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Recovery and reuse of ionic liquid cholinium glycinate in the treatment of brewery spent grain

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ABSTRACT

Recently, the ability of the biocompatible ionic liquid cholinium glycinate (N_{1112OH}Gly) for waste vegetable delignification like brewery spent grain (BSG) has been proved. However, the technical viability should march hand in hand with economic competitiveness. Therefore, the first step must address the reuse of the ionic liquid to mitigate the high cost associated with the synthesis of this kind of neoteric solvents. Different antisolvent-based strategies have been considered, in the present research work, to produce a delignified carbohydrate rich material (CRM) and reuse the ionic liquid. It was checked that the addition of a mixture of water: acetone (1:1 v/v) was able to efficiently precipitate carbohydrates with high levels of delignification, while about 97 % of the ionic liquid is recovered at a purity near 72%. The use of this strategy allowed reusing N_{1112OH}Gly for 5 cycles and FTIR and XRD spectroscopy, scan electron microscopy (SEM) and, quantitative acid hydrolysis evidenced the suitability of the proposed process for the competitive sequential BSG delignification. Liquid chromatography sheds light on the recovery percentages and purity of the ionic liquid after each use.

Keywords: ionic liquids; cholinium glycinate; brewery spent grain; reuse, delignification

1. Introduction

The concepts of Bioeconomy and Biorefinery are currently prioritized at EU level through the "The New Waste Framework Directive" 2008/98/EC [1], where new processes focused on the reuse and recycling of food waste are encouraged. Among the food wastes, brewery spent grain (BSG), the main waste of beer industry [2], can be highlighted. This lignocellulosic material is obtained after cereal (usually barley) mashing, which represents over 30% of the initial malted grains [3]. An estimated world production of this waste of 38.6 million tons in 2014 [4] has turned it into a relevant raw material. In fact, this waste has triggered a great research interest in different areas (e.g. animal feed, food, pharmaceutics, cosmetics, or biotechnology, among others) [3] due to its interesting chemical composition. More specifically, lignin, cellulose, hemicellulose, minerals, proteins and lipids content has put this waste in the limelight for biorefinery-based research works [5].

However, lignin removal in lignocellulosic materials must be unavoidably tackled, as its presence hinders the enzymatic accessibility to the structure of carbohydrates. This fact urges the scientific community to propose solutions for efficiently delignify this kind of wastes [6,7]. Pretreatment is often the most costly step for biomass transformation [8], because of this, efforts in this field focus on reducing costs and improving the production of sugar monomers during hydrolysis [9]. Different alternatives have been proposed to this end, namely, steam or ammonia fiber explosion, acid or alkaline hydrolysis (using H_2SO_4 or NaOH, respectively), ferric chloride-based or solvent-based (methanol, ethanol, acetone, hexane and ethyl acetate) options and ultrasound and/or microwave-assisted processes [2,5,7]. The most common chemical treatments are based in dilute acids or alkalis and organic solvents, however these treatments concern due to their dangerousness and toxicity [8]. Despite the existence of this array of treatment alternatives, a careful analysis should be made depending on the composition of the lignocellulosic biomass. The rationale lays in the harsh conditions that may cause sugars degradation and secondary reactions leading to toxic or inhibitory compounds for both microbial and biocatalytic reactions [7,10].

Although processes such as steam explosion or supercritical CO_2 explosion can be considered green, the ionic liquids have proven to be advantageous for their versatility, being able to dissolve biomass completely or its components selectively and, in many cases, improve the formation of final products [11,12]. In this way, ionic liquids have emerged as a viable option due to they allow an *à la carte* design combining specific cations and anions to produce new compounds with the desired properties [13]. Taking into account this variety of opportunities, the first investigations that tackling the dissolution of cellulose with ionic liquids bet on the most commercial family, based on imidazolium cation [14]. Years later, the suitability of acetate anion paired with 1-ethyl-3-methylimidazolium cation for complete dissolution and delignification of wood was demonstrated by the same research group [15]. Nevertheless, some members of this family have already been registered under REACH regulation, as their toxicity, biodegradability and persistence in both the aquatic and the soil environments have made the scientific community consider their “green brand” [16], for example, IL 1-alkyl-3-methylimidazolium is not sustainable or biodegradable and has relatively high toxicity [17].

In this scenario, a recent booming interest in biocompatible, non-toxic and biodegradable cholinium amino acid ionic liquids has been detected, and their suitability for biomass delignification has been researched [17–20]. The exploitation of these biocompatible ionic liquids in fields such as biomass pretreatment and food industry remains limited [18]. Current environmental concerns and economic constrains compel us to reuse these ionic liquids prior to implement the process at greater scale. Different recovery methods should be applied for different pretreatments based on ionic liquids [21]. Therefore, an efficient ionic liquid recycling approach must be investigated.

In this work, three different antisolvent-based alternatives have been tested, including acetone, ethanol and NaOH as key compounds for carbohydrate separation from BSG previously pretreated with cholinium glycinate (N_{1112OH}Gly). Similar methods have been investigated for this purpose, however, no direct comparison has been made between such different methods in the same system. The delignification efficiency and BSG composition have been qualitatively monitored for different reuses employing valuable spectroscopy and microscopy tools like FTIR, XRD or SEM. The purity of the ionic liquid after each treatment and reuse has been checked by high performance liquid chromatography (HPLC). Additionally, quantitative acid hydrolysis was performed to follow the changes on carbohydrate rich material (CRM) to understand changes in carbohydrates composition throughout the different antisolvent-based strategies and in the successive cycles of the selected procedure.

2. Experimental approach

2.1. Materials

BSG (about 80% water content) from the artisanal production of beer, was kindly provided by Letra (Vila Verde, Braga, Portugal). BSG humidity content was reduced in a laboratory oven (Celsius 2007, Memmert, Schwabach, Germany) at 50 °C for approximately 48 h to prevent microbial contamination during storage. Finally, BSG was also washed with water at 50ºC for 4 h and 160 rpm and dried at 50ºC to avoid errors caused by carbohydrates derived from the brewing process [22].

N_{1112OH}Gly was synthetized following the procedure reported elsewhere [23], although choline hydroxide was purchased as an aqueous solution (46 wt%) from Sigma-Aldrich (Steinheim, Germany) just like glycine (99%). The synthesized ionic liquid was vacuum-dried at reduced pressure and 50 ºC for 3 days and stored in an amber glass vial with screw cap.

2.2. BSG fractionation

BSG was fractionated according to a previous work [20] with slight modifications: 0.5 g of BSG were treated in a 100 mL glass bottle with 10 g of ionic liquid (5% w/w) at 90ºC during 16 h in a sand bath with orbital stirring (120 rpm) in the presence of glass pearls.

Three antisolvent-based methods for BSG fractionation were evaluated:

Treatment A (TA): it was based on the previous work of this group [20] with slight modifications. Briefly, 50 ml of an acetone/water solution (1:1 v/v) was added and stirred for 30 min at room temperature, thus leading to the CRM precipitation, which was removed by centrifugation (Ortoalresa, Consul 21, EBA 20, Hettich Zentrifugen. Germany) at 2755 x g for 30 min and filtration with a nylon membrane (pore size 0.45 µm). The CRMs were washed with deionized water until they became colorless (6 washes) in order to remove all remaining ionic liquid. Finally, they were dried for 24 h at 50°C in an oven (Celsius 2007, Memmert, Schwabach, Germany) and stored for later analysis. The acetone present in the liquid phase was evaporated at 50°C in the oven, causing the precipitation of lignin-rich material that was separated and washed as was done for the CRMs. Finally, the remaining liquid phase was combined with the washing water, and this mixture was submitted to a rotary evaporation (40 mbar, 50°C).

