
Sustainable Valorization of Rice Straw Using Deep Eutectic Solvents

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May 2019

Abstract This work is aimed at the development of a sustainable valorization process for rice straw, consisting in two stages. Firstly, a liquid-liquid gamma-oryzanol (OZ) extraction was carried out using hydrophobic deep eutectic solvents (DES). High performance liquid chromatography analyses of the extracted phases allow to conclude that the extraction of this compound (OZ) was not successful, probably due to the very small concentration of this compound in the chosen biomass. The second stage involved a treatment of biomass with hydrophilic DESs based on choline chloride (CC). This process managed the best solubilizing performance with a DES composed of CC and urea (U) in a molar ratio of 1:2, recovering 48% of the added biomass, conducting to a solubility of 25.9 mg of rice straw/g DES. In respect to the selectivity towards glucose and xylose, the DES comprised of CC and acetic acid (AA) in a 1:2 molar ratio achieved the best results. The water's effect in the biomass treatment was also studied and an optimization of this stage warranted the use of the CC and AA DES in a 4 hr biomass incubation for achieving the most promising results.

Keywords: Deep eutectic solvent (DES), rice straw, gamma-oryzanol, valorization, green chemistry

Introduction

With the growing necessity to achieve «sustainable development», which is the organizing principle for meeting the demands of both human development and the ability of natural systems to sustain themselves, in order to provide the resources upon which the economy and society depends[1], the birth of biorefineries has been prompted to answer these problems mainly due to their renewable nature. The objective is to manufacture high-value low-volume products (HVLV), that are largely profitable, and simultaneously to add value to the industrial process with low-value high-volume (LVHV) products that can be converted into energy to meet the global energy demand through a network of processes[2]. Furthermore, the recent development of alternative solvents to those commonly called volatile organic solvents namely ionic liquids (ILs) and deep eutectic solvents (DESs), has been a very dynamic field, as regulations became stricter and the implementation of more sustainable processes has become paramount. The latter have quickly grown in importance since they have a simpler and more economical production compared to ILs while maintaining their process flexibility, high selectivity and also offsetting their major drawbacks, specifically high toxicity and nonbiodegradability[3], therefore helping in the preservation of the environment by respecting green chemistry's principles. A DES can be defined as a mixture of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), differing from ILs that only contain discreet anions[4] in a 1:1 ratio, while in DESs the ratio of HBA (typically a salt, although neutral

compounds can also be used) and HBD can be any. These compounds build on the eutectic point concept to develop low temperature melting compounds, with a melting temperature lower than that of the two parent compounds. Some «major families» consist in the combination in pairs of a carbohydrate, like C6 sugars, an urea derivative and a chloride salt or even in the mixture of several natural occurring acids with menthol or quaternary ammonium salts that show a hydrophobic behavior unlike the previous hydrophilic DESs[5]. In regards to the applications presented in this work, choline chloride (CC) based-DESs and octanoic acid (C8) based-DESs were chosen as their low cost and biodegradability are characteristics of the utmost importance. CC is produced in large scale and used as a nutrient in chicken feed[5] and C8 is used as an antimicrobial pesticide[6] also attesting for its large-scale availability and low price.

As for the used resource, rice straw is one of the most abundant available lignocellulosic biomass residues, with an average composition of 34, 28, 18, 7 and 13% of cellulose, hemicellulose, lignin, ash and extractives, respectively[7]. It's worldwide annual production amounts to over 950 million tons and whereas most of it is used to feed cattle, another large portion is burnt across agricultural fields with little usefulness apart from adversely affecting nature's already susceptible fate[8] creating an urgent need for its valorization. It is also important to remark that it is estimated that this agricultural residue can potentially produce around 205 billion liters of bioethanol annually, making it the

largest amount from a single biomass feedstock[8] and as it is a by-product in the production of rice, rice straw should also contain added-value compounds such as antioxidants that would include vitamin E and particularly gamma-oryzanol (OZ) that will be our HVLV product and on which we will center our attention. OZ was first extracted and isolated from rice bran oil in the 1950s[9], and while it was thought to be composed of one single component, it was later determined to be a group of sterol esters of ferulic acid[10]. The three major compounds are cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesterol ferulate[11] that together account for 80% of OZ[12]. It has many biological properties that include hypolipidemic[10][11], antioxidant, anti-inflammatory, antineoplastic, hypoallergic, antidiabetic and antiulcerogenic[13] among other proposed health benefits like antitumor activity[11]. Overall, OZ is a very interesting set of compounds with promising applications in the pharmaceutical[14], nutraceutical[9] and cosmetic[15][16] fields. It must be mentioned that as no OZ extraction from rice straw has ever been reported in the literature, moreover with a DES, the chosen combinations of C8:Menthol (C8:M) and C8:Dodecanoic acid (C8:C12) were elected due to their hydrophobicity, an important factor since it is the common denominator in all of the studied articles wherein OZ was extracted.

