Pretreatment of waste biomass in deep eutectic solvents: Conductive heating versus microwave heating

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ABSTRACT

Deep Eutectic Solvents (DESs) have recently emerged as an environmentally-friendly way to separate lignin from cellulose and hemicellulose in recalcitrant biomass prior to enzymatic hydrolysis to enhance glucose yields. Rice hulls, a widely-available secondary agricultural residue was pretreated with FA: CC (formic acid and choline chloride in a 2:1 mol ratio) using conductive heating and microwave heating. Pretreated biomass using either heating technique gave glucose titers 100 times higher than raw rice hulls after enzymatic hydrolysis. FTIR analysis shows similar findings for both heating techniques. For biomass pretreatment using DESs, microwave heating requires less than half of the energy (0.10 kW-h) compared to conductive heating (0.31 kW-h). Conductive heating requires more residence time of biomass for the pretreatment process. Thus, this work shows the potential benefits of a microwave heating technique for deconstruction of biomass using DESs.

1. Introduction

In a planet nearing 8 billion people, new environmentally-friendly ways are needed to produce food, fuel, and other products. Biomass can sustainably provide these necessities. However, food like corn is frequently converted into fuel, rather than being used for its intended purpose. Such use provokes "food vs. fuel" conflicts, which increase when food stocks are assigned to produce fuel (Thompson, 2012). One method to avoid such conflicts is to convert agricultural byproducts like the leaves and stalks of corn (corn stover) into biofuels or other bioproducts. Corn stover, like other primary agricultural residues, must be transported to a biorefinery for conversion. Transportation of low density biomass is costly in terms of fuel and emissions of pollutants. Thus, conversion of secondary agricultural residues is a more environmentally-friendly option. Secondary agricultural residues are waste biomass required for food production that have already been transported to processing centers. Some secondary agricultural residues can be used for animal feed, such as soybean hulls, peanut skins or peanut hulls (Cranston et al., 2006; Hill, 2002). Other secondary agricultural residues are not suitable for animal feed due to high inorganic content or indigestibility (Zadrazil and Puniya, 1995). Such waste biomass includes rice hulls.

Rice hulls have what is in essence silica "armor" on their outside surfaces, so they are not suitable as compost materials or as animal feed (Ouyang, 2001). Rice hulls are generally landfilled or even burned in the open field, therefore polluting the environment (Lim et al., 2012; Wang et al., 2011). In the United States, about 3 million acres are used for rice production (United States Department of Agriculture, 2017). Rice hulls are approximately 40% cellulose, a component that could be converted into glucose as a feedstock for biofuel or chemical production. They also contain ~30% lignin Fig. 1.

The significant challenge of commercialization of underutilized agriculture byproducts to biofuels or other products is cost-effective, safe, environmentally-healthy deconstruction. This deconstruction separates cellulose and hemicellulose from lignin, so that the two components can be converted separately. Using acids, alkalis, volatile organic compounds, or high pressure processes for this separation presents dangers to both human health and the environment. In addition, the transport of whole, low-density biomass over great distances for processing by such methods generates carbon dioxide, while wasting fuel. If a safe, low-pressure, and low-cost process could be developed for rice milling processing centers, the valuable cellulosic component could be shipped to a biorefinery and the recovered lignin to a separate or local facility for use. One potential use for lignin so separated from the agricultural byproduct rice hulls is in asphalt binder to improve its asphalt's resistance to aging. Arafat et al. (2019) have found improved asphalt binder properties with replacement of petroleum-based asphalt binder with lignin from rice hulls separated using

Fig. 1. Rice hulls or husks (Picture taken by corresponding author).

deep eutectic solvents (DESs) (Arafat et al., 2019).

DESs are alternate solvents for environmentally-friendly biomass deconstruction. Many DESs are biocompatible and nontoxic, while also having low volatility and a wide liquid range. They are also biodegradable and non-flammable (Francisco et al., 2012). Preparation of high purity DESs is relatively simple and the starting materials are low cost and easily procured, compared to most ionic liquids, which are other low volatility solvents. Most DESs do not deactivate enzymes, making DES advantageous in biomass conversion (Gorke et al., 2010). They have low sensitivity to water content, allowing their use with undried biomass, which can have a high moisture content (Francisco et al., 2012). Drying biomass can be costly and high moisture content biomass is expensive to transport (Lamers et al., 2015). Comprised of a homogeneous mixture of solid-phase chemicals, a eutectic system forms a joint super-lattice at a certain molar ratio that is called the eutectic composition. The eutectic melting temperature at this composition is a temperature lower than the melting points of each of the components. While ionic liquids have ionic bonding, DESs are created with hydrogen bonding (Yiin et al., 2016).