Treatment B (TB): After pretreatment with ionic liquid, 50 mL of deionized water was added and stirred for 30 min at room temperature, thus precipitating the CRMs that were separated and washed as described in the previous treatment. The liquid fraction was concentrated by removing water and a subsequent addition of 150 mL of 96% ethanol (v /v) allowed the precipitation of the hemicellulose-rich material [24]. Ethanol was then removed in a rotary evaporator in order to trigger lignin rich material precipitation that was separated and washed as described in the previous treatment. Finally, the liquid phase was mixed with the washing water and the rotatory evaporator was again employed to concentrate it, as described previously in TA.

Treatment C (TC). This alternative is based on the protocol reported elsewhere [21] with some modifications. 50 mL of NaOH (0.1 M) was added to the ionic liquid-pretreated BSG and stirred for 30 min to precipitate the CRMs, which were submitted to the same process described in treatments A and B. pH of the liquid fraction was adjusted to 2 with HCl to foster lignin-rich material precipitation, as previously described. Again, the liquid fraction was mixed with the washing water and neutralized with NaOH prior to be submitted to rotatory evaporation (where NaCl and other residues precipitate). Finally, methanol was added to the ionic liquid mixture to ease NaCl removal by filtration, prior to eliminate methanol by rotatory evaporation.

All experiments were performed in triplicate.

2.3 Analytical methods

2.3.1 BSG and CRM characterization

Moisture content of BSG and CRM was determined by drying in an oven at 105 ºC up to a constant weight. The composition of BSG and CRMs was analyzed by quantitative acid hydrolysis following the previously described procedure [20].

2.3.2 Statistical analysis

The BSG and CRMs (lignin, glucan, xylan and arabinan) composition determined by quantitative acid hydrolysis were subjected to analysis of variance (ANOVA) with Statgraphics Centurion XVI.I software using Tukey's test at a significance level of $P < 0.05$ to determine statistically significant differences.

2.3.3 ATR-FTIR analysis.

Infrared spectroscopy equipment (Thermo Nicolet 6700 FTIR Spectrometer, Thermo Fisher Scientific Inc., Madison, WI, USA) coupled with an attenuated total reflection (ATR) accessory equipped with a diamond crystal (Smart Orbit Diamond ATR, Thermo Fisher, USA) was used to characterize BSG and CRM fractions at room temperature. Dry samples were analyzed in the range 4000 to 400 cm^{-1} at 4 cm^{-1} resolution and 20 scans using a deuterated triglycine sulfate (DTGS) KBr detector.

Lateral order index (*LOI*) [25] was calculated by means of the absorbance (*A*) to evaluate changes in cellulose crystallinity, following the expression:

$$LOI=\frac{A_{1437\text{cm}^{-1}}}{A_{898\text{cm}^{-1}}} \tag{1}$$

2.3.4 X-ray diffraction analysis.

The crystallinity of BSG caused by treatment with the ionic liquid was followed by X-ray spectroscopy (Siemens D500) employing diffraction angles spanning from $2\theta = 2 – 45°$, with a step size of 0.02° and a step time of 0.5 s [26]. The crystalline index (*CrI*) was calculated as follows [27]:

$$CrI=\frac{I_{\text{cry}} - I_{\text{am}}}{I_{\text{cry}}} \text{ x } 100 \tag{2}$$

where I_{cry} is the intensity of the crystalline region at $2\theta = 22.35$ and I_{am} is the intensity in the amorphous region at $2\theta = 16.17$.

2.3.5 Field Emission Scanning Electron Microscopy (FE-SEM).

The dry samples were mounted onto aluminum stubs and coated with gold in Sputter Coater (Sputtering Emitech K550X, Quorum Technologies, Kent, UK) for 3 min. Finally, a FE-SEM system (Model JSM-6700 F, Jeol, Japan) was employed to observe and photograph morphological changes of BSG after each treatment.

2.3.6 Ionic liquid purity.

An HPLC equipped with a conductivity detector (Dionex ICS-3000) and an ion exchange column (Dionex IonPac CS12, 250 x 4 mm) working in isocratic mode was employed for N_{1112OH}Gly quantification. A macroporous (100 Å) divinyl benzene/ethyl vinyl benzene polymer was used as stationary phase and 2 mM HNO_3 as mobile phase at a flow rate of 1 mL/min at 30ºC. The limit of detection (LOD) and limit of quantification (LOQ) was 1 ppm and 2.5 ppm, respectively.

3. Results and discussion

3.1 Antisolvent selection for ionic liquid recovery

The demonstrated suitable delignification [18] capacity of the selected cholinium aminoacid ionic liquid stresses the necessity of addressing the recovery after the BSG pretreatment. To do that, different antisolvent-based strategies have been proposed, including acetone, ethanol and NaOH (0.1 M) as key compounds, as detailed in the experimental section. Then, the delignification and composition of the obtained CRM fractions have been monitored, together with the amount of recovered ionic liquid.

The first step for BSG delignification in order to obtain CRM requires its characterization, as the bagasse composition is dependent on numerous factors such as the barley variety, harvesting place and climate conditions, malting and maceration operation, among others. Usually, this raw material displays cellulose (glucan), xylan, arabinan and lignin composition intervals of (17-26)%, (12-20)%, (6-9)% and (8-28)%, respectively [4]. In the present case, the characterization of BSG sample showed values within these ranges (Table 1), being xylan and arabinan slightly higher.

The application of the different antisolvent-based strategies for ionic liquid recovery led to statistically significant differences in glucan and xylan content, as can be checked in Table 1. In general, TA, TB and TC led to similar improvements in xylan content (up to about 30%) compared to raw BSG (22%). On the contrary, the application of TA involved ameliorations in glucan content, significantly higher than the values recorded with TB and TC.

The reason for the increase in these polysaccharides content is the drastic reduction of lignin caused by the N_{1112OH}Gly pretreatment. In this regard, the statistical analysis of the values displayed in Table 1 evidences significant differences between Klason and soluble lignin of BSG and CRMs. Hence, TA, TB and TC led to delignification percentages about 71, 60 and 56 %, respectively. It is clear that TA is the most efficient alternative for this aim, as the values surpasses those found in previous research works focused on the treatment of other lignocellulosic biomass like rice straw with cholinium aminoacid ionic liquids [21,28].

The rationale for the high delignification potential may be the existence of hydrogen bonding sites since these type of bonds have been seen as the main forces ruling the lignin dissolution process [29]. In this sense, the interesting results may be due to the fact that glycinate anion has three hydrogen bonding sites (two from carboxylate and one from amino groups) while the acetone has an additional one, which surpasses the hydrogen bonding capacity provided by ethanol and NaOH used in TB and TC, respectively.