Finally, in order to grasp the mechanisms and techniques that are commonly used in this valorization, the literature[17] provided the fractionation methodology that was used to obtain the LVHV products as it implements it in an effective way. It showed the need of a treatment to enhance the lignocellulosic biomass's conversion into fermentable sugars as its complex heteromatrix structure makes it a hard to process matter. It is remarked that this treatment, when done with DESs composed of CC and acid, fares well in the processing of several biomasses but when weakly basic DES like CC and U or neutral DES, for instance CC and glycerol, are used, we observe only moderate sugar yields comparable with saccharification efficiencies of the untreated sources. Thus the selection of the DESs to use in this production of LVHV residues is inspired on the results of the literature but it must be observed that some other DESs were added to this list due to their functional groups that can partake in the key interactions of the lignocellulosic biomass dissolution; they are all CC based-DESs that are hydrophilic as the presence of water also plays an important part in the hydrolysis of the polymeric biomass.

Therefore this work will delve in this field of biomass processing with the goal of harnessing a HVLV product and the prospects of producing a LVHV product in order to increase the process value.

Experimental Section

Reagents All of the employed compounds were commercial grade and used without further purification apart from CC and U that were dried in an oven at 45°C for at least 24 hrs prior to use and polyethylene glycol 200 (PEG 200) that was also dried under vacuum. The dried rice straw was obtained from COTARROZ, Centro Operativo e Tecnológico do Arroz, Salvaterra de Magos. The rice straw was cut and grinded using a regular coffee mill into particles with a size of 0.5-1 mm approximately, and then stored in a sealed plastic container that was kept under atmospheric conditions preceding its utilization.

Material and Equipment Common laboratory glasswork like beakers, pipettes and glass flasks, was used as well as regular laboratory material and small equipment. Moreover, a Karl-Fischer apparatus, 831 KF Coulometer from Metrohm, coupled with a 728 magnetic stirrer, from Metrohm as well, was used to determine the water content in the selected solvents. A UV-vis spectrophotometer, UV-1800, from Shimadzu was used for the preliminary analysis of the extraction step's experiments with a UVProbe v2.34 software from Shimadzu. A HPLC composed of a Phenomenex Luna Silica (2) 100 Å 250 mm x 4.6 µm column coupled with a UV detector was used to determine the OZ content in the extraction step's experiments and an HPLC apparatus composed of a CarboPac PA 10 4x250 mm column preceded by an Aminotrap and coupled with a pulsed amperometric detector was used to determine the sugar content in the liquid extract phases obtained from the fractionation step.

Methods

Preparation of DESs The methodology used to prepare the DESs was based on the heating method that was described in the literature[18]. CC and U were dried for at least 24 hrs in an oven at around 45°C, while PEG 200 was dried for at least 24 hrs under vacuum. All the other starting materials were used without further processing.

The HBA and HBD composing the DES were then mixed at 80°C under stirring. After one hour, the mixture was let to cool down until it reached room temperature. Only the hydrophilic DES aqueous solutions were prepared with later addition of water.

The DESs were prepared with selected molar ratios present in the literature[19][20][21][22][17][18][23][24].

Extraction of OZ from Rice Straw The extraction protocol of OZ from rice straw was carried out according to Bergman et al.[11]. The same

protocol was applied when the two hydrophobic DESs were used, with two exceptions: instead of using 5 mL of hexane, the corresponding mass of DES was used and the final evaporation step of the extract phase's solvent was not conducted as the samples were analyzed after being filtered. The residual biomass obtained from these experiments with hydrophobic DESs was dried in an oven at 45°C for at least 72 hrs to assess if the solvent could be evaporated.

The extraction matrix was analyzed with UV-Vis spectroscopy and by HPLC coupled with a UV detector set for a wavelength of 330 nm. The mobile phase was composed of hexane, isopropanol, ethyl acetate and acetic acid in a 94:5:0.5:0.5 volumetric proportion and 20 µL were injected at a flow rate of 1.5 mL/min at 30°C. The standards were prepared using the purchased OZ in hexane at different concentrations in order to obtain a calibration curve.

Hydrophilic DES Treatment: Fractionation of Rice Straw Biomass After an initial screening where the cloud point method was employed, the best performing DESs were selected and used to achieve the fractionation goal following the procedure present in the literature[17]. An optimization of this fractionation step was achieved by shortening the incubation period to 4 and 8 hrs.

The content of dissolved sugars in the residual DES aqueous solution obtained in the fractionation procedure was also analyzed by an HPLC system with a pulsed amperometric detector. The mobile phase was composed of an 18 mM NaOH solution and 10 µL were injected at a flow rate of 1 mL/min at 25°C. The standards were prepared using ultrapure water with the addition of monomeric sugars in order to obtain a calibration curve.

Results & Discussion

Extraction of OZ from Rice Straw Solubility tests were initially conducted with the two hydrophobic DESs and the experiments showed that both C8:M and C8:C12 managed to completely dissolve the added OZ, with no trace of any undissolved particle or any turbidity, thus making their application in the extraction procedure appropriate since the concentration of OZ in the solubility test was over fourfold higher than the hypothetical one that would be obtained in the real extractions. Following this step, the effect of the chosen solvents on the OZ detection by UV-vis spectrophotometry was studied by analyzing the solubility tests and the extraction assays made with DESs with a UV-1800 spectrophotometer. This analysis also served to

validate the location and the characteristic shape of the OZ's peak in these solvents.