Some DESs can solubilize lignin well, but only disrupt the cellulose without dissolving it, suggesting that DES pretreatment could prepare recalcitrant biomass for further saccharification. DESs that have this property include mixtures of lactic acid and betaine; lactic acid and choline chloride; formic acid and choline chloride; and lactic acid and proline (Francisco et al., 2012).

For use in deconstruction, DESs are biodegradable, have low volatility, and are low-cost. Lynam et al. (2017) have found that the density of a DES pretreated loblolly pine sample was increased by 40% compared to the untreated biomass. Shipping a more dense, cellulose-concentrated product to a biorefinery would decrease the need for storage space. DESs have been used on wheat straw for lignin removal (Francisco et al., 2012; Jablonský et al., 2015; Xu et al., 2016a). The DES choline chloride:formic acid, in a 1:2 mol ratio, displayed excellent performance in the pretreatment of corn stover by removal of hemicellulose and lignin (Xu et al., 2016a). Alessandra et al. (2018) has investigated DES pretreatment of apple residues, potato peels, coffee silverskin, and brewer's spent grains (Alessandra et al., 2018). These biomass have potential to be used to produce fermentable sugars (Gebrechristos and Chen, 2018; Liu and Wolfe, 2003). Corn cobs have also been treated by DESs, by Zhang et al. (2016), giving improved delignification and enzymatic hydrolysis efficiency (Zhang et al., 2016). Zhang and Wang, 2017 found that DESs could extract polysaccharides using ultrasound treatment from Chinese yam (Zhang and Wang, 2017). Kumar et al., 2016a found that rice straw could be treated with choline chloride based DES to increase saccharification with enzymes, and the enzymes were not significantly affected by the presence of the DES (Kumar et al., 2016b). Kumar et al. (2016) also were able to generate pure lignin from rice straw and found a slight reduction in cellulose crystallinity from XRD with DES treatment (Kumar et al., 2016a). Hou et al. (2018) used choline chloride DESs on rice straw and reported

xylan removal (Hou et al., 2018). Kandanelli et al. (2018) worked with rice straw and rice husks using a combination of butanol with DESs and reported that up to 49% delignification could be achieved (Kandanelli et al., 2018). Palm oil trunk fibers are another biomass that has been investigated by Zulkefli et al. (2017). These researchers reported that the DES ethylammonium chloride: ethylene glycol was effective in removing lignin and hemicellulose from palm oil trunk fibers (Zulkefli et al., 2017). Corn stoverwas subjected to DES treatment by Xu et al., 2016a. These researchers found that choline chloride: formic acid DES was effective at removing lignin and xylan from the stover (Xu et al., 2016b). These biomass that have been researched for DES pretreatment are either primary agricultural residues, meaning that they must be transported from the fields for use, or can be used for animal feed (Kamphayae et al., 2017). We have not found data for rice hulls, which are not animal feed and are already transported to process centers, treated with DES alone available in the literature. This work has investigated the separation of the cellulosic component from lignin in rice hulls using a DES.

If DESs are to be used in secondary agricultural residue pretreatment, methods must be developed to increase the temperature to promote the bond-breaking reaction that permits separation of lignin from biomass. The standard method of conduction (e.g. a hot plate) can be used in this pretreatment (Francisco et al., 2012; Lynam et al., 2017; Xu et al., 2016a). Convection (e.g. a drying oven) can also be used to heat the biomass-DES mixture (Jablonský et al., 2015).

An alternative method to promote the pretreatment reactions is using microwaves. Ionic liquids have been used with microwave pretreatment to perform pretreatment of eucalyptus (Xu et al., 2015). Liu et al. (2017) were able to effectively cleave lignin-carbohydrate bonds for poplar wood flour with DESs using a microwave pretreatment (Liu et al., 2017). Muley et al. (2019) found that microwave pretreatment using a DES of oxalic acid-choline chloride (1:1 mol ratio) gave a high lignin yield from pine sawdust in less than an hour (Muley et al., 2019).

This work attempts to compare DES pretreatment of rice hulls with conductive heating to that with microwave heating. The glucose titer from enzymatic hydrolysis of the original and pretreated biomass, as well as FTIR results from these and the precipitate from the DES, are described. Fiber analysis and X-ray Diffraction (XRD) of the untreated and pretreated rice hulls were performed. Differences in time of pretreatment and energy consumption from pretreatment are also discussed.

2. Material and methods

2.1. Material

Louisiana-harvested rice hulls were generously donated by Falcon Rice Mill (Crowley, La, USA). Formic acid (95%) was obtained from DudaDiesel (Decatur, AL). Formic acid (reagent grade, < 95%), choline chloride (98%), cellulase (powder) from Trichoderma reesei ATCC 26921, hemicellulase (powder) from Aspergillus niger, and cellobiase (liquid) from Aspergillus niger were purchased from Sigma-Aldrich (St. Louis, Missouri). Sodium citrate dehydrate, 99.0% and sodium azide 99% min, were purchased from Alfa Aesar (Ward Hill, MA, USA).

