{-1}. The maximum load for initial deformation was noted and the machine stopped applying force when the subsequent load reached half of the maximum load.

Enzymatic Hydrolysis. Enzymatic hydrolysis of the various sample were performed by slightly modifying and scaling down to the standard method of enzymatic hydrolysis for lignocellulosic biomass.[28] Enzymatic hydrolysis was performed in centrifuge vials (1.5 mL) using 973.3 μL of 50 mM acetate buffer of pH 4.8, 40 μg of cycloheximide (4 μL from 10 mg/mL in 70% ethanol solution), 30 μg of tetracycline hydrochloride (3 μL from 10 mg/mL in distilled water solution) and 25 FPU/g cellulase (7.14 μL of as received Cellucast 1.5L). The sample vials were placed in a heating block to maintain a

DOI: 10.1021/acssuschemeng.5b00126
ACS Sustainable Chem. Eng. 2015, 3, 992−999

constant temperature of 50 °C and the whole block was then placed onto a plate shaker (Maxi rotator, USA). Moreover, the entire sample was vigorously shaken 5 min before measuring the glucose concentration. The converted glucose concentration was measured over time using a portable glucose meter (Accuchek Active, Roche) for selective quantification and which was calibrated earlier in lab for accuracy. Each experiment was performed in triplicate.

Compositional Analysis. The compositional analysis (glucose, lignin and ash) was performed for macadamia nut shell flour before and after pretreatment with [Emim][OAc] according to NREL standard procedures.[27] The extractive content in untreated shell flour was determined by a one-step 24 h Soxhlet extraction with dichloromethane as the solvent, according to standard procedure.[29] To a pressure vessel with a screw cap was added together 300 mg of oven-dried extractive free solid with 3 mL of H_2SO_4 (72% v/v), and the solution was stirred at 30 °C for 2 h. 84 mL of distilled water was then added, and the pressure vessel was incubated at 121 °C for 1 h. After the solution cooled down to room temperature, the solid residue was filtered using a Millipore filter (10 μm pore size) and sent for TGA analysis to determine the acid insoluble lignin and ash content. The acid soluble lignin in the filtrate was determined by measuring the UV absorbance at 205 nm, applying an extinction coefficient of 110 g^{-1} L cm^{-1}.[27] 1 mL of the filtrate was neutralized to pH 6 with $CaCO_3$. The supernatant layer was filtered, and its glucose content was measured using a portable glucose meter (Accuchek Active, Roche), which had been calibrated prior to use and demonstrated to be selective to glucose over other sugars.

RESULTS AND DISCUSSION

Pretreatment and Cracking of Whole Macadamia Nuts-in-Shell. Ionic liquids are recognized for their ability to dissolve relatively large quantities of biomass, but nut shells in general and macadamia nuts in particular have not been previously investigated. We therefore screened a range of common ionic liquids for their ability to dissolve macadamia nut shell (solubility values reported in Table 1). At 110 °C and open to the atmosphere, [Emim][OAc] was found to dissolve the greatest quantity of macadamia nut shell flour (5.5 ± 0.5 wt %), as is consistent with prior observations for rice husks[30] and various wood-based biomass.[13] At 110 °C and open to the atmosphere the water content in [Emim][OAc] equilibrated at 7.2 wt % (compared to 30.8 wt % at 25 °C).

Table 1. Solubility Values Recorded for Various ILs, with the Addition of 1 wt % Macadamia Flour to 1 g of IL every 24 h, until Flour Visually Remained Undissolved[a]

ionic liquid	% wt dissolved
[Bmim][PF_6]	≪1
[Emim][BF_4]	≪1
[Emim][$N(CN)_2$]	<1
[Emim][CF_3SO_3]	<1
[Emim][SCN]	1.5 ± 0.5
[Emim][DEP]	1.5 ± 0.5
[Emim][$EtSO_4$]	2.5 ± 0.5
[Emim][HSO_4]	2.5 ± 0.5
[Emim][OAc]	5.5 ± 0.5

[a]Constantly heated at 110 °C in a heater block, open to the atmosphere. [Emim] = 1-ethyl-3-methylimidazolium, [Bmim] = 1-butyl-3-methylimidazolium, [DEP] = diethylphosphate, [$EtSO_4$] = ethylsulfate and [OAc] = acetate.

Dissolution was confirmed visually by microscope (Figure 1) that confirmed complete dissolution of the macadamia nut shell upon heating, except for the inner layer of the shell (vide infra).

Given that [Emim][OAc] could almost completely dissolve the shell, we therefore investigated the effect of [Emim][OAc] upon whole shells, to observe if the ionic liquid could dissolve and thus compromise the structural integrity of the whole shell, facilitating cracking.