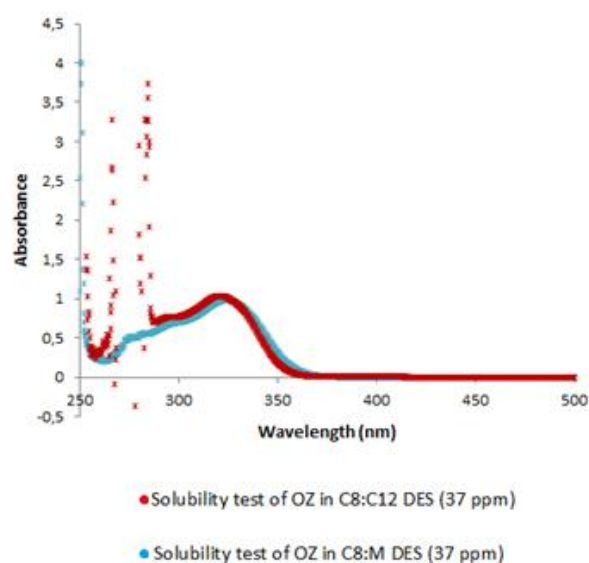


Figure 1 - Comparison of the solubility tests' UV-vis spectra.

It can be observed that in both cases, the absorption bands present a maximum at a wavelength of approximately 330 nm, as confirmed by the literature[11][25]; this is the wavelength corresponding to ferulic acid[26] that is one of the architecting blocks of the compounds present in OZ. With this in mind, it can be seen as well that both spectrums are similar, with only a slight deviation of 10 and 5 nm in the C8:C12 DES and the C8:M DES, respectively, from the 330 nm reported maximum. This wavelength shift of the maximum is due to the specific interactions of each solvent with OZ, allowing us to conclude that although similar, both solvents have different affinities for OZ. Moreover, it must also be stated that there is some signal interference of the solvent. It is present in both DESs below 250 nm, where a sudden drop in absorbance is witnessed but further interference is noted between approximately 260 and 285 nm in the C8:C12 DES. The obtained disturbance below 250 nm can only be due to the presence of C8 since it is the only common denominator in both used baselines and meanwhile the interference between the specified wavelengths in the C8:C12 DES can only be due to the presence of C12 because the HBD is the only altered variable in the used baselines as well as in the solubility tests.

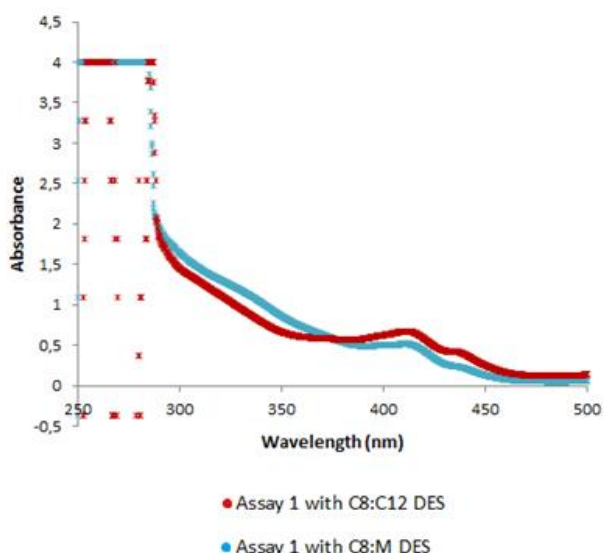


Figure 2 - Comparison of the extraction step's experiments' spectrums.

The obtained UV-vis spectra of the OZ extraction assays using both DESs show that the ferulic acid characteristic absorption band cannot be identified and therefore the use of an HPLC is mandatory. This is probably due to the simultaneous extraction of other compounds present in the rice straw besides OZ. Nevertheless an inflexion point can be noticed in figure 2 around the 330 nm mark, indicating the possible existence of OZ in the extracts as both DESs lead to similar results. However, it must be stated that above approximately 380 nm, the C8:M DES extract has a lower absorbency than the C8:C12 DES, implicating a lower extraction efficiency, while in the region between 380 to 290 nm, the opposite is verified, therefore suggesting that the former DES has a higher extraction efficiency of OZ. Moreover, the saturation observed below 290 nm attests for the extraction of other lipophilic material contained in the

rice straw. In fact over 60 compounds fitting this description exist in this lignocellulosic biomass and the literature reports the use of a similar wavelength for detecting them[27], suggesting that their absorption bands have maximums around this region which could in turn explain the observed spectrum saturation. It must also be stated that the extraction of one or more rice straw's constituents responsible for its color was achieved, as was verified by the yellowish color of all assays after the extraction procedure; this is indeed validated by the presence of two observed peaks in the 415 to 430 nm region, associated with the visible region, that should correspond to these compounds.