2.2. Methods

2.2.1. Synthesis of DES

The synthesis of the DES was carried out by adding choline chloride to formic acid in a 2:1 mol ratio. The mixture was then vortexed for 1 min. The vortexed mixture was then placed in an orbital shaker at 200 RPM at 60 °C for 20 min. If a cloudy solution was obtained, the mixture was placed in the orbital shaker for an additional 20 min at 60 °C. Once a homogeneous and transparent solution was obtained with no evidence of solid particles, the solution was then stored at room temperature. Karl-Fischer titration showed the percentage of water in the

DES to be 6.81%.

2.2.2. Conductive heating pretreatment of rice hull biomass with the DES

Rice hulls were dried at 105 °C for 24 h and milled to mesh 14–28 (1.168-0.589 mm in diameter) before undergoing DES pretreatment. A rice hull concentration of 9.1 wt% (3 g of dry rice hulls and 30 g of FA:CC in a flask for a 1:10 mass ratio) was used for pretreatments. The flask was immersed in an oil bath heated by a hot plate with magnetic stirring. The temperature control of the hot plate kept the oil bath within 2 °C of the desired temperature. A condenser with tap water running on its outside was connected above the flask to cool and return as liquid the vapor coming from the slightly volatile DES., This technique kept the solvent volume constant. Standard errors for results are reported from 5 replicates of the 155 °C, 2 h experiments. Energy required per hour for conductive heating was 0.139 kW-h for 145 °C experiments, 0.157 kW-h for 155 °C experiments, and 0.180 kW-h for 165 °C experiments.

2.2.3. Microwave heating pretreatment of rice hull biomass with the DES

Rice hulls were also pretreated in a microwave unit and the results were compared with conventional heating. Dried and milled rice hulls (3 g) were mixed with 30 g of FA:CC DES. The mixture was heated in a fully controlled Ethos EZ microwave digestion unit (Milestone Inc. Shelton, CT, USA) with a maximum power of 1.6 kW. The mixture was added to 100 ml (maximum volume) Teflon sample holders. The sample holders were rotating at a central axis of a carousel to ensure temperature uniformity. The beakers were placed in a pressurized Teflon unit with maximum pressure of 10 bar. Teflon magnetic stir bars were placed in each beaker for continuous stirring. The sample temperature inside the unit was monitored using a fiber optic temperature probe. Standard errors for results are reported from 4 replicates of the 155 °C, 30 min experiments. Energy required per hour for microwave heating was 0.160 kW-h for 145 °C experiments, 0.196 kW-h for 155 °C experiments, and 0.243 kW-h for 165 °C experiments.

The pretreatment of rice hull with the DES was conducted at three different temperatures: 145, 155, and 165 °C each with three different times: 1, 2, and 3 h for conductive heating and 20, 30, and 40 min for microwave heating. After microwave pretreatment and conductive pretreatment, the procedures in sections *2.2.4* through *2.2.7* were followed.

2.2.4. Separation and filtering of biomass and the DES

Vacuum filtering with a coarse nylon net filter separated solid rice hull residue from DES. The pretreated rice hull residues were then magnetically stirred with 70 ml deionized (DI) water at 50 °C for 20 min to remove DES remaining on the residues. The DI water was decanted, and the rinsing process was repeated for another 20 min at 50 °C. Finally, the water rinsing process was repeated for another 24 h at 50 °C to ensure the complete removal of the DES. A 0.45 μm nylon filter was used to separate the pretreated rice hull from water by vacuum filtering. The pretreated rice hulls were dried in an oven at 105 °C for 24 h prior to weighing. Ethanol (25 mL) was added to the used solvent to precipitate the dissolved biomacromolecules.

2.2.5. Enzymatic hydrolysis

Raw and pretreated rice hulls were dried for 24 h at 105 °C in a drying oven. Samples of raw and pretreated rice hulls of 0.1 g were placed in a vial with 5 ml of pH 5.05 sodium citrate buffer, followed by 100 μl of a 2% sodium azide solution and DI water was added to give a total volume of 10 ml, as described in NREL's enzymatic Saccharification of Lignocellulosic Biomass LAP 009 protocol (Selig et al., 2008). Cellulase, hemicellulase, and cellobiase were added at a concentration of 5, 14, and 50 units per 0.1 g sample respectively. Samples were placed in an orbital shaker at 200 RPM at 50 °C. Aliquots were taken at 24, 48, and 72 h, underwent filtration through a 0.45 μm syringe filter, and were stored at 4 °C prior to analysis.