The analysis of the recovery percentages in terms of CRM, glucan, xylan, arabinan and N_{1112OH}Gly could also shed more light on the suitability of each antisolvent-based treatment, so these data were measured and the values obtained have been compiled in

Table 2. The data confirm the best performance attained after applying TA, as both glucan and ionic liquid recovery are higher with this treatment (60.74 and 96.68% respectively). Regarding the ionic liquid, its recovery is crucial for allowing its subsequent reutilization and reaching a true competitive process, so both TA and TB can be considered suitable alternatives. An et al [21] reported a recovery of 75% of the IL N_{1112OH}Arg used for the pretreatment of rice straw using a regeneration method based on NaOH. Analogously, a recovery of another cholinium-based ionic liquid over 90% was reported by Nimomiya et al [30] when using an acetone-based treatment. This pattern was also recorded for the treatment of wheat straw with imidazolium-based ionic liquids [31].

3.2 Ionic liquid reuse

Given the interesting results provided by TA, this method was chosen for further research on the viability of using the recycled ionic liquid in five sequential cycles. The ionic liquid purity and recovery have been monitored by HPLC throughout the cycles and these data are included in Table 3. Although the ionic liquid purity decreased from 72% in the first cycle to 33% in the subsequent cycles (from the 3rd cycle on), it is remarkable that the proposed treatment allowed recovering N_{1112OH}Gly at levels higher than 80%, which confirms the potential of this strategy for BSG treatment. A possible explanation behind the purity reduction may be the occurrence of secondary reactions between the ionic liquid and the solvent, accumulation of compounds derived from the biomass degradation like organic acids, or depolymerized lignin and water. Despite this, the treatment efficiency in terms of glucan and ionic liquid recovery, and delignification seem to not to be altered. A similar behavior was checked by Lee et al. [32], who demonstrated the lignin solubilization capacity of $C_2C_1imCH_3OOO$ when treating wood flour.

In the same line, the CRM composition and delignification data were also monitored after each cycle and the data obtained are included in Table 3. It is interesting to highlight that the CRM recovery is quite constant (about 25%), while the delignification is slightly decreased after the first cycle (lowering from 71% to about 60%). The values are higher than those reported previously by Ravindran et al [2] for other physicochemical treatments like ammonia fiber explosion (7.0%), steam explosion (5.2%), dilute acid hydrolysis (14.1%), organosolvent-based alternatives (42.3%), ferric chloride treatment (13.7%) and alkaline microwave-assisted methods (61.1%). Therefore, the advantage of the present strategy is clear not only in terms of process efficiency but also in environmental sustainability. Similarly, the values attained in the present research work outperform those reported previously for other cholinium-based ionic liquids with rice straw (50%) and mulberry stem (65%), when using choline acetate and choline aminoacids (N_{1112OH} Ala and N_{1112OH}Gly) , respectively [6,30].

Regarding the CRM composition, glucan recovery keeps constant throughout the whole cycles (50%), while arabinan and xylan recovery ranges between 24 and 32% and between 31 and 40%, respectively. In brief, the high glucan content in CRM and delignification yield evidence the interest of the proposed treatment while maintaining the ionic liquid effectiveness.

3.2.1 Monitoring BSG structural alterations through SEM and FTIR

After having demonstrated the suitability of the reused strategy in terms of CRM composition, delignification and ionic liquid recovery, SEM and FTIR were used to further characterize the BSG after each cycle.

SEM images at 1000 magnifications presented in Fig. 1 reveal clear changes in BSG cell walls after the five cycles. Initially, the raw BSG (Fig. 1a) shows a compact structure made up by fibers and non-fibrous components (hemicellulose and lignin) [22]. However, the treatment with N_{1112OH}Gly entails an extensive lignin removal, which allows microfiber visualization and pore formation, which favors enzymatic hydrolysis, as reported previously in literature [33,34]. The comparison between cycles leads to the conclusion that the morphological changes are very similar no matter the number of reuses of the ionic liquid, thus confirming the abovementioned quantitative results.

To avoid misinterpretations, Fourier-transform infrared (FTIR) was employed as a valuable tool often used to characterize the chemical structure and chemical changes of lignocellulosic materials [2,35]. Therefore, FTIR spectra between 1800 and 400 cm^{-1} of raw BSG and those obtained after the first and the fifth cycle (as the most representative) are shown in Fig. 2.

The analysis of the data reveals some differences in the intensity of some peaks, especially after the first cycle. Thus, it is possible to identify the characteristic cellulose and hemicellulose peaks at 1437 cm^{-1} (CH_2 scissoring motion in cellulose) [36], 1370 cm^{-1} (C-H bending vibration) [30,35], 1151 cm^{-1} (C-O-C antisymmetric bridge stretching vibration) [20], 1074 cm^{-1} (C-O stretching of secondary alcohol in cellulose, hemicellulose and lignin) [37], and 1024 cm^{-1} (C-O or C-C in cellulose and hemicellulose) [20,38]. Furthermore, new peaks also appear at 1731 cm^{-1} (C=O stretching vibration in acetyl groups on hemicelluloses) [20], and 1318 cm^{-1} (CH_2 wagging crystalline cellulose) [39]. It can be also highlighted an increased intensity of the peak at 990 cm^{-1} (C-O ring stretching of cellulose) [40] and 898 cm^{-1} (C-H bending vibration in cellulose and hemicelluloses characteristic of β-glycosidic bonds in carbohydrates) [24,30,33,41].

On the other hand, the analysis of lignin bands by FTIR spectra in the region 4000 to 400 cm^{-1} (Fig. 3) shows representative peaks at 2920 cm^{-1} (C-H stretching vibrations of methyl and methylene groups), 2852 cm^{-1} (C-H stretch O-CH_3 group) [20,42] and 1454 cm^{-1} (asymmetric bending in CH_3 lignin) [30,33,41], although its intensity is reduced. Several peaks of low intensity appear between 1574 and 1503 cm^{-1} (C=C stretching of the aromatic ring in syringyl and guaiacyl units) [35,43] and at 1462 cm^{-1} (C-H bending vibration) [43,44]. The decrease in intensity regarding the same peaks in BSG allows confirming the lignin content reduction caused by N_{1112OH}Gly treatment. It should be noted that the peak at 1737 cm^{-1} (C=O stretching ester-linked acetyl, feruloyl and *p*-cumaroyl groups between hemicellulose and lignin) [20,45] almost vanishes after the first reuse, which may indicate the rupture of this bond. This also happens with the peak at 1240 cm^{-1} (syringyl ring and C-O stretching vibration in lignin, xylan and ester groups) [41]. In summary, the decreased peaks intensity between 1460 and 1600 cm^{-1} is associated to an effective lignin removal, and only residual lignin amounts may be present as inferred from the low intensity peaks that remain in this region.

These results are consistent with those reported by Costa Lopes et al. [31] who concluded a decrease in lignin bands and the disappearance of the peak at 1737 cm^{-1} when wheat straw biomass was treated with $C_2C_1imCH_3COO$. Analogously, Ravindran et al. [2] and Outeiriño et al. [33] reported similar changes when applying several physicochemical treatments or N_{1112OH}Gly, respectively, for an effective BSG delignification.