A number of nuts-in-shell were cracked uniaxially by diametral compression between two parallel plates using an Instron Universal Testing System (orientation demonstrated in Figure S1 of the Supporting Information). Figure 2 displays a typical force vs deformation plot, where the cracking of the shell is clearly indicated by a sudden drop in the force required to cause deformation. All were loaded such that the shell sat naturally on its hilum, with the micropyle facing upward. This has previously been described as the polar orientation[18] or compression on the longitudinal axis through the hilum,[24] and noted as the orientation that requires the least force to crack the shell.

The ca. 7 g, 25 mm wide nuts-in-shell, as received in this study, were found to require, on average, 2240 ± 430 N (ca. 0.23 ton force) per shell in order to be cracked, typically after ca. 1.5 mm deformation. Our measured values are slightly higher than literature values reported for macadamia nut-in-shell cracking along the polar orientation, which were reported as ca. 1500 N[24] and 1800 ± 200 N[18] for Brazilian- and Hawaiian-cultivated macadamia nuts, respectively. Higher values of ca. 2500 N[24] and 2400 ± 500 N[18] have been reported for equatorial (as opposed to polar) cracking orientations. The observation that the Australian macadamia nuts possess stronger shells than Brazilian- and Hawaiian-cultivated nuts is not unexpected. The latter have undergone relatively greater horticultural selection and development, stemming from a small original population selected for export from Australia.

Nuts-in-shell were tested for the force required to crack them after 0, 4, 24, 48 and 72 h in (i) a convection oven at 110 °C, and (ii) a convection oven at 110 °C in contact with a small quantity of [Emim][OAc]. The overall force required to crack the shells as a function of time in the oven is displayed in Figure 3.

For shells heated in the absence of the IL, the average force required dropped from 2240 ± 430 N to 1720 ± 340 N (a ca. 23% reduction) after 4 h, likely due to the shells becoming brittle after drying. After 4 h, the force required reached a plateau, indicating the shells were rapidly dried at this temperature and no significant further drying or embrittlement of the shells occurred. Mass loss at this point was ca. 8.1 ± 0.8 wt %.

The IL-treatment of the shell entailed several large glass petridishes, into which five macadamia nut-in-shell (ca. 35 g) and 20 g of [Emim][OAc] were placed, followed by heating for the required time. Approximately one-third of the macadamia nut-in-shell was immersed into the [Emim][OAc]. The samples were rotated manually every hour for the first 4 h, then subsequently every 8 h. This was to ensure the relatively uniform treatment of each part of nut shell with [Emim][OAc].

The force required to crack the shells after exposure to [Emim][OAc] was found to decrease in a linear manner with respect to heating time (Figure 3). This force reached 760 ± 240 N at 72 h, representing only ca. 34% of the force required to crack the shells relative to as-received (ripe) shells; a ca. 3-fold decrease. This also corresponds to a ca. 2.3-fold greater decrease in shell strength relative to heating in the absence of [Emim][OAc]. There was no evidence of a rapid drop in the