2.2.6. Glucose analysis

A YSI 7100 MBS (multiparameter bioanalytical system from YSI Life sciences) with a system buffer (YSI 2357), calibrator standard, YSI 7147 (1.80 g/l glucose), was used to measure the glucose concentrations in the enzymatic hydrolyzed samples. Results reported for glucose titer are those analyzed directly by the YSI without any further dilution of the samples.

2.2.7. Fourier transform infrared spectroscopy (FTIR)

A Mattson Genesis II FTIR (Mattson Technology, Fremont, CA, USA) with a KBR Pellet holder was used to perform FTIR on KBr pellets prepared containing the samples. For each sample, 32 scans were performed from 4000 to 400 cm^{-1}. FTIR analysis was performed on both raw and pretreated samples, as well as the precipitate formed from ethanol addition to the used DES.

2.2.8. NREL fiber analysis

For raw and pretreated rice hull samples, fiber analysis was carried out using NREL's Determination of Structural Carbohydrate and Lignin in Biomass Laboratory Analytical Procedure 2008 protocol (Sluiter et al., 2008). An Ultimate 3000 HPLC (ThermoFisher Scientific, Waltham, MA, USA) equipped with refractive index detector and Aminex HPX-87 P column from Bio-Rad was used for sugar analysis in hydrolyzed samples using the HPLC method described in the above-mentioned protocol. It should be noted that neither this fiber analysis method nor any other is considered to be completely valid for biomass with greater than 10% ash, such as rice hulls. The central condition (155 °C and intermediate time) was used to compare the conductive and microwave pretreated rice hulls to untreated rice hulls.

2.2.9. X-ray diffraction (XRD)

The cellulose crystallinity of raw and pretreated rice husk samples was measured using Bruker D8 Discover diffractometer (WI, USA). The rice husk sample was placed on Bragg-Brentano geometry optics on a flat plate sample holder. Copper kα radiation, 40 kV of voltage, 40 mA of current and a 2Θ continuous scan from 3° to 60° were applied. XRD analysis was performed on conductive and microwave samples pretreated at the central condition (155 °C and intermediate time), as well as untreated rice hulls. A Crystallinity Index (CI) was calculated as the peak area of the crystalline peak centered at a 2Θ of 22° divided by the combined peak areas of all peaks (at 2Θs of 16°, 22°, and 35°) (Park et al., 2010).

3. Results and discussion

3.1. Glucose titers change with time and temperature

Table 1 shows the glucose titers from various temperatures and times of pretreatment in FA:CC 2:1 after 72 h of enzymatic hydrolysis.

3.1.1. Glucose titers change with time and temperature – conductive heating

For conductive heating, pretreatment at 145 °C for 2 h gave the highest glucose titer compared to the other temperatures and times (Table 1). Enzymatic hydrolysis of untreated (raw) rice hulls gave a much lower glucose titer than any of the pretreated rice hulls. Since all pretreatment conditions gave similar mass yields, the highest amount of the product glucose could be obtained using 145 °C and a 2 h pretreatment time. Since a shorter reaction time is preferable to a longer reaction time, and more glucose was produced after 2 h of pretreatment, a 2 h at 145 °C pretreatment would be optimal for conductive heating.

3.1.2. Glucose titers change with time and temperature – microwave heating

Table 1 shows the glucose titers from various temperatures and times for microwave pretreatment in FA:CC 2:1 after 72 h of enzymatic hydrolysis. For 30 min of pretreatment, a temperature of 145 °C gave

Table 1

Glucose Yields; and the Vibration Area Ratios from FTIR for Different Experiments Temperatures and Times.

T (°C)	time (min)	Glucose titer (g/L)	Ratio cellulose:lignin vibration areas	Ratio amorphous cellulose: crystalline cellulose vibration areas
Conductive heating				
145	60	4.58	3.41	3.28
145	120	5.28	3.33	7.47
145	180	4.82	2.61	2.05
155	60	3.94	2.79	4.68
155	120	4.34	3.04	4.04
155	180	4.15	3.52	3.96
165	60	4.24	2.99	7.66
165	120	3.87	2.64	1.68
165	180	4.66	2.01	2.91
Microwave heating				
145	20	3.88	2.40	4.31
145	30	4.59	2.68	3.53
145	40	4.47	3.64	12.13
155	20	4.35	2.75	2.75
155	30	4.03	3.20	5.80
155	40	3.97	3.22	3.94
165	20	4.77	2.75	5.73
165	30	4.01	2.11	5.31
165	40	5.06	2.52	7.44
No pretreatment		0.03	0.80	2.25

Values are averages for replicated samples. Standard error for the central condition 155 °C, 120 min conductive sample, replicated 5 times, for glucose titer is 0.36 g/L.