Finally, it is evident the presence of a wide band at about 3330 cm^{-1} associated with the presence of O-H bonds, which together with the peak at 1650 cm^{-1}, make it clear the presence of retained water. Besides, the comparison between raw BSG and that obtained after the cycles reveals a decrease of this signal, thus indicating the reduction of the amount of water in the CRM. Normally, the amount of water that can be retained by a certain material is closely related to crystallinity, since more amorphous materials usually houses more water molecules, which lead to greater intensity in these bands [46]. Following this assumption, an increase in the crystallinity of the samples may be occurring, so X-ray diffraction (XRD) was thus employed to confirm this hypothesis.

3.2.2 Monitoring BSG crystallinity modifications through XRD and FTIR

Cellulose, hemicellulose and lignin content allows classifying lignocellulosic biomass in crystalline or amorphous, as only the first include crystalline structures [2,36]. In this sense, XRD analysis was employed to measure the crystallinity of the material as a whole, while FTIR data helps us to unravel the changes in the relative crystallinity of the cellulose [36].

Therefore, the crystalline index (*CrI*) was calculated from XRD data using the equation described in the material and methods section. The data presented in Table 4 evidence high *CrI* for the CRM obtained after treatment with the reused ionic liquid (between 60.58% to 80.05%) and in the diffractograms presented in Fig.4, the BSG shows a predominantly amorphous structure, this is due to the presence that amorphous components such as lignin and hemicellulose, on the other hand, in all CRMs, since lignin has been extensively removed, it can be noted the crystalline structure of this materials, because of the increase in cellulose content, which confirms the effectiveness of the treatment [2,47].

On the other hand, *LOI* was calculated from FTIR data in order to deeper study the cellulose structure, by dividing the absorbance at 1437 cm^{-1} (associated with crystalline cellulose) over the absorbance at 898 cm^{-1} (related to amorphous cellulose) [36,48], and the data after each BSG treatment are also compiled in Table 4. It becomes patent the *LOI* reduction from 2.67 of the raw BSG to about 1 after the ionic liquid reuse in five cycles, which may indicate a decrease in crystallinity caused by a disordering of cellulose structure, which would ultimately boost the enzymatic hydrolysis [49].

All in all, N_{1112OH}-based pretreatment of BSG allows removing amorphous fractions such as lignin, while increasing the cellulose content. In turn, the crystallinity of cellulose is reduced, which may favor further treatments such as enzymatic hydrolysis [36].

4. Conclusions

Three methods have been studied for BSG delignification and recovery of carbohydrates and N_{1112OH}Gly. The method based on the use of a mixture acetone / water (1: 1 v / v) as an anti-solvent led to the greatest delignification, glucan and ionic liquid recovery at greater purity. Additionally, the feasibility of reusing the ionic liquid-based treatment for five cycles was demonstrated, obtaining a CRM fraction with low lignin content (always lower than 10%). At the same time, the ionic liquid recovery and purity after each cycle reached values greater than 80% and 30%, respectively.

A deeper analysis of the BSG biomass using SEM images and FTIR spectroscopy confirmed the changes in the N_{1112OH}Gly-treated BSG regarding the raw one. On the other hand, the crystallinity indices by FTIR and XRD turned out to reveal an increased cellulose content and a reduced crystallinity, thus verifying the viability of the proposed strategy for BSG conditioning prior to enzymatic hydrolysis.

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References

[1] Directive 2008/98/EC on waste (Waste Framework Directive) - Environment - European Commission, (n.d.). https://ec.europa.eu/environment/waste/framework/ (accessed January 20, 2020).

[2] R. Ravindran, S. Jaiswal, N. Abu-Ghannam, A.K. Jaiswal, A comparative analysis of pretreatment strategies on the properties and hydrolysis of brewers' spent grain, Bioresour. Technol. 248 (2018) 272–279. doi:10.1016/J.BIORTECH.2017.06.039.

[3] A.C. Fărcaş, S.A. Socaci, F. V. Dulf, M. Tofană, E. Mudura, Z. Diaconeasa, Volatile profile, fatty acids composition and total phenolics content of brewers' spent grain by-product with potential use in the development of new functional foods, J. Cereal Sci. 64 (2015) 34–42. doi:10.1016/j.jcs.2015.04.003.

[4] A. Paz, S. da Silva Sabo, M. Vallejo, E. Marguet, R. Pinheiro de Souza Oliveira, J.M. Domínguez, Using brewer's spent grain to formulate culture media for the production of bacteriocins using Patagonian strains, LWT. 96 (2018) 166–174. doi:10.1016/j.lwt.2018.05.027.

[5] S.I. Mussatto, Brewer's spent grain: A valuable feedstock for industrial applications, J. Sci. Food Agric. 94 (2014) 1264–1275. doi:10.1002/jsfa.6486.

[6] W. Pakdeedachakiat, N. Phruksaphithak, A. Boontawan, Pretreatment of mulberry stem by cholinium-based amino acid ionic liquids for succinic acid fermentation and its application in poly(butylene) succinate production, Bioresour. Technol. 291 (2019) 121873. doi:10.1016/j.biortech.2019.121873.

[7] R. Ravindran, A. Jaiswal, Microbial Enzyme Production Using Lignocellulosic Food Industry Wastes as Feedstock: A Review, Bioengineering. 3 (2016) 30. doi:10.3390/bioengineering3040030.

[8] W.C. Tu, J.P. Hallett, Recent advances in the pretreatment of lignocellulosic biomass, Curr. Opin. Green Sustain. Chem. 20 (2019) 11–17. doi:10.1016/j.cogsc.2019.07.004.

[9] A. Abraham, A.K. Mathew, H. Park, O. Choi, R. Sindhu, B. Parameswaran, A. Pandey, J.H. Park, B.I. Sang, Pretreatment strategies for enhanced biogas production from lignocellulosic biomass, Bioresour. Technol. 301 (2020) 122725. doi:10.1016/j.biortech.2019.122725.

[10] L.J. Jönsson, C. Martín, Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects, Bioresour. Technol. 199 (2016) 103–112. doi:10.1016/j.biortech.2015.10.009.

[11] M. Mohammadi, M. Shafiei, K. Karimi, A. Abdolmaleki, J.P. Mikkola, C. Larsson, Improvement of ethanol production from birch and spruce pretreated with 1-H-3-methylmorpholinium chloride, Electron. J. Biotechnol. 41 (2019) 95–99. doi:10.1016/j.ejbt.2019.07.004.

[12] Z. Usmani, M. Sharma, P. Gupta, Y. Karpichev, N. Gathergood, R. Bhat, V.K. Gupta, Ionic liquid based pretreatment of lignocellulosic biomass for enhanced bioconversion, Bioresour. Technol. 304 (2020) 123003. doi:10.1016/j.biortech.2020.123003.

[13] M. FREEMANTLE, DESIGNER SOLVENTS, Chem. Eng. News. 76 (1998) 32–37. doi:10.1021/cen-v076n013.p032.

[14] R.P. Swatloski, S.K. Spear, J.D. Holbrey, R.D. Rogers, Dissolution of Cellose with Ionic Liquids, J. Am. Chem. Soc. 124 (2002) 4974–4975. doi:10.1021/ja025790m.

[15] N. Sun, M. Rahman, Y. Qin, M.L. Maxim, H. Rodríguez, R.D. Rogers, Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate, Green Chem. 11 (2009) 646. doi:10.1039/b822702k.