In respect to the HPLC analysis of the extraction assays, carried out to quantitatively infer on the OZ extraction yield of each used solvent as the preliminary UV-vis spectrophotometry was inconclusive, it showcased the absence of OZ's characteristic peak. This was later confirmed by a posterior addition of the OZ standard to an extraction assay with each DES since the solvent's matrix can influence the retention time of OZ in the column. This addition led to the appearance of the OZ peak as is witnessed in figures 3 and 4. Indeed, the presence of the OZ's peak at 4.92 and 4.90 min in C8:M and C8:C12 chromatograms, respectively, after the addition of the pure OZ, leads to the conclusion that despite the matrix interference, it was not the reason justifying the previous absence of its peak. Thus, in conclusion, the extraction of OZ from rice straw was not effective as no OZ peak could be identified in the HPLC analyses. This could either be due to the very small concentration of this compound in the rice straw or to the fact that the protocol's experimental conditions were not optimized. Nonetheless the failure on extracting OZ with hexane, points to the former.

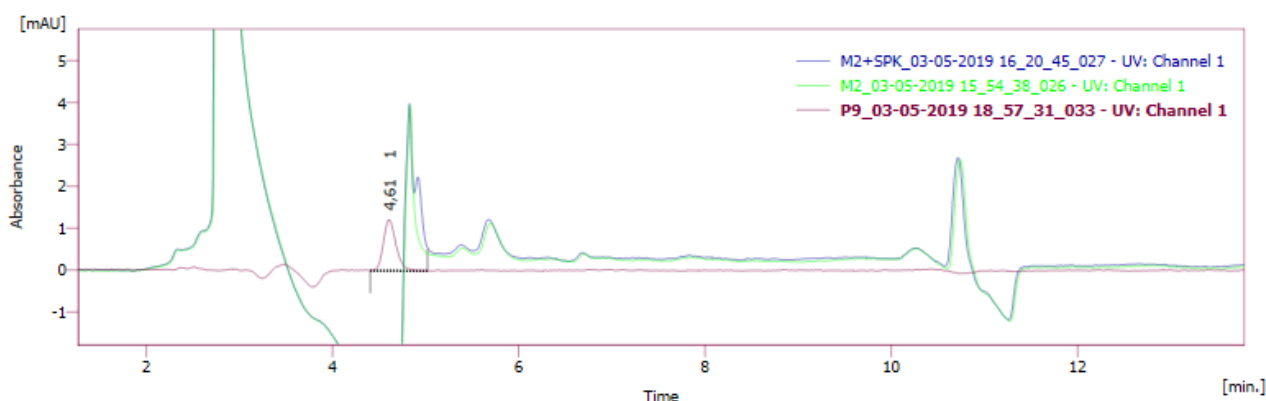


Figure 3 - Comparison of HPLC chromatograms obtained from an extraction assay with C8:M DES with (in blue) and without (in green) the addition of OZ standard.

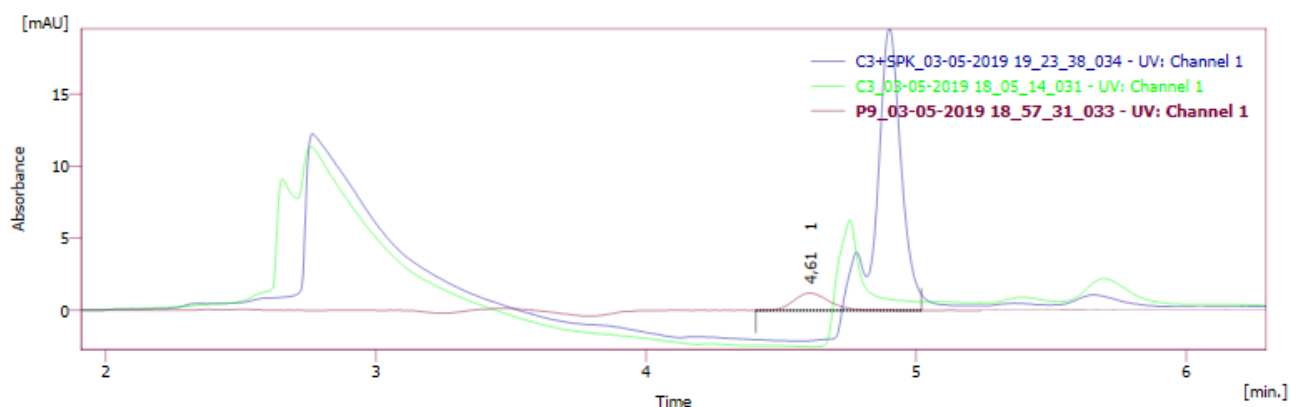


Figure 4 - Comparison of HPLC chromatograms obtained from an extraction assay with C8:C12 DES with (in blue) and without (in green) the addition of OZ standard.