the highest glucose titer, while 155 °C and 165 °C pretreatments give lower glucose titer. However, at 165 °C, 20 min and 40 min of pretreatment gave higher glucose titers than 30 min. A monotonic increase or decrease in glucose titer might be expected, rather than a minimum for 165 °C pretreatment at 30 min. One explanation for this phenomenon is that more lignin can be removed at the higher temperature and longer microwave time. The presence of lignin is known to inhibit enzymatic hydrolysis (Brandt et al., 2013; Ioelovich, 2014). If lignin is more effectively solubilized by the severity (higher temperature and longer time) of the 40 min 165 °C microwave pretreatment, then enzymatic hydrolysis may give higher titers of glucose. Another effect that can increase glucose titers from biomass with enzymatic hydrolysis is conversion of crystalline "native" cellulose to amorphous "disordered" cellulose (Papa et al., 2012). Further research is required to determine if lignin removal or change from crystalline to amorphous cellulose increases the effectiveness of enzymatic hydrolysis for biomass pretreated with DES.

Amorphous cellulose is more available to enzymes, increasing the glucose titer. The high glucose titer for the 40 min 165 °C microwave pretreatment may show this effect, as confirmed by FTIR as described in section *3.2.2*. The 30 min 165 °C microwave pretreatment shows lower glucose titer. A possible explanation for this is decomposition of amorphous cellulose without a high degree of conversion of crystalline cellulose. The same trend is found for conductive heating at 165 °C, with 2 h showing a minimum glucose titer. As discussed in the FTIR section *3.2.1*, decomposition of amorphous cellulose without a high degree of conversion of crystalline cellulose may cause this effect.

3.2. FTIR results

Table 2 shows vibrations identified in these spectra that are relevant to cellulose, hemicellulose, and lignin. Table 1 has area ratios for selected vibrations. The ratio of the combined areas of the cellulose vibrations to those of the lignin vibrations can give insight into the effects of the pretreatments. In addition, a grasp of the effects of pretreatment on cellulose can be found from the ratio of the area of the amorphous cellulose vibration to that of the crystalline cellulose vibration.

3.2.1. FTIR of pretreated rice hulls – conductive heating pretreatment

As seen in Fig. 2, the FTIR spectra of pretreated rice hulls are different than that of raw rice hulls. Table 2 shows vibrations identified in these spectra that are relevant to cellulose, hemicellulose, and lignin. Overall, area for cellulose-related vibrations increased in the pretreated samples compared to area for lignin vibrations, compared to the same ratio for untreated rice hulls. Lignin in the hulls thus appears to be removed by the pretreatment. For the conductive heating pretreatments, the vibration area ratio of amorphous cellulose (896 cm^{-1}) to crystalline cellulose (1415 cm^{-1}) was greatly increased by pretreatments compared to the same area for untreated rice hulls (Table 2). This suggests that crystalline cellulose in the rice hulls is being disordered by the pretreatment. Amorphous cellulose is more amenable to enzymatic hydrolysis (Papa et al., 2012) to produce glucose. As seen in Table 1, the pretreatments increased glucose titers.

The 165 °C for 2 h pretreatment shows somewhat lower area ratio for cellulose vibrations: lignin vibrations, compared to 1 and 3 h 165 °C pretreatment. This particular pretreatment also gave lower glucose titers. One possible explanation is that amorphous cellulose is decomposed by this relatively severe condition, but more of the crystalline cellulose is not sufficiently disordered to compensate for the loss of amorphous cellulose. The 1 and 3 h 165 °C pretreatments show a higher ratio for amorphous vibration area/crystalline vibration area, compared to the 2 h pretreatment. The 1 h 165 °C pretreatment may not decompose amorphous cellulose, while the 3 h 165 °C pretreatment may disorder more of the native cellulose, as suggested by lower cellulose vibration area for cellulose-related peaks, compared to lignin. Mass yield for the 165 °C for 2 h pretreatment was also slightly (~5%) lower than for the other 165 °C treatments. Recondensation of dissolved lignin precipitating from solution is one possible reason that mass yield was higher for the 3 h 165 °C pretreatment compared to the 2 h 165 °C pretreatment. Donohoe et al. (2008) has found lignin droplets to form with thermochemical pretreatment of biomass (Donohoe et al., 2008). Such recondensation may not block enzymatic hydrolysis but would decrease the cellulose to lignin ratio. As shown by Table 1, the cellulose to lignin vibration area ratio is low for the 3 h 165 °C pretreated biomass, which would suggest that recondensed lignin droplets may have adhered to the biomass surface.

3.2.2. FTIR of pretreated rice hulls – microwave heating pretreatment

As seen in Fig. 2, the FTIR spectra of the microwave pretreated samples show similar trends to those of the conductively pretreated samples. The ratio of the cellulose-related vibrations area to lignin vibrations area for the samples pretreated at 165 °C and the 30 min time (corresponding to 2 h conductive treatment) is low, just as was seen for the 2 h conductive treatments. This particular condition also showed lower glucose titers compared to pretreatment for longer or shorter times at 165 °C. Compared to the 20 min 165 °C pretreatment, mass yield for the 30 min 165 °C pretreatment was ~10% lower. As described in the previous section *3.2.1*, more amorphous cellulose may be decomposed by this severe condition. The 30 min 165 °C pretreatment also showed a smaller ratio for amorphous vibration area/crystalline vibration area, compared to that of the 20 min 165 °C pretreatment. This again suggests amorphous cellulose has been decomposed at this more severe condition.