DOI: 10.1021/acssuschemeng.5b00126
ACS Sustainable Chem. Eng. 2015, 3, 992−999

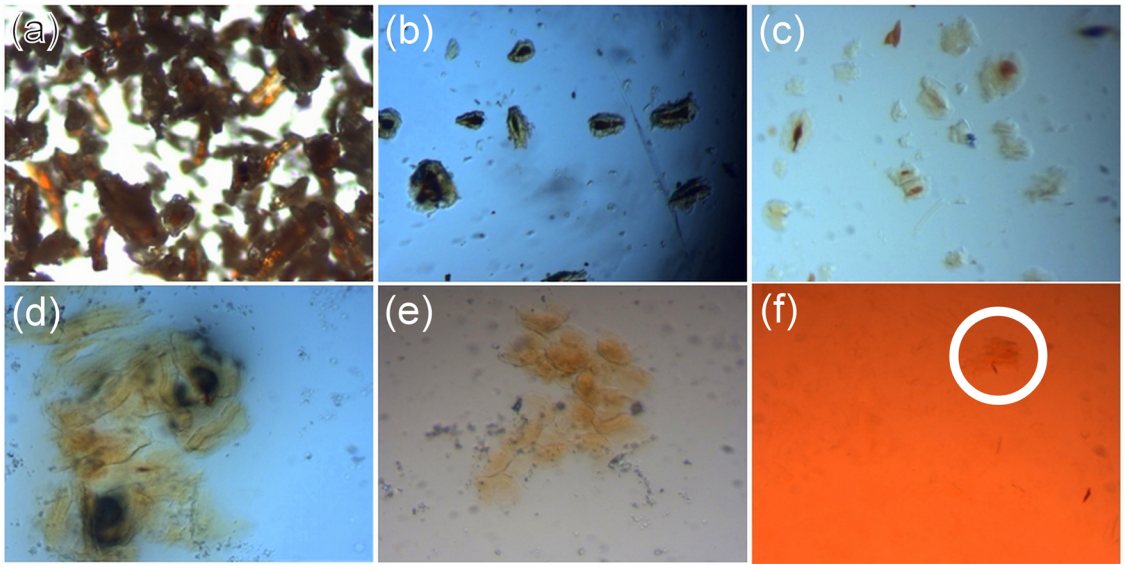


Figure 1. Images of (a) macadamia nut shell flour on a glass slide, and (b) after the addition of [Emim][OAc]. The heated microscope stage was susequently heated up to 110 °C, and images recorded after (c) 2 h, (d) 4 h and (e) 6 h. Also shown is (f) a sample where 5 wt % macadamia nut shell flour was dissolved in [Emim][OAc] (18 h stirring at 110 °C). The barely observable undissolved material has been highlighted by the white circle.

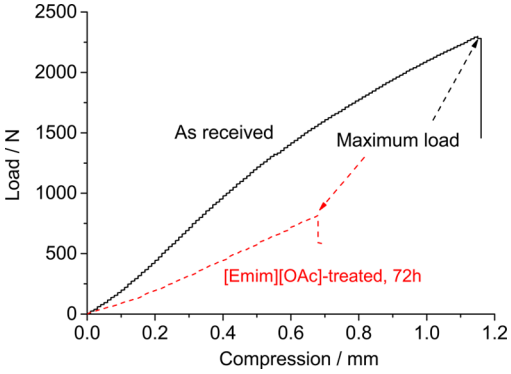


Figure 2. Compression vs load plots up to the cracking point for an average macadamia nut-in-shell, as received (black) and after being treated with some [Emim][OAc] for 72 h at 110 °C (red), displaying a reduction in both force and displacement required to crack the shell post-treatment.

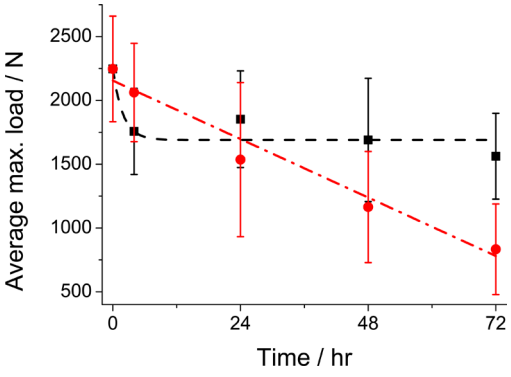


Figure 3. Plot of the average maximum load required to crack whole macadamia nut-in-shells as a function of time in a convection oven at 110 °C, in both the presence (red ●) and absence (■) of [Emim][OAc]. Each data point represents 8 to 14 distinct measurements, with the error bars corresponding to the 95% confidence interval.

force required, as observed when heating in the absence of the IL, which is attributed to the slow dehydration of the IL relative to that of the shell. Indeed, the water content of the IL itself when equilibrated in the oven under the same conditions (ca. 7 wt %) is very similar to the initial water content of the shells prior to heating (ca. 8 wt %).

Although the error bars appear relatively large in Figure 3, this corresponds to the heterogeneous nature of real biomass samples, and each data point corresponds to the average for 8 to 14 evaluated macadamia nuts-in-shell. A one-tailed, equal variance Student's *t*-test was performed to investigate the effect of IL during the heating, relative to heating in the absence of the IL. The *t*-test clearly indicated that the force required to crack the shells was reduced by the presence of [Emim][OAc] treatment after 48 h ($P = 3 \times 10^{-4}$) and after 72 h ($P = 3 \times 10^{-8}$).

The deformation required in order to crack the shell was also recorded (c.f. *x*-axis in Figure 2). The average deformation decreased from 1.47 ± 0.37 mm to 0.71 ± 0.15 mm after 72 h heating in the oven. After 72 h in the presence of [Emim][OAc], a statistically similar reduction (0.67 ± 0.16 mm) was observed. Therefore, the IL treatment achieves the same embrittlement of the shell as the heating treatment, a significant factor in terms of isolating whole kernels postcracking. The energy required in J to crack the shell can be approximated by the product of 0.5 × deformation (m) × maximum force (N). The approximate average energy required to crack the as-received shells was 1.64 ± 0.52 J. Values of ca. 1 J have been reported for cracking Brazilian macadamia nuts longitudinally.[24] After 72 h of heating in the presence of [Emim][OAc], this energy requirement dropped to 0.25 ± 0.10 J.