An analysis on the DESs' recyclability was also conducted as it would ease the residual biomass sample handling by providing an efficient method to recover the solvent. It was observed that only the biomass sample treated with C8:M DES completely dries under the experiment's conditions, demonstrating the feasibility of applying a simple drying step to the processual residue obtained with this solvent. Nonetheless it was not evaluated if the DES could indeed be evaporated or solely part of its constituents. Further investigation must be conducted, although the similar boiling points of C8 and M suggest that the whole DES is evaporated, presenting an exception to the general consensus found in the literature about DESs, stating that they are not volatile.

Hydrophilic DES Treatment: Fractionation of Rice Straw Biomass

The water fraction in the DES aqueous solution is paramount in order to achieve a greater solubilization of rice straw and in turn a better depolymerization yield of the lignocellulosic biomass sample. The rice straw dissolution tests that were performed at 60 and 90°C allowed to conclude on this matter. It was observed that a small water fraction leads, in general, to a higher biomass dissolution, for the tested DESs and both studied temperatures, justifying the choice of a 10 wt% water content in the DES aqueous solutions in the next stages of this work. Furthermore, the results for the CC:AA DES indicate that the dissolution process carried out in this solvent is less affected by the water content, unlike the CC and fructose (FRU) DES, where the increase in the water fraction manages a twofold increase of rice straw solubility at 60°C. This is due to the fact that these DESs are very viscous and the addition of water facilitates the mass transport. Moreover the solubility results, for all DESs, obtained at the two temperatures are very different from each other indicating the relevant role this variable also plays in the dissolution of rice straw biomass. On another note, it is hypothesized that the dissolution mechanisms of both DESs are different. While the CC:FRU DES may form more hydrogen bonds with the

polymeric saccharides present in the sample due to its hydroxyl groups, the CC:AA DES's acidity should allow for a better digestion of the rice straw, thus freeing its components and leading to a higher solubility as well. Nonetheless, the results showed that the CC:FRU mixture is more efficient at solubilizing rice straw than the CC:AA DES, suggesting that its mechanistic interaction with the biomass is more favorable.

Once the optimal water fraction in the DES aqueous solution was determined, a screening of 8 different DESs at 25 and 50°C was performed as well. Observations made during a one month period led to the conclusion that the CC:AA, CC:U, CC and lactic acid (LA) and CC and oxalic acid (OA) DESs achieved the best results and were subsequently chosen to carry out the fractionation step of the biomass with the goal of obtaining two LVHV products. Nonetheless, the CC:OA DES was replaced by the CC and malic acid (MA) DES because of the degradation of OA into hydrogen and carbon dioxide at the process temperature[28], making the employment of this DES unfeasible in this step.

The employed protocol involved the use of a high process temperature for 18 hrs to not only guarantee that the solvent saturation is reached but also to allow the thermal degradation of the biomass sample. It was based on a methodology employed in the literature[17] where it could be observed that for the proposed one step treatment, higher processing times led to lower residue recovery fractions and therefore higher rice straw concentrations in the DESs. This is exactly what can be observed in our experiments (figure 5). An even higher solubility of rice straw in the DESs is achieved in this presented method thus implicating a further depolymerization of its constituents since their polymeric form hinders their dissolution in these hydrophilic solutions. Furthermore, the drastic solubility increase achieved in this process outperforms the other comparable literature result of the dissolution of rice straw in the CC:FRU DES at 120°C, where a solubility of 6.5 mg/g of DES was afforded in a simple dissolution test[18].

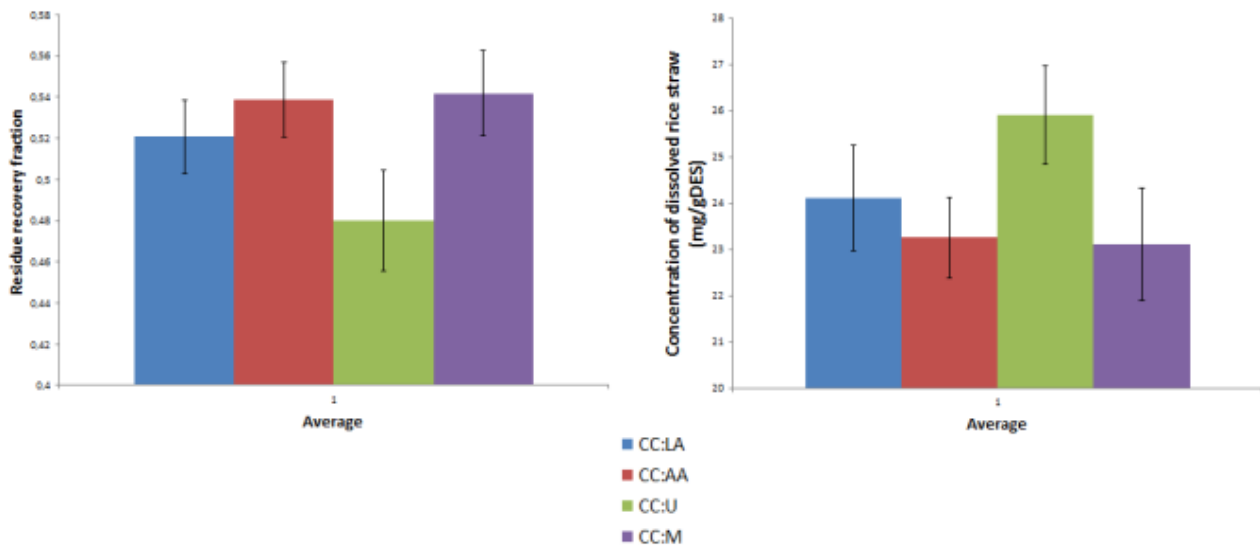


Figure 5 - Residue recovery fraction and concentration of dissolved rice straw with each tested DES for incubation at 120°C, 18 hrs (+/- 1 S.D.).