#### 3.2.3. FTIR of the precipitate from the spent deep eutectic – conductive heating pretreatment

Table 3 shows the area ratios for cellulose vibrations: lignin vibrations for the precipitates from the spent DES. These are all lower than the same area ratio for the untreated rice hulls, thus they are higher in lignin. The 2 h pretreatments showed the lowest cellulose to lignin area ratios, suggesting that this time is optimal for obtaining lignin that is purer (except for 145 °C, where the two shorter times give similar

Table 2

Wavenumber Vibration Assignments and References. 896, 1060, 1162, and 1425 cm^{-1} are cellulose-related peaks. 858, 1600, and 1718 cm^{-1} are lignin-related peaks.

Wavenumber (cm^{-1})	Band Assignment	Reference
858	C-H out of plane positions 2,5,6 of lignin (guaiacyl) ring	Boeriu et al. (2014)
896	C1 − H deformation with a ring vibration contribution for amorphous cellulose	Raj et al. (2015)
1060	C-O stretching vibration for cellulose and hemicellulose	Shi and Li (2012)
1162	C-O-C vibration in cellulose	Shi and Li (2012)
1425	Crystalline cellulose	Åkerholm et al. (2004); Mood et al. (2013); Oh et al. (2005); Raj et al. (2015)
1515	Lignin aromatic ring skeletal stretch	Raj et al. (2015)
1600	Lignin C = O stretching conjugated to the aromatic ring, aromatic ring vibration	Li et al. (2015); Lin et al. (2015)
1718	C = O stretching in unconjugated ketone, carbonyl and ester groups related to lignin	Lopes et al. (2013)
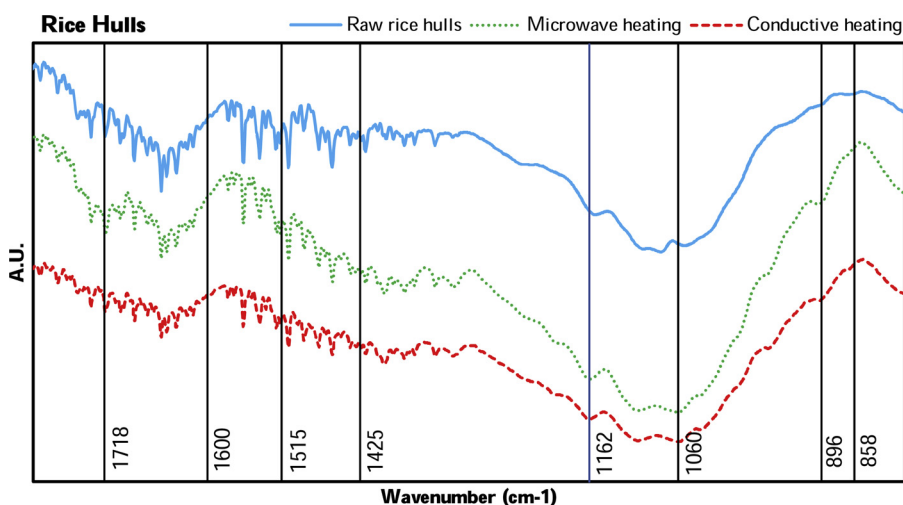
Fig. 2. FTIR spectra of untreated and DES pretreated (155 ℃, 2 h conductive or 0.5 h microwave) rice hulls. Solid line is untreated rice hulls, dotted line is microwave DES pretreated rice hulls, dashed line is conductively DES pretreated rice hulls.

Table 3

Precipitate from spent DES after pretreatment.

Pretreatment conditions		
T (°C)	time (min)	Ratio cellulose:lignin vibration areas
Conductive heating		
145	60	0.32
145	120	0.34
145	180	0.48
155	60	0.43
155	120	0.35
155	180	0.53
165	60	0.35
165	120	0.29
165	180	0.41
Microwave heating		
145	20	0.42
145	30	0.48
145	40	0.50
155	20	0.35
155	30	0.43
155	40	0.40
165	20	0.39
165	30	0.41
165	40	0.45
No pretreatment		0.80

Values are averages for replicated samples. Standard error for the ratio is 0.02.

results). The 3 h pretreatments tended to give higher area ratios, suggesting that more of the cellulosic component had been solubilized, along with lignin.