The response of the nut shells is different from that reported for wood. Brandt et al. have reported that exposure of wood to IL results in the swelling of wood chips, and subsequent grinding of the IL-expanded wood was more facile.[10] However, this was related to lubricating properties of the IL rather than any weakening of the integrity of the anisotropic wood.[10] The

DOI: 10.1021/acssuschemeng.5b00126
ACS Sustainable Chem. Eng. 2015, 3, 992–999

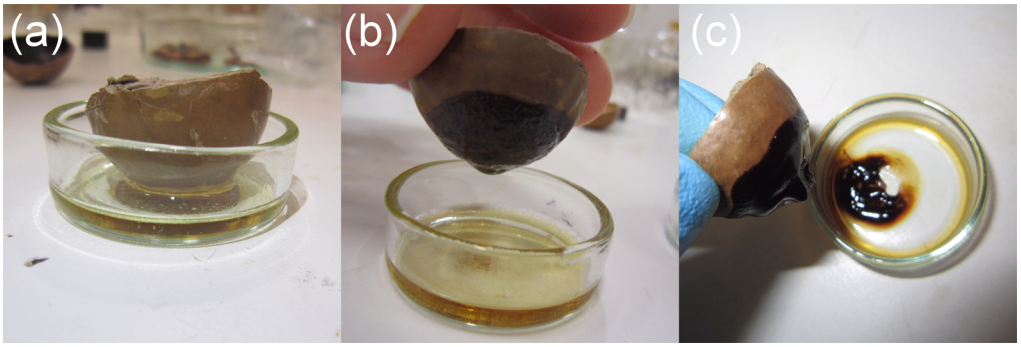


Figure 4. Photos of half a macadamia nut shell after being placed into 0.5 g [Emim][OAc], (a) before heating, (b) after 4 h at 110 °C in a convection oven and (c) after 20 h, displaying signficant dissolution of the shell exterior.

extraordinary strength of macadamia shells can be related to the ca. 160 μm thick dense outer shell,[13] isotropic bundles of lignified cells,[6,18] and the presence of crack-terminating fractures.[17] In the presence of [Emim][OAc] shell swelling was observed (see the Supporting Information for full details), which would significantly disrupt the isotropic structure and potentially fill the strain-dissipating fractures in the shell. Shell dissolution was also apparent, which would rapidly remove the tough, dense exterior of the shell. The observation that IL treatment dramatically reduces the mechanical strength of (isotropic) macadamia nut shells yet does not weaken (anisotropic) wood can be related to the different degrees of organization in these structures, as well as the different roles such organization has upon the physical properties of the biomass.

Experiments with Half-Shells: Interior vs Exterior of the Shell. If ionic liquids can successfully be used to weaken the shell, they should do so without penetrating the shell and thus contacting the edible nut inside. Half of a shell was placed such that the exterior part of the shell was in contact with [Emim][OAc] (Figure 4a). After heating for 4 h at 110 °C, clear darkening of the exterior of the shell had occurred (Figure 4b) and pieces of the shell exterior were visible at the bottom of the IL. After 20 h, the IL was essentially saturated with dissolved shell exterior, and formed a viscous mass largely adhered to the partially dissolved shell exterior (Figure 4c). Cutting the shell in half revealed the IL had not penetrated all the way to the interior of the shell.

An experiment was therefore performed by deliberately sawing a shell in half and filling the shell halves with [Emim][OAc] (Supporting Information, Figures S3 and S4). These were then heated to 110 °C in a convection oven. The IL became extremely dark, likely a result of high tannin and sugar content in these layers, which can be followed by nonenzymatic browning of the latter. Removal of the IL revealed a blistered-looking epidermis layer which was otherwise intact; even after extended exposure, the [Emim]-[OAc] failed to penetrate the shell beyond the epidermis layer (Supporting Information, Figure S5).

These experiments indicate that the [Emim][OAc] can gradually dissolve the tough smooth exterior of the shell, thus weakening the shell. Experiments on smaller shell fragments (see the Supporting Information for full details) demonstrate that IL can rapidly penetrate the porous middle section of the shell and result in significant expansion of the thickness of the shell, likely accompanied by partial dissolution of the interior lignin-rich structure. The inner epidermis layer provides a final IL-impervious barrier which should prevent contamination of any nut inside the shell, due to the barrier that it provides between the nut and the external world, including the shell.

Saccharification Yield of Macadamia Nut Shell Flour after Pretreatment with [Emim][OAc]. As demonstrated above, ionic liquid pretreatment can weaken the shells, but results in shell fragments as a waste product. Pretreatment of biomass with ionic liquids has been demonstrated to enhance the release of glucose during downstream enzymatic saccharification for a variety of biomasses such as wood, switchgrass or corn stover.[11,31] Macadamia nut shell, can also be utilized in this procedure to produce glucose.

A solution of macadamia nut shell flour was prepared in [Emim][OAc] (5 wt % loading, 110 °C and 24 h), and then "fractionated" into cellulose-rich and lignin-rich precipitates.[13] This entailed the addition of water-acetone or methanol as antisolvents for the cellulose-rich fraction, and the evaporation of acetone to precipitate the lignin-rich precipitate, with all conditions as previously reported.[13] The cellulose-rich fractions were subjected to enzymatic saccharification according to standard procedures,[28] and the glucose released was monitored over time.