In respect to the above presented charts, it can be seen that the CC:U DES showcased the best performance in terms of both residue recovery fraction, with an average recovery of 48% of the originally added rice straw, and biomass concentration in the solution, amounting to an average of 25.9 mg/g of DES. The obtained solid residue with this DES's employment was less than with other solvents thus inferring a greater dissolution of rice straw in it. As for the other DESs, no statically significant difference in these factors was observed between them. An explanation for these observed results may be the fact that U can be both a HBA and a HBD; it could be hypothesized that this characteristic ability permits a greater interaction with the lignocellulosic biomass's constituents.

the performance of the CC:AA DES as no statistically significant difference was observed in the residue recovery fraction and in the concentration of rice straw in this DES. This could be due to the fact that the minimum water fraction threshold to warrant an efficient solubilization of rice straw is attained and this was particularly apparent in the study of the effect of the water fraction with the CC:U DES. The water content in this DES aqueous solution does indeed influence the results as a significantly higher degree of biomass solubilization is achieved in the 2 and 10 wt% water content assays when compared with the 1 wt% solution suggesting that this threshold is situated around this value and giving an economical and eco-friendly advantage to this developed process. This is due to the decrease in viscosity in the DES aqueous solution that was visually noticeable during the conduction of these experiments.

The water fraction's effect was also studied. It showed that the water content of the solution did not affect

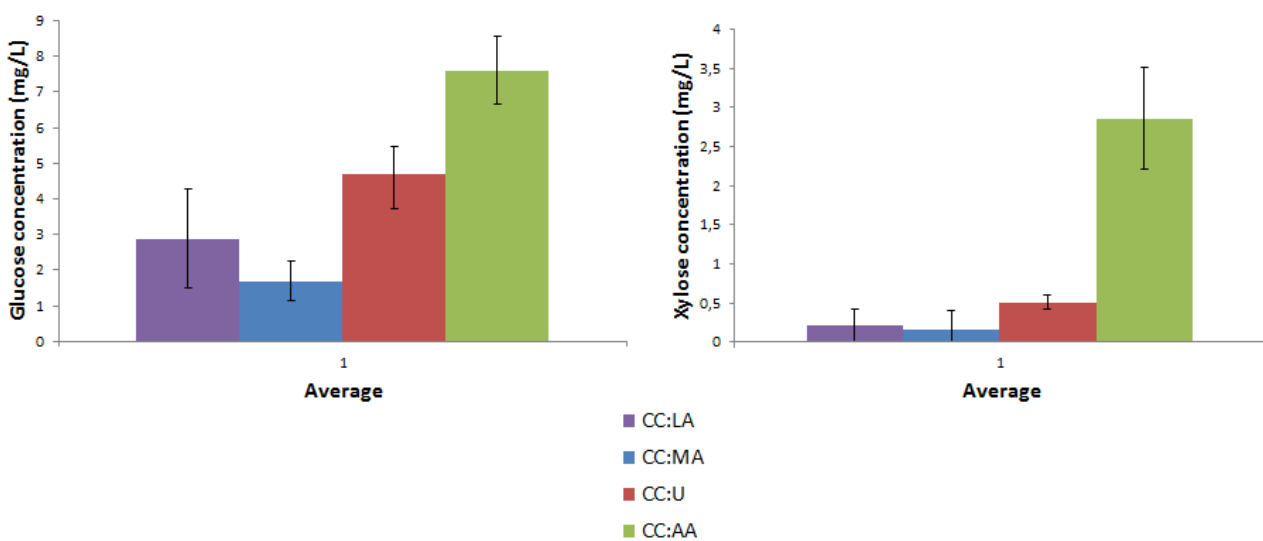


Figure 6 - Glucose and xylose yields in the liquid phase residues obtained from the fractionation step (+/- 1 S.D.).

In what concerns the glucose yields (figure 6), it can be seen that there is a statistically significant difference between the CC:AA DES and all the other DESs, with this mixture managing the best result of 7.6 mg of glucose/L of filtrate. As for the other DESs, the CC:U appears to be better at depolymerizing the rice straw than CC:MA and regarding the CC:LA DES's performance, the uncertainty makes it comparable to that of the CC:MA and CC:U DESs. It is interesting to note that the aforementioned mechanism of the CC:AA DES for solubilizing the rice straw, involving its acidic character, does not appear to justify the observed results as the CC:LA DES that has a more acidic nature, due to the lower pK_A of the LA in comparison with the AA, does not achieve the same degree of depolymerization of the rice straw that the CC:AA DES did. It could be hypothesized that the additional hydroxyl group of the lactic acid could actually stabilize the lignocellulosic biomass's constituents by creating hydrogen bonds with them.