The vibration band for amorphous cellulose (896 cm^{-1}) was essentially absent for the spent DES precipitate samples. Apparently,

either little amorphous cellulose was dissolved from the biomass by DES pretreatment or amorphous cellulose was not precipitated from the spent DES by ethanol.

As seen in Fig. 3, a new vibration not detected in untreated or pretreated rice hulls is detectible in the spent DES precipitate samples. A vibration for C–H out of plane positions 2,5,6 of the guaiacyl ring (858 cm^{-1}) was prominent in all spent DES precipitate samples (Boeriu et al., 2014). Presumably, this vibration was masked by larger vibrations that relate to cellulose in untreated rice hulls. Its presence suggests that lignin has been precipitated from the spent DES.

3.2.4. FTIR of the precipitate from the spent deep eutectic for microwave heating

Microwave heating also gave lower area ratios for cellulose vibrations: lignin vibrations for the precipitates from the spent DES compared to the untreated or pretreated biomass. Little difference could be seen for the different time periods of heating, although a trend towards higher cellulose to lignin ratio with longer time may be discernable. Cellulose: lignin vibration areas tended to be higher than those found for 1 and 2 h conductive heating. These findings suggest that the reactions that separate lignin from cellulose may be sequential, requiring a longer time than that used for microwave pretreating. Nevertheless, the differences are slight.

As found for conductive heating, no vibration band for amorphous cellulose (896 cm^{-1}) was seen in the precipitate spectra. The same prominent vibration for C–H out of plane positions 2,5,6 of the guaiacyl ring (858 cm^{-1}) that was seen for conductive heating was found for all the spent DES precipitate samples from microwave pretreating (Fig. 3). Further investigation into the characteristics and possible uses of this lignin-rich product should be pursued.
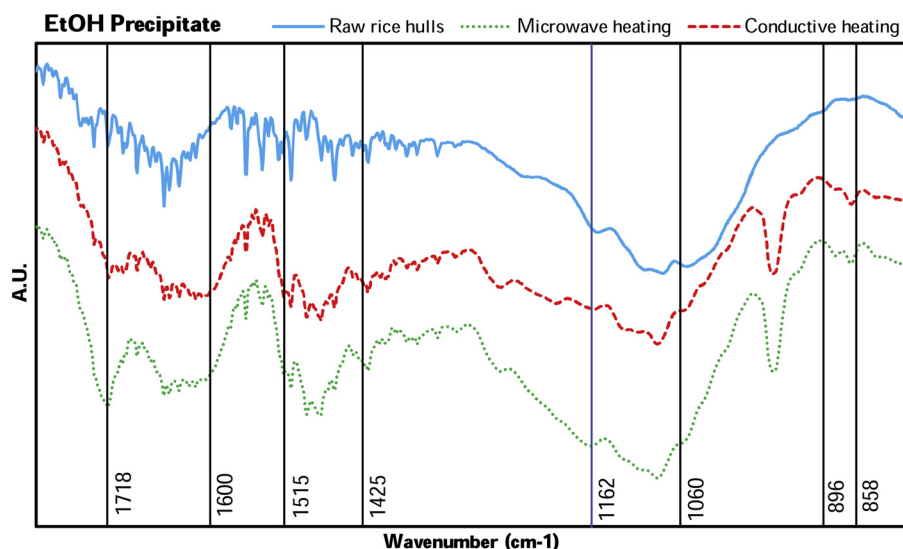
EtOH Precipitate — Raw rice hulls — Microwave heating — Conductive heating

A.U. vs Wavenumber (cm-1)

1718, 1600, 1515, 1425, 1162, 1060, 896, 858

Fig. 3. FTIR spectra of material precipitated by ethanol from the DES after rice hull pretreatment at 155 °c, 2 h conductive or 0.5 h microwave. Solid line is untreated rice hulls, dotted line is material precipitated from DES after microwave pretreatment, dashed line is material precipitated from DES after conductive pretreatment rice hulls.

3.3. Changes in cellulose and lignin content from pretreatment as measured by fiber analysis

As can be seen in Fig. 4, the cellulose content of the rice hulls pretreated at the central temperature and time conditions increases compared to the raw rice hulls for both the conductive and microwave pretreatments. Also, for these pretreatments, lignin content decreases compared to the raw rice hulls. Thus, the results from the FTIR analysis are confirmed by fiber analysis. Delignification of 45% was found for conductive heating and 36% for microwave heating. The slightly higher delignification found for conductive heating may be due to the longer time period involved. Since no other studies have been performed with DES alone on rice hulls, which are an unusual biomass, these results cannot be compared with other studies.

3.4. XRD of the conductive and microwave pretreated and raw rice hulls

Fig. 5 shows the XRD of conductive and microwave pretreated rice hulls, along with untreated rice hulls. The crystallinity index for conductive heating was 90.3% and for the microwave heating was 90.2%, while for untreated rice hulls it was 94.4%. The reduction in crystallinity index suggests that crystalline cellulose may become disordered by the pretreatments. Further research is required to determine if a change from crystalline to amorphous cellulose actually occurs for rice hulls, an atypical biomass, when pretreated with DES.