Figure 5 shows the glucose yield over a period of 48 h. The glucose yields are calculated based on the total glucose content found in the as-received macadamia flour, which was

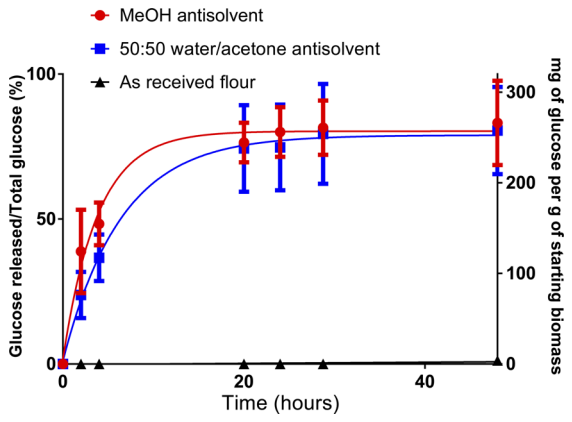


Figure 5. Saccharification yield over time during cellulase enzymatic treatment of the as received macadamia nut shell flour (▲), shell flour that has been pretreated with [Emim][OAc] and precipiated with water/acetone as the antisolvent (blue ■) and with methanol as the antisolvent (red ●). Each data point represents triplicate measurements, with the error bars corresponding to the 95% confidence interval. The error bars for ▲ correspond to ±1% glucose, which is smaller than the data points themselves.

DOI: 10.1021/acssuschemeng.5b00126
ACS Sustainable Chem. Eng. 2015, 3, 992−999

determined according to standard procedures.[27] Treatment with [Emim][OAc] allowed enzymatic release of 80 ± 15% of the available glucose from the macadamia flour. Conversely, the as-received flour released negligible glucose, eventually reaching only ca. 1.3 ± 1.0% of the available glucose after 48 h. There was no significant difference between the use of water-acetone mixture or methanol as antisolvents. The fact that only 80 ± 15% of the theoretical glucose available from the initial macadamia flour was released into the enzymatic broth likely corresponds to some hemicellulose loss into the IL during pretreatment; hemicellulose is known to partially degrade during IL treatment.[11]

Effect of [Emim][OAc] Treatment upon the Macadamia Nut Shell Composition and Structure. Compositional analysis on the as-received macadamia nut shell flour and after treatment with [Emim][OAc] was performed according to standard procedures.[27,29] The structure of the shell flour was investigated using thermogravimetric analysis (TGA), powder X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FT-IR). The IL-assisted fractionation of the holocellulosic and lignin components was also investigated. The disruption of crystalline cellulose I into amorphous cellulose II is important for the rapid enzymatic digestion of the cellulose fractions.[9] Delignification is also beneficial.[3]

A solution of 5 wt % macadamia nut shell flour was prepared in [Emim][OAc], and then "fractionated" as described in the previous section. The isolated cellulose-rich fractions recovered represented the consistent recovery (dry weight) of ca. 68.5 wt % of the flour initially added. However, after evaporating the acetone only a small quantity of "lignin-rich" material was isolated (~1 wt %), an order of magnitude less than that reported previously reported for southern yellow pine.[13] Acetonitrile has been patented at a suitable antisolvent for IL-lignocellulosic processing.[14] In this study the addition of acetonitrile was most effective at removing the residual dissolved material (additional 25.3 wt % precipitated, resulting in ca. 93.8% total weight recovered), compared to dichloromethane (4.2 wt %) and tetrahydrofuran (~0 wt %). The IL could be recovered by evaporation of the antisolvent.

TGA and XRD data was measured for the as-received macadamia nut shell flour, and compared against the cellulose-rich isolated fraction. TGA (Figure 6a) demonstrated the earlier onset of thermal decomposition postfractionation, suggesting that dissolution and precipitation of the macadamia nut shell flour from [Emim][OAc] successfully disrupted the biomass.[5] This was confirmed by XRD (Figure 6b). Filter paper displayed all of the classical XRD features for cellulose I, which could also be observed for the as-received macadamia nut shell flour; the broader peaks and the higher ratio between the I_{am} (intensity at $2\theta = 18°$) and the I_{main} (intensity at the maximum of the 002 peak) also highlighted the expected higher degree of sample amorphousness and cellulose crystallinity in the biomass sample.[32] After regeneration from the [Emim][OAc], the loss of the (040) feature, separation of the (101) and (10$\bar{1}$) features and the dramatically increased ratio between the I_{am} and the I_{main} all indicate reduced crystallinity and transition of cellulose I to cellulose II.[32]