Considering the obtained xylose concentrations, it can be seen that once again, the CC:AA DES manages the best performance with a result of 2.9 mg of xylose/L of filtrate followed by the CC:U DES that afforded a much more modest 0.51 mg of xylose/L of filtrate. The xylose concentrations resulting from the two other DESs' use is even lower, only amounting to approximately 0.18 mg of xylose/L of filtrate. Consequently, although the CC:AA DES is not the best at solubilizing the rice straw as was previously seen, it does manage to selectively depolymerize more of the biomass's components hence creating a higher monomeric sugar content liquid residue that can then be directly fermented. Subsequently, the application of an enzymatic hydrolysis step to further refine these mentioned residues, both the liquid and solid, is feasible since their remaining saccharides are under a polymeric form thus hindering the organisms' fermentation activity.

When comparing these results with the literature's reports on the carbohydrate content of rice straw's pre-fermented hydrolysates obtained via conventional methods, where 41 g of glucose/L of hydrolysate and 21 g of xylose/L of hydrolysate were secured[29], it can be seen that both the glucose and xylose yields of this process are substantially lower. However, to truly compare these results, the obtained liquid fraction's enzymatic hydrolysis must be conducted because a non-negligible amount of polysaccharides may still exist in this residue and the employed analytical method only detects their monomeric form. This underlines the need of applying another treatment step to the obtained residue.

The water fraction's effect was once again studied in the CC:AA and CC:U assays. In both DESs, the increasing water content leads to an increase in the glucose and xylose yields, evidencing a clear improvement. While in the former there is an increase of almost fourfold and approximately sixfold in the glucose and xylose yields, respectively, in the CC:U DES, a drastic thirtyfold improvement in the glucose production is observed when water is present at a 10 wt% compared with a 1 wt%. This is due to the fact that water partakes in the hydrolysis of hemicellulose and cellulose, from where these sugars are obtained, as one of this depolymerization main reaction's reagents as well as its already mentioned effect on lowering the viscosity of the solvent. Finally, an optimization of the fractionation stage was conducted and its results are showcased in figure 7 and 8.

It can be seen that while the residue recovery fraction and the concentration of dissolved rice straw with the CC:U DES show significant improvements with the increase of the duration of this step, leading to the best results for the 18 hrs incubation, these studied parameters do not showcase the same tendency for the CC:AA DES. In fact, it would seem that the best results with this solvent are attained at the 8 hrs mark

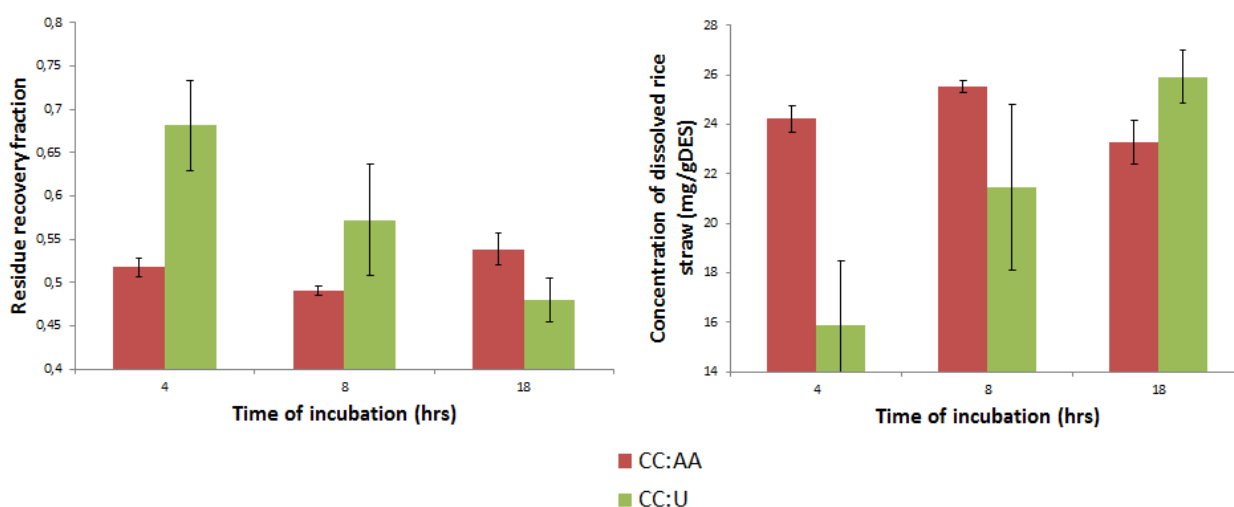


Figure 7 - Residue recovery fraction and concentration of dissolved rice straw with the tested DESs for incubation at 120°C during 4, 8 and 18 hrs (+/- 1 S.D.).

since a reduction of the residue recovery fraction and a subsequent augmentation of the solubilized biomass are noted in the passage from the 4 to 8 hrs incubation with the opposite being remarked when increasing the duration of this step. This could be due to the thermal degradation of the biomass sample, over this longer period, inhibiting the action of the solvent thus, conducting to the worst results during the 18 hrs incubation.