3.5. Energy consumption comparison

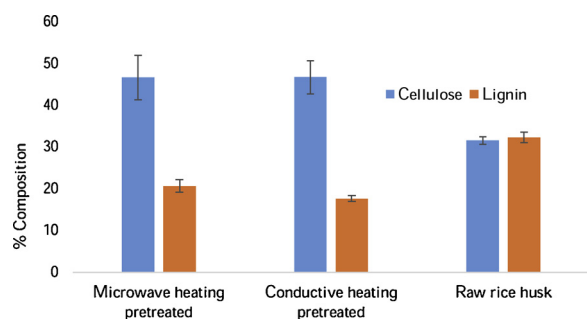For this study, 20 min of microwave heating time was considered to

Fig. 4. Cellulose and lignin content from pretreated and raw rice husk as measured by fiber analysis for the central condition of 155 °C and 2 h for conductive heating and 0.5 h for microwave heating.
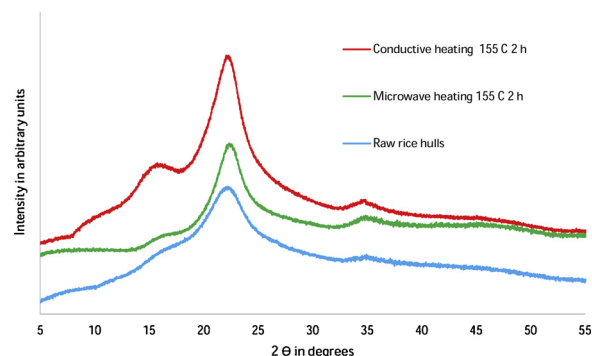

Fig. 5. XRD of raw and pretreated rice hulls for the central condition of 155 °C and 2 h for conductive heating and 0.5 h for microwave heating. (Top spectra is conductive heating, middle is microwave heating, bottom is untreated rice hulls.).

Table 4
Energy used per experiment for each temperature and time.

T (°C)	time (min)	Energy used (kW-h)
Conductive heating		
145	60	0.14
145	120	0.28
145	180	0.42
155	60	0.16
155	120	0.31
155	180	0.47
165	60	0.18
165	120	0.36
165	180	0.54
Microwave heating		
145	20	0.05
145	30	0.08
145	40	0.11
155	20	0.07
155	30	0.10
155	40	0.13
165	20	0.08
165	30	0.12
165	40	0.16

be comparable to 1 h of conductive heating time, 30 min comparable to 2 h, and 40 min comparable to 3 h. These durations were chosen as a result of the experience of Dr. Boldor's research team in microwave pretreatment (Chen et al., 2012). Table 4 compares the energy

requirements for conductive and microwave pretreatments.

As can be seen in Table 4, for comparable heating, the microwave experiments required less than half of the energy required for conductive experiments. In addition, microwave pretreatments were much shorter in duration. Anyone who has made a cup of tea using a microwave and using a stove should not be surprised at the lower amount of time required for the microwave experiments. DESs are electric dipoles, similar to water in that they have a slight positive charge in one part and a slight negative charge at another. The molecules therefore rotate as they try to align themselves with the alternating electric field of the microwaves. Rotating molecules strike other molecules and causing them to move, thus dispersing energy. This energy raises the temperature of the solvent, in a process similar to heat transfer by conduction. However, the movement of individual molecules may enhance penetration of solvent into biomass pores and provide energy to activate the lignin bond breaking required for lignin solvation.

Less waste heat is also generated by microwaves since the heat source is inside the solvent molecules, rather than being external. For conductive heating, the heat gradient outside the hotplate on the noncontact sides also causes heat loss that does not heat the solvent. If adapted for industrial purposes, low temperature steam at 180 ˚C might be sufficient to heat such a process, thus the higher cost of using electric energy could be avoided. Nevertheless, using microwave technology allows a reduction in residence time of biomass for the process. Such an advantage should encourage further investigation into its use to perform the lignin –cellulose separation using DESs.

4. Conclusions

Deep eutectic solvents can be used at relatively low temperatures and pressures to deconstruct rice hulls into cellulose-rich and lignin-rich products. The cellulose-rich product, which now contains disordered amorphous cellulose, can be enzymatically hydrolyzed to produce glucose. With DES pretreatment, up to 100 times more glucose can be obtained with enzymatic hydrolysis compared to untreated rice hulls. The lignin-rich product has potential to be used in asphalt binders to improve aged asphalt properties. Using microwaves for heating in DES pretreatment gives similar results to conductive heating and requires less time and energy.

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