There are also changes in the chemical composition of the macadamia flour before and after treatment. The lignin content of the as-received shell flour was found to be 35.5 wt % by dry weight (full details in the Supporting Information in Table S1). This value is higher than contents typically associated with wood[3,13,33] but is consistent with the greater strength of the

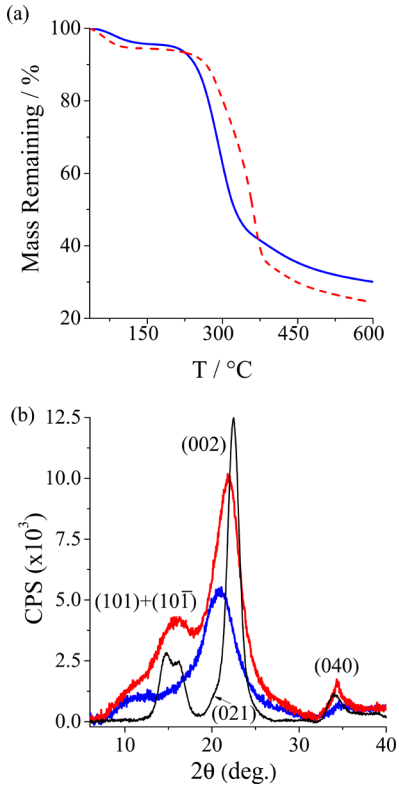


Figure 6. (a) Thermogravimetric plot of the thermal decomposition of ca. 10 mg biomass samples in a Pt crucible (30 mL min^{-1} Argon flow, 10 °C min^{-1}). Plot displays the earlier thermal decomposition of the cellulose-rich fraction regenerated from a 5 wt % solution in [Emim][OAc] (blue —) relative to the as-received macadamia nut shell flour (red ---) indicating an associated decrease in crystallinity. (b) XRD plot of ca. 15 mg samples of filter paper (black, intensity of *y*-axis scaled down by a factor of 3, for ease of comparison with other samples), untreated nut shell flour (red) and biomass regenerated from [Emim][OAc] (blue), again showing decreased crystallinty.

macadamia nut shell. A higher value of 47.6 wt % was previously reported by Toles et al. based upon unknown proximate analysis.[34] The fraction isolated by water-acetone precipitation contained reduced lignin content (from 35.5 wt % down to 29.2 wt % lignin) indicating partial delignification of the macadamia nut shell flour had occurred (full details in the Supporting Information, Table S1). This was further supported by FT-IR analysis (Supporting Information, Figure S2), notably by the loss of a peak at ca. 1739 cm^{-1} (C═O stretch) and reduction in a peak at ca. 1512 cm^{-1} (C═C stretch) in the regenerated material relative to the untreated material. The ash content was also similar (~2% for both as-received and treated flour).

DISCUSSION

This work has demonstrated that ILs can be used to chemically pretreat tough lignocellulosic biomass, in this case by facilitating the cracking of extraordinarily strong nut shells. Using this protocol, a small quantity of hot IL could be used to significantly swell and weaken the shell, such that shell cracking is more facile. This could replace hot-air drying by combustion of fossil fuels, given that heating of a liquid is relatively more facile (e.g., basic solar concentrators can easily boil water). Complete dissolution of the shell allows biomass disruption and

DOI: 10.1021/acssuschemeng.5b00126
ACS Sustainable Chem. Eng. 2015, 3, 992–999

fractionation, enhancing enzymatic saccharification yield, as well as subsequent IL recycle.

However, a key issue to note is that after 72 h, ca. 10% of macadamia nuts displayed evidence of IL contamination. Contamination was obvious, due to the dark coloration of the IL after contact with the macadamia nut shells. The dark-colored IL was clearly observed against the pale nut. This contamination was localized at one specific point on the nut, which corresponded to the location of the micropyle. This occurred due to dissolution enlarging the micropyle, through which the IL could then penetrate. Another key issue is that the (arbitrarily) utilized temperature of 110 °C is too hot for aesthetic and taste reasons; nonenzymatic browning at these temperatures results in premature coloration and sugar level reduction in the nut.[24] Others have noted that IL−water mixtures promote biomass swelling over dissolution.[11,31] Work will therefore progress on moving toward nontoxic ILs for biomass processing[12] that can be effective at significantly lower temperatures,[35] potentially by the incorporation of significant fractions of water.[11]

CONCLUSIONS

The extraordinary mechanical properties of macadamia nut shells have encouraged specialized cracking technology, often involving the combustion of fuels. Ionic liquids can potentially open up a novel route to macadamia nut shell processing. Heating the nuts-in-shell in the presence of [Emim][OAc] for 72 h results in a significant reduction in both the force (ca. 3-fold decrease) and the energy (ca. 7-fold decrease) required to crack the shells, as well as the important embrittlement of the shell (ca. 2-fold decrease in displacement required to crack the shell). If more liquid is added, then the macadamia nut shell can almost entirely dissolve in [Emim][OAc] (up to 5.5 ± 0.5 wt %), allowing both biomass pretreatment and subsequent ionic liquid recovery. Macadamia nut shell flour pretreated with [Emim][OAc] showed changes in its structure, which consequently enhances its glucose yield when subjected to enzymatic hydrolysis, from 1.3 ± 1% of the possible glucose released up to 80 ± 15% release.