[16] Regulation (EC) No 1907/2006 of the European Parliament and of The Council of 18 December 2006). https://eur-lex.europa.eu/legal-content/ES/TXT/PDF/?uri=CELEX:32006R1907&from=EN (accessed July 21, 2020).

[17] S. Kirchhecker, D. Esposito, Amino acid based ionic liquids: A green and sustainable perspective, Curr. Opin. Green Sustain. Chem. 2 (2016) 28–33. doi:10.1016/j.cogsc.2016.09.001.

[18] J.M. Gomes, S.S. Silva, R.L. Reis, Biocompatible ionic liquids: fundamental behaviours and applications, Chem. Soc. Rev. 48 (2019) 4317–4335. doi:10.1039/C9CS00016J.

[19] X.-D. Hou, J. Xu, N. Li, M.-H. Zong, Effect of anion structures on cholinium

ionic liquids pretreatment of rice straw and the subsequent enzymatic hydrolysis, Biotechnol. Bioeng. 112 (2015) 65–73. doi:10.1002/bit.25335.

[20] D. Outeiriño, I. Costa-Trigo, A. Paz, F.J. Deive, A. Rodríguez, J.M. Domínguez, Biorefining brewery spent grain polysaccharides through biotuning of ionic liquids, Carbohydr. Polym. 203 (2019). doi:10.1016/j.carbpol.2018.09.042.

[21] Y.X. An, M.H. Zong, H. Wu, N. Li, Pretreatment of lignocellulosic biomass with renewable cholinium ionic liquids: Biomass fractionation, enzymatic digestion and ionic liquid reuse, Bioresour. Technol. 192 (2015) 165–171. doi:10.1016/j.biortech.2015.05.064.

[22] A. Paz, D. Outeiriño, N. Pérez Guerra, J.M. Domínguez, Enzymatic hydrolysis of brewer's spent grain to obtain fermentable sugars, Bioresour. Technol. 275 (2019) 402–409. doi:10.1016/j.biortech.2018.12.082.

[23] F.J. Deive, D. Ruivo, J. V. Rodrigues, C.M. Gomes, M.Á. Sanromán, L.P.N. Rebelo, J.M.S.S. Esperança, A. Rodríguez, On the hunt for truly biocompatible ionic liquids for lipase-catalyzed reactions, RSC Adv. 5 (2015) 3386–3389. doi:10.1039/C4RA15021J.

[24] A.M. da Costa Lopes, K.G. João, D.F. Rubik, E. Bogel-Łukasik, L.C. Duarte, J. Andreaus, R. Bogel-Łukasik, Pre-treatment of lignocellulosic biomass using ionic liquids: Wheat straw fractionation, Bioresour. Technol. 142 (2013) 198–208. doi:10.1016/J.BIORTECH.2013.05.032.

[25] R.T. O'Connor, E.F. DuPré, D. Mitcham, Applications of Infrared Absorption Spectroscopy to Investigations of Cotton and Modified Cottons, Text. Res. J. 28 (1958) 382–392. doi:10.1177/004051755802800503.

[26] A.K. Kumar, B.S. Parikh, M. Pravakar, Natural deep eutectic solvent mediated pretreatment of rice straw: bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue, Environ. Sci. Pollut. Res. 23 (2016) 9265–9275. doi:10.1007/s11356-015-4780-4.

[27] L. Segal, J.J. Creely, A.E. Martin, C.M. Conrad, An Empirical Method for Estimating the Degree of Crystallinity of Native Cellulose Using the X-Ray Diffractometer, Text. Res. J. 29 (1959) 786–794. doi:10.1177/004051755902901003.

[28] X.-D. Hou, T.J. Smith, N. Li, M.-H. Zong, Novel renewable ionic liquids as highly effective solvents for pretreatment of rice straw biomass by selective removal of lignin, Biotechnol. Bioeng. 109 (2012) 2484–2493. doi:10.1002/bit.24522.

[29] A. Casas, J. Palomar, M.V. Alonso, M. Oliet, S. Omar, F. Rodriguez, Comparison of lignin and cellulose solubilities in ionic liquids by COSMO-RS analysis and experimental validation, Ind. Crops Prod. 37 (2012) 155–163. doi:10.1016/j.indcrop.2011.11.032.

[30] K. Ninomiya, K. Inoue, Y. Aomori, A. Ohnishi, C. Ogino, N. Shimizu, K. Takahashi, Characterization of fractionated biomass component and recovered ionic liquid during repeated process of cholinium ionic liquid-assisted pretreatment and fractionation, Chem. Eng. J. 259 (2015) 323–329. doi:10.1016/j.cej.2014.07.122.

[31] A.M. da Costa Lopes, K.G. João, A.R.C. Morais, E. Bogel-Łukasik, R. Bogel-Łukasik, Ionic liquids as a tool for lignocellulosic biomass fractionation, Sustain. Chem. Process. 1 (2013) 3. doi:10.1186/2043-7129-1-3.

[32] S.H. Lee, T. V. Doherty, R.J. Linhardt, J.S. Dordick, Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis, Biotechnol. Bioeng. 102 (2009) 1368–1376. doi:10.1002/bit.22179.

[33] D. Outeiriño, I. Costa-Trigo, R. Pinheiro de Souza Oliveira, N. Pérez Guerra, J.M. Domínguez, A novel approach to the biorefinery of brewery spent grain, Process Biochem. (2019). doi:10.1016/j.procbio.2019.06.007.

[34] J. Zhao, H. Chen, Correlation of porous structure, mass transfer and enzymatic hydrolysis of steam exploded corn stover, Chem. Eng. Sci. 104 (2013) 1036–1044. doi:10.1016/j.ces.2013.10.022.

[35] C.-M. Popescu, M.-C. Popescu, G. Singurel, V. Cornella, A. Dimitris S., W. Stefan, Spectral characterization of Eucalyptus wood, Appl. Spectrosc. 61 (2007) 1168–1177. https://pdfs.semanticscholar.org/a67f/939dc68f904c7eff91273a3236fc7820f2c0.pdf (accessed September 24, 2019).

[36] A.R.C. Morais, J.V. Pinto, D. Nunes, L.B. Roseiro, M.C. Oliveira, E. Fortunato, R. Bogel-Łukasik, Imidazole: Prospect Solvent for Lignocellulosic Biomass Fractionation and Delignification, ACS Sustain. Chem. Eng. 4 (2016) 1643–1652. doi:10.1021/acssuschemeng.5b01600.

[37] J. de O. Lopes, R.A. Garcia, N.D. de Souza, Infrared spectroscopy of the surface of thermally-modified teak juvenile wood, Maderas Cienc. y Tecnol. 20 (2018) 737–746. doi:10.4067/S0718-221X2018005041901.

[38] C. Zhou, W. Jiang, B.K. Via, O. Fasina, G. Han, Prediction of mixed hardwood lignin and carbohydrate content using ATR-FTIR and FT-NIR, Carbohydr. Polym. 121 (2015) 336–341. doi:10.1016/j.carbpol.2014.11.062.