Furthermore, although the best performance can still be credited to the CC:U DES with a 48% residue recovery fraction and a solubility of 25.9 mg of rice straw/g of DES during the 18 hrs incubation, the fact that the CC:AA DES manages similar results, with a 49% residue recovery fraction and a solubility of 25.5 mg of rice straw/g of DES, in less than half the time, during the 8 hrs incubation, makes it more suitable for achieving the sustainability goal of this thesis as the energy expenditure will be considerably inferior.

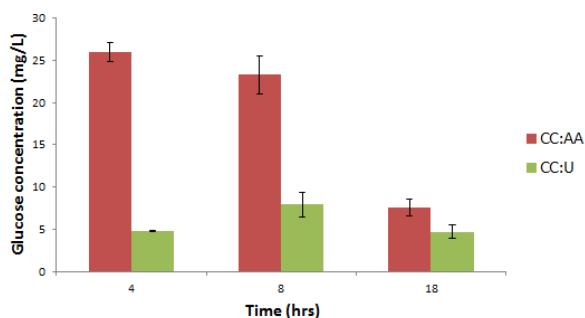


Figure 8 - Glucose yields in the liquid phase residues obtained from the optimization of the fractionation step (+/- 1 S.D.).

From the monomeric sugar analysis presented in figure 8, a drastic increase in the glucose concentration of the filtrate can be observed when using the CC:AA DES with a shorter biomass incubation of the lignocellulosic biomass sample. As a matter of fact, an almost fourfold increase in this parameter is remarked between the 18 and 4 hrs step, with this latter attaining a maximal concentration of 26 mg of glucose/L of filtrate.

As for the CC:U DES, a maximum is attained for the 8 hrs incubation, managing a concentration of 7.9 mg of glucose/L of filtrate, slightly higher than the ones obtained with the other durations of this step. The increase of process efficiency witnessed with this solvent in the passage of the 4 to 8 hrs mark can simply be explained by the longer processing time of the solvent.

However, for the further decline of the glucose content with the longer incubation periods of the assays, both cases may be explained by the degradation of glucose into hydroxymethyl furfural

(HMF) by the DESs' activity, as is suggested in the literature[17].

In what concerns the xylose concentration of the obtained filtrates, the same exact tendency as remarked before for the glucose content is noted once again for both tested DESs and the same previously mentioned reasons could explain these observations. Nonetheless, it must be mentioned that the product of the xylose degradation would be furfural and not HMF[17].

In conclusion, the choice of the CC:AA DES with a 4 hr biomass incubation leads to the most promising results and it is important to underline that the literature's statement, acknowledging that DESs displaying an acidic nature fare better in these kinds of treatment, is validated[17].

Conclusion

As the urgent need to find further uses to rice straw, an abundant agricultural residue, is of the utmost importance to positively affect nature's susceptible fate, this thesis envisaged to tackle this problem through the development of a sustainable process that aimed at the extraction of OZ from rice straw as well as focusing on a biomass treatment to produce products rich in saccharides and polysaccharides that can be fermented and transformed into bioethanol.

The extraction experiments allowed to infer that no OZ was successfully extracted from the rice straw with the hydrophobic DESs and hexane, hence leading to the discovery that the use of rice straw as a source of this compound is not feasible. Nonetheless, this extraction procedure with these novel solvents managed to extract a multitude of other chemicals that have yet to be identified, showing that rice straw has indeed the potential to be a source of added value compounds and as such, valorized.

Additionally, the fractionation experiments, where the high solubility of rice straw in the CC:U DES alongside the selectivity for glucose and xylose of the CC:AA DES were observed, conducted to the finding that the tested DESs are candidates for treating biomass with the goal of creating fermentable products to obtain bioethanol while once again reinforcing the idea that rice straw can be valorized and its employment useful to face the global energetic crisis. Moreover, the optimization of the fractionation step warranted the use of the CC:AA DES in a 4 hr biomass incubation for achieving the most promising results thus ensuring that the end goal of this project that focused on sustainability was respected. As for the study on the effect of the water fraction in these hydrophilic DESs, it determined that a 10 wt% of water in the used DES

aqueous solutions leads to better results over lower concentrations, although a further investigation on the use of higher concentrations of water must yet be performed. The use of severe treatment conditions also justifies an optimization of the temperature at which the process is conducted and it should be mentioned as well that further compositional analysis of the products obtained in these experiments must still be carried out.

Thus, for a brighter future to be secured, many changes in today's society must happen and

harnessing the total potential of lignocellulosic biomasses is a key factor to turn the tide on the environmental degradation that is witnessed on a global scale. The development of rice straw valorization processes is only a small part of this field of science as the need for new, clean and efficient biomass conversion technologies still persists but it can be said that, besides this presented investigation, the fact that researches are being conducted to harness this biomass's full potential[30][31] seems to point at a hopeful prospect for humanity's fate.

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