ASSOCIATED CONTENT

Supporting Information

Total lignin content, FT-IR spectra, photographic data relating to half-shell treatments, as well as data (photographs and graphs) and discusstion relating to the swelling and dissolution of shell fragments. This material is available free of charge via the Internet at http://pubs.acs.org/.

AUTHOR INFORMATION

Corresponding Author

*L. Aldous. E-mail: l.aldous@unsw.edu.au. Tel.: +61 2 9385 4752.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The Australian Research Council (ARC DECRA DE130100770) is acknowledged for funding. Bill Joe is thanked for training and assistance in the cracking experiments and Benjamin Lau for measuring the XRD data. Micro Milling Pty Ltd. is thanked for the kind donation of the macadamia nut shell flour, and Macadamias Direct for donation of a generous quantity of macadamia nuts-in-shell and shell fragments.

REFERENCES

(1) Aparicio, S.; Atilhan, M.; Karadas, F. Thermophysical properties of pure ionic liquids: Review of present situation. *Ind. Eng. Chem. Res.* **2010**, *49* (20), 9580−9595.

(2) George, A.; Tran, K.; Morgan, T. J.; Benke, P. I.; Berrueco, C.; Lorente, E.; Wu, B. C.; Keasling, J. D.; Simmons, B. A.; Holmes, B. M. The effect of ionic liquid cation and anion combinations on the macromolecular structure of lignins. *Green Chem.* **2011**, *13* (12), 3375−3385.

(3) Hossain, M. M.; Aldous, L. Ionic liquids for lignin processing: Dissolution, isolation, and conversion. *Aust. J. Chem.* **2012**, *65* (11), 1465−1477.

(4) Hart, W. E. S.; Harper, J. B.; Aldous, L. The effect of changing the components of an ionic liquid upon the solubility of lignin. *Green Chem.* **2015**, *17*, 214−218.

(5) Swatloski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D. Dissolution of cellose with ionic liquids. *J. Am. Chem. Soc.* **2002**, *124* (18), 4974−4975.

(6) Kilpelainen, I.; Xie, H.; King, A.; Granstrom, M.; Heikkinen, S.; Argyropoulos, D. S. Dissolution of wood in ionic liquids. *J. Agric. Food Chem.* **2007**, *55* (22), 9142−9148.

(7) Li, W. Y.; Sun, N.; Stoner, B.; Jiang, X. Y.; Lu, X. M.; Rogers, R. D. Rapid dissolution of lignocellulosic biomass in ionic liquids using temperatures above the glass transition of lignin. *Green Chem.* **2011**, *13* (8), 2038−2047.

(8) Deetlefs, M.; Seddon, K. R. Ionic liquids: Fact and fiction. *Chim. Oggi* **2006**, *24* (2), 16−+.

(9) Rollin, J. A.; Zhu, Z. G.; Sathitsuksanoh, N.; Zhang, Y. H. P. Increasing cellulose accessibility is more important than removing lignin: A comparison of cellulose solvent-based lignocellulose fractionation and soaking in aqueous ammonia. *Biotechnol. Bioeng.* **2011**, *108* (1), 22−30.

(10) Brandt, A.; Erickson, J. K.; Hallett, J. P.; Murphy, R. J.; Potthast, A.; Ray, M. J.; Rosenau, T.; Schrems, M.; Welton, T. Soaking of pine wood chips with ionic liquids for reduced energy input during grinding. *Green Chem.* **2012**, *14* (4), 1079−1085.

(11) Brandt, A.; Ray, M. J.; To, T. Q.; Leak, D. J.; Murphy, R. J.; Welton, T. Ionic liquid pretreatment of lignocellulosic biomass with ionic liquid-water mixtures. *Green Chem.* **2011**, *13* (9), 2489−2499.

(12) Pinkert, A.; Goeke, D. F.; Marsh, K. N.; Pang, S. S. Extracting wood lignin without dissolving or degrading cellulose: Investigations on the use of food additive-derived ionic liquids. *Green Chem.* **2011**, *13* (11), 3124−3136.

(13) Sun, N.; Rahman, M.; Qin, Y.; Maxim, M. L.; Rodriguez, H.; Rogers, R. D. Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. *Green Chem.* **2009**, *11* (5), 646−655.