[39] A. Carballo-Meilan, A. Goodman, M. Baron, J. Gonzalez-Rodriguez, An specific case in the classification of 4 woods by FTIR and chemometrics: 5 Discrimination of Fagales from 6 Malpighiales 7, 2014.

[40] R.S. Dassanayake, P.T. Wansapura, P. Tran, A. Hamood, N. Abidi, Cotton cellulose-CdTe quantum dots composite films with inhibition of biofilm-forming S. aureus, Fibers. 7 (2019). doi:10.3390/fib7060057.

[41] N. Labbé, T. Rials, S. Kelley, Z.M. Cheng, J.Y. Kim, Y. Li, FT-IR imaging and pyrolysis-molecular beam mass spectrometry: New tools to investigate wood tissues, Wood Sci. Technol. 39 (2005) 61–76. doi:10.1007/s00226-004-0274-0.

[42] N.M. Stark, D.J. Yelle, U.P. Agarwal, Techniques for Characterizing Lignin, in: Lignin Polym. Compos., Elsevier, 2016: pp. 49–66. doi:10.1016/B978-0-323-35565-0.00004-7.

[43] A. Casas, M.V. Alonso, M. Oliet, E. Rojo, F. Rodríguez, FTIR analysis of lignin regenerated from Pinus radiata and Eucalyptus globulus woods dissolved in imidazolium-based ionic liquids, J. Chem. Technol. Biotechnol. 87 (2012) 472–480. doi:10.1002/jctb.2724.

[44] J.C. De Andrade Neto, A. De Souza Cabral, L.R.D. De Oliveira, R.B. Torres, A.D.A. Morandim-Giannetti, Synthesis and characterization of new low-cost ILs based on butylammonium cation and application to lignocellulose hydrolysis, Carbohydr. Polym. 143 (2016) 279–287. doi:10.1016/j.carbpol.2016.01.069.

[45] H. Ren, M.H. Zong, H. Wu, N. Li, Efficient Pretreatment of Wheat Straw Using Novel Renewable Cholinium Ionic Liquids to Improve Enzymatic Saccharification, Ind. Eng. Chem. Res. 55 (2016) 1788–1795. doi:10.1021/acs.iecr.5b03729.

[46] H.J. Contreras, H.A. Trujillo, G. Arias, J.L. Pérez, E. Delgado, ESPECTROSCOPIA ATR-FTIR DE CELULOSA: ASPECTO INSTRUMENTAL Y TRATAMIENTO MATEMÁTICO DE ESPECTROS ATR-FTIR spectroscopy of cellulose: instrumental aspect and mathematical treatment of spectra, (2010) 14. www.e-gnosis.udg.mx/vol8/art9.

[47] P. Binod, K. Satyanagalakshmi, R. Sindhu, K.U. Janu, R.K. Sukumaran, A. Pandey, Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse, Renew. Energy. 37 (2012) 109–116. doi:10.1016/j.renene.2011.06.007.
[48] M. Poletto, H.L. Ornaghi Júnior, A.J. Zattera, Native cellulose: Structure, characterization and thermal properties, Materials (Basel). 7 (2014) 6105–6119. doi:10.3390/ma7096105.
[49] Z. Ling, S. Chen, X. Zhang, K. Takabe, F. Xu, Unraveling variations of crystalline cellulose induced by ionic liquid and their effects on enzymatic hydrolysis, Sci. Rep. 7 (2017). doi:10.1038/s41598-017-09885-9.

Table 1

Chemical composition of BSG and CRMs after the application of different antisolvent-based treatments for ionic liquid recovery

	BSG	TA	TB	TC
Humidity (%)	6.38 ± 0.05	7.52 ± 0.23	4.90 ± 0.18	5.64 ± 0.23
Klason Lignin (%)	17.94 ± 0.30*	6.31 ± 0.06^{A}	8.53 ± 0.29^{B}	9.38 ± 0.41^{B}
Soluble Lignin (%)	7.32 ± 0.03*	0.98 ± 0.00^{A}	1.48 ± 0.10^{B}	1.65 ± 0.02^{B}
Total Lignin (%)	25.26	7.29	10.01	11.03
Glucan (%)	22.74 ± 0.80*	57.44 ± 1.19^{A}	49.48 ± 0.78^{B}	50.25 ± 0.37^{B}
Xylan (%)	21.97 ± 0.07*	29.31 ± 0.93	30.66 ± 0.83	30.56 ± 0.67
Arabinan (%)	9.71 ± 0.20	9.45 ± 0.81^{A}	11.45 ± 0.65^{B}	10.08 ± 0.09

* Statistically significant differences between BSG and CRMs.
Different letters show significant differences between CRMs.
BSG: brewery spent grain; TA, TB and TC are the CRMs of treatments A, B and C respectively

Table 2

Percentages of recovery after treatment

	TA	TB	TC
CRM Recovered	24.05 ± 0.75	23.85 ± 0.13	24.01 ± 0.35
Glucan Recovered	60.74 ± 1.89	51.91 ± 0.28	53.06 ± 0.77
Xylan Recovered	32.08 ± 1.00	33.29 ± 0.18	33.40 ± 0.48
Arabinan Recovered	23.40 ± 0.73	28.23 ± 0.15	24.93 ± 0.36
N_{1112OH}Gly Recovered	96.68 ± 0.45	96.71 ± 1.59	85.92 ± 2.06

Table 3
Chemical composition of the CRM after five treatments

	TA	TA-T1	TA-T2	TA-T3	TA-T4	TA-T5
IL recovery (%)		96.68 ± 0.45	85.52 ± 1.17	81.82 ± 2.16	88.43 ± 1.81	87.87 ± 2.93
IL purity (%)		72.01	50.17	33.22	33.56	33.17
CRM Recovery (%)	24.05 ± 0.75	25.79 ± 0.78	26.39 ± 1.52	25.60 ± 1.27	25.17 ± 0.23	23.67 ± 0.65
Klason Lignin (%)	6.31 ± 0.06	7.56 ± 0.08	7.63 ± 0.52	8.60 ± 0.32	8.96 ± 0.22	8.45 ± 0.25
Soluble Lignin (%)	0.97 ± 0.00	1.19 ± 0.00	1.91 ± 0.00	1.31 ± 0.06	1.43 ± 0.06	1.12 ± 0.02
Total Lignin (%)	7.28	8.75	9.54	9.91	10.39	9.57
Delignification (%)	71.18	65.36	62.23	60.76	58.86	62.11
Glucan (%)	57.44 ± 1.19	47.45 ± 0.66	43.10 ± 0.95	46.21 ± 0.41	44.27 ± 0.06	46.90 ± 0.53
Glucan Recovery (%)	60.74 ± 1.89	53.81 ±1.65	50.03 ± 2.88	52.03 ± 2.59	49.01 ± 0.44	48.83 ± 1.34
Xylan (%)	29.32 ± 0.93	32.01 ± 0.38	33.71 ± 0.13	30.01 ± 0.39	32.50 ± 0.22	28.65 ± 0.46
Xylan Recovery (%)	32.08 ± 1.00	37.58 ± 1.14	40.50 ± 2.33	34.97 ± 1.74	37.24 ± 0.34	30.87 ± 0.85
Arabinan (%)	9.45 ± 0.81	10.64 ± 0.14	11.97 ± 0.22	10.78 ± 0.21	10.88 ± 0.19	9.84 ± 0.56
Arabinan Recovery (%)	23.40 ± 0.73	28.26 ± 0.86	32.54 ± 1.87	28.43 ± 1.41	28.21 ± 0.25	23.99 ± 0.66

T1 to T5 represent the cycles

Table 4
Crystallinity indices values

Sample	***CrI* (%)**	***LOI***
BSG	-	2.67
TA	79.38	1.36
TA-T1	67.20	1.07
TA-T2	80.05	1.30
TA-T3	67.01	1.81
TA-T4	75.92	1.22
TA-T5	60.58	1.13

T1 to T5 represent the cycles

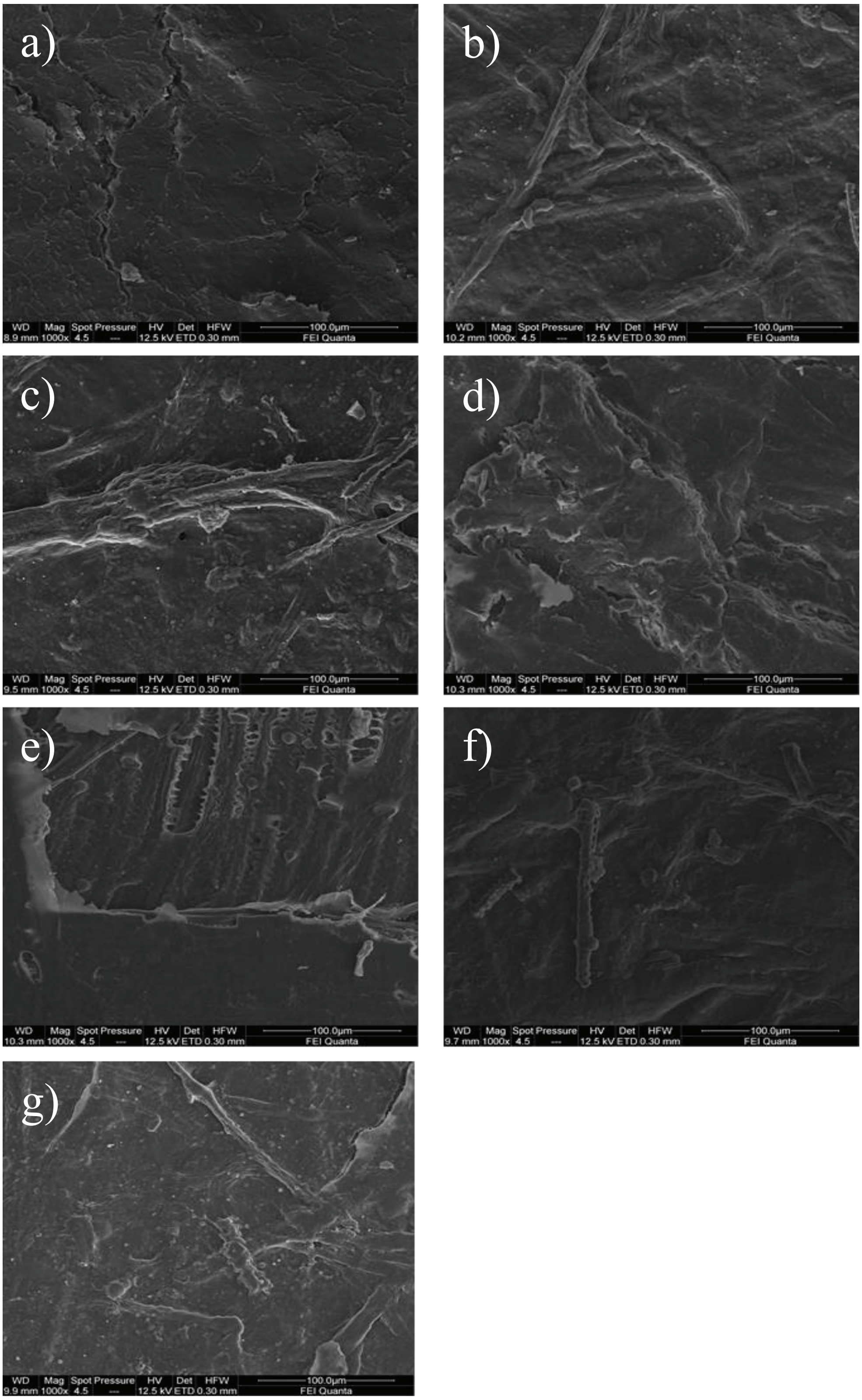

Fig. 1. SEM photographs showing the morphology of lignocellulosic surface of a) BSG, b) TA, c) TA-T1, d) TA-T2, e) TA-T3, f) TA-T4, g) TA-T5.

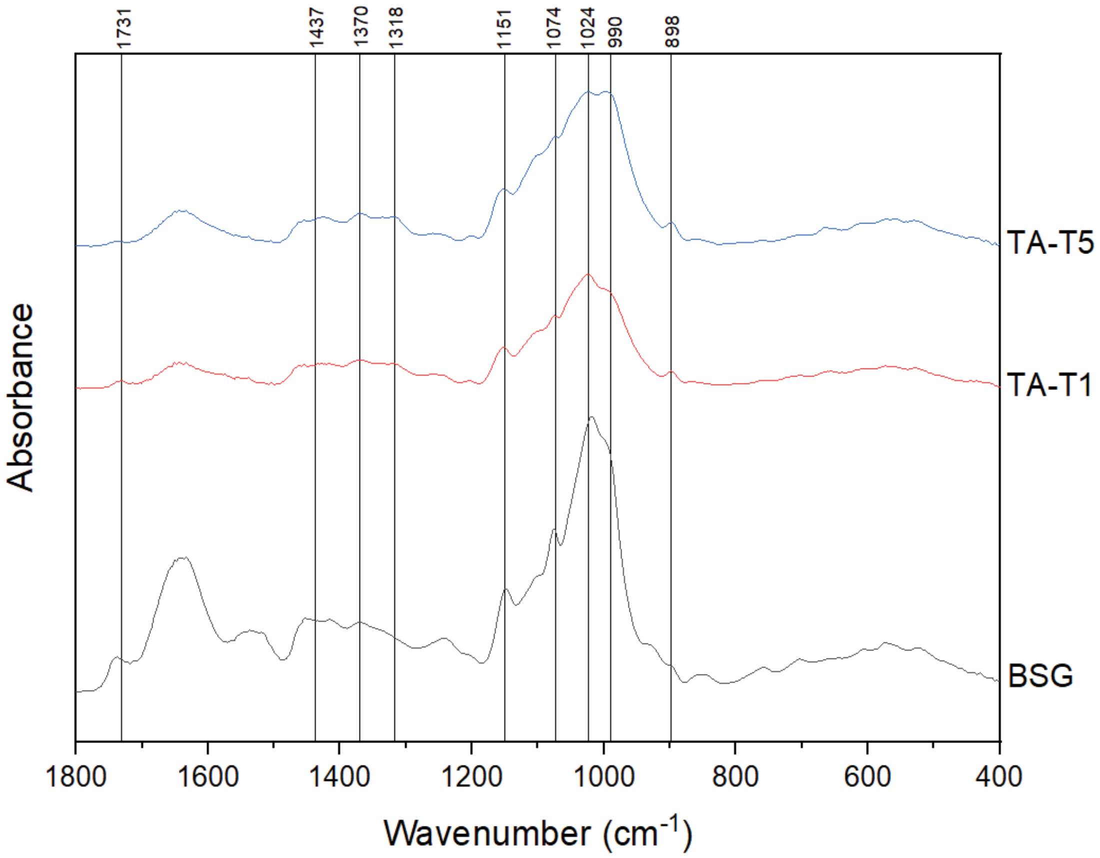

Fig. 2. FTIR spectra of BSG, TA-T1 and TA-T5 in the region 1800-400 cm^{-1} with carbohydrates characteristic peaks.