(14) Argyropoulos, D. S. Use of lignocellulosics solvated in ionic liquids for production of biofuels. U.S. Patent US 2008 0190013 A1, August 14, 2008.

(15) Walton, D. A. Anatomy and handling: Implications for macadamia nut quality. Ph.D. Thesis, University of the Sunshine Coast, Sippy Downs, Australia, 2005.

(16) International Nut Council. *International Nut & Dried Fruit Council Foundation: Nuts and Dried Fruits Global Statistical Review 2006−2011*; International Nut Council: REUS, Spain, 2010.

(17) Wang, C. H.; Mai, Y. W. Deformation and fracture of macadamia nuts 0.2. Microstructure and fracture-mechanics analysis of nutshell. *Int. J. Fract.* **1994**, *69* (1), 67−85.

(18) Jennings, J. S.; Macmillan, N. H.; Tough, A. Nut to crack. *J. Mater. Sci.* **1986**, *21* (5), 1517−1524.

(19) Fleck, C.; Schüler, P.; Meinel, D.; Zaslansky, P.; Currey, J. D. Microstructural features influencing failure in macadamia nuts. *Bioinspired, Biomimetic Nanobiomater* **2012**, *1* (2), 67−75.

DOI: 10.1021/acssuschemeng.5b00126
ACS Sustainable Chem. Eng. 2015, 3, 992−999

(20) Kaupp, G.; Kaupp, M. Advanced fullerene-type texture and further features of the macadamia nutshell as revealed by optical 3D microscopy. *Sci. Res. Essays* **2007**, *2* (5), 150−158.
(21) Price, W. M. Nutcracker for cracking hard-shell nuts such as black walnuts and macadamia nuts. U.S. Patent 4,520,719 A, June 4, 1985.
(22) Wakelam, H. G. Macadamia nut opening machine. US Patent 5,303,470 A, April 19, 1994.
(23) Patel, C. K. N. Method for shelling of nuts with a laser beam. U.S. Patent 4,358,467 A, November 9, 1982.
(24) Braga, G. C.; Couto, S. M.; Hara, T.; Neto, J. T. P. A. Mechanical behaviour of Macadamia nut under compression loading. *J. Agric. Eng. Res.* **1999**, *72* (3), 239−245.
(25) Liang, T.; Mehra, S. K.; Khan, M. A.; Macadamia Nut, A. Curing system for improving kernel recovery. *J. Agric. Eng. Res.* **1989**, *43* (2), 103−111.
(26) Murphy, T.; Jones, G.; Vanclay, J.; Glencross, K. Preliminary carbon sequestration modelling for the Australian macadamia industry. *Agroforest. Syst.* **2013**, *87* (3), 689−698.
(27) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass*; Technical Report (NREL/TP-510-42618); National Renewable Energy Laboratory: Golden, CO, 2011.
(28) Selig, M.; Weiss, N.; Ji, Y. *Enzymatic Saccharification of Lignocellulosic Biomass*; Technical Report NREL/TP-510-42618; National Renewable Energy Laboratory: Golden, CO, 2008.
(29) Sluiter, A.; Ruiz. R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of Extractives in Biomass*; Technical Report NREL/TP-510-42619; National Renewable Energy Laboratory: Golden, CO, 2005.
(30) Lynam, J. G.; Toufiq, R. M.; Vasquez, V. R.; Coronella, C. J. Pretreatment of rice hulls by ionic liquid dissolution. *Bioresour. Technol.* **2012**, *114*, 629−636.
(31) Brandt, A.; Hallett, J. P.; Leak, D. J.; Murphy, R. J.; Welton, T. The effect of the ionic liquid anion in the pretreatment of pine wood chips. *Green Chem.* **2010**, *12* (4), 672−679.
(32) Segal, L.; Creely, J. J.; Martin, A. E.; Conrad, C. M. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Text. Res. J.* **1959**, *29*, 786−794.
(33) Zakzeski, J.; Bruijnincx, P. C. A.; Jongerius, A. L.; Weckhuysen, B. M. The catalytic valorization of lignin for the production of renewable chemicals. *Chem. Rev.* **2010**, *110* (6), 3552−3599.
(34) Toles, C. A.; Marshall, W. E.; Johns, M. M. Phosphoric acid activation of nutshells for metals and organic remediation: Process optimization. *J. Chem. Technol. Biotechnol.* **1998**, *72* (3), 255−263.
(35) Fukaya, Y.; Sugimoto, A.; Ohno, H. Superior solubility of polysaccharides in low viscosity, polar, and halogen-free 1,3-dialkylimidazolium formates. *Biomacromolecules* **2006**, *7* (12), 3295−3297.

DOI: 10.1021/acssuschemeng.5b00126
ACS Sustainable Chem. Eng. 2015, 3, 992−999