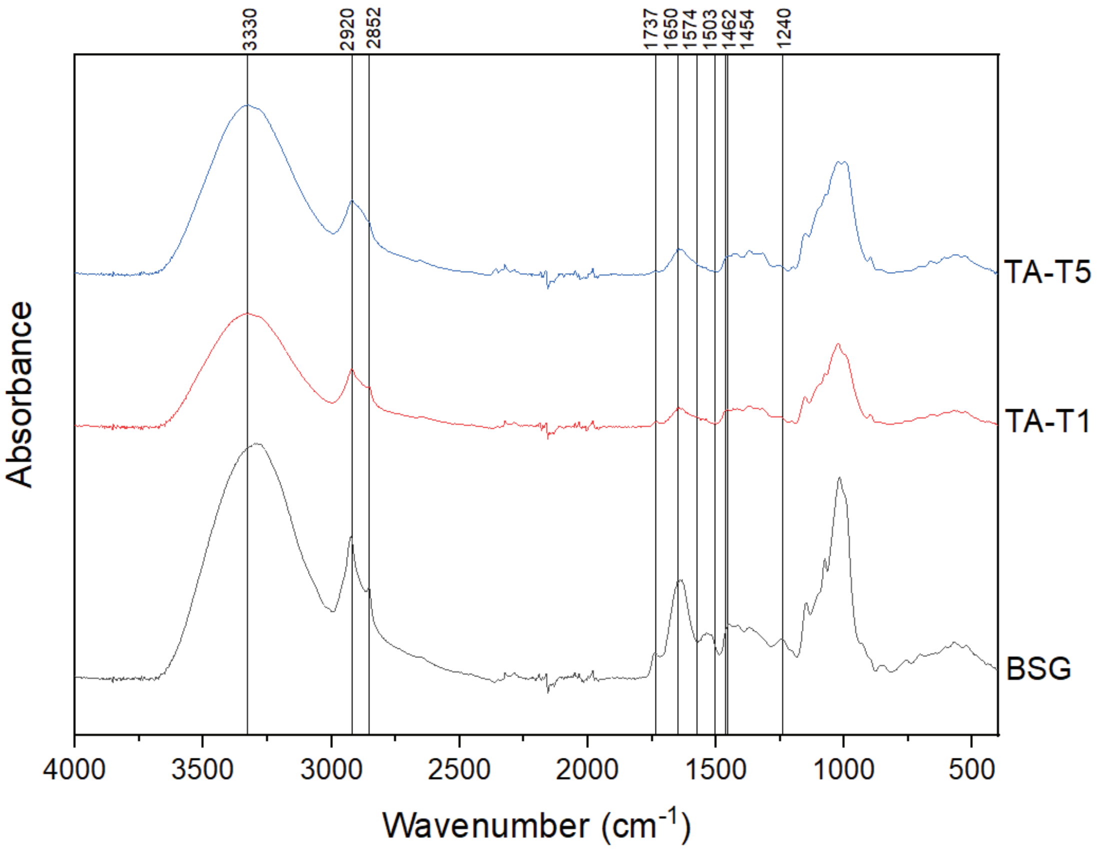

Fig. 3. FTIR spectra of BSG, TA-T1 and TA-T5 in the region 4000-400 cm^{-1} with lignin characteristic peaks.

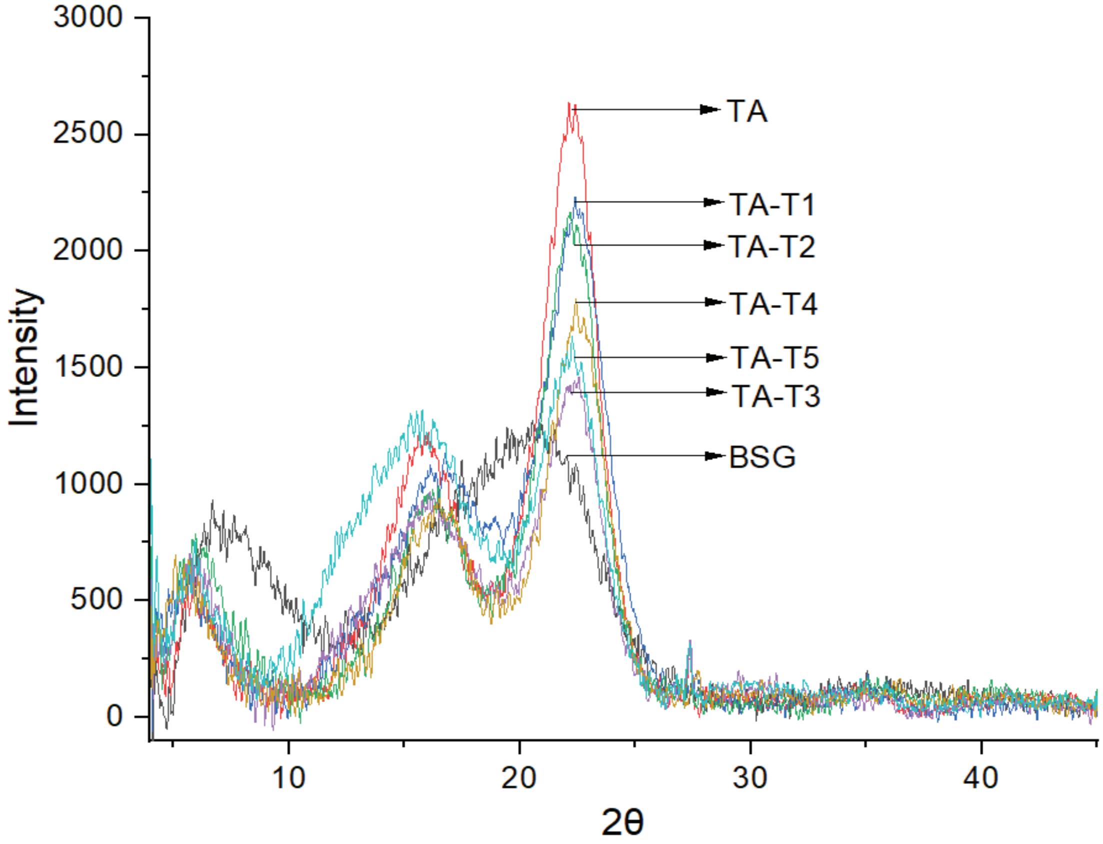

Fig. 4. XRD diffractogram of BSG and TA to TA-T5.

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Conflict of interest Authors declare that they have no conflict of interest.

Author statement

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