Aromatics extraction from pyrolytic sugars using ionic liquid to enhance sugar fermentability

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HIGHLIGHTS

- Aromatics were effectively extracted from pyrolytic sugars by $P_{666,14}[N(CN)_2]$.
- Sugars were not extracted at all.
- Regenerated IL exhibited similar aromatics extraction efficiency.
- Pure 40 g L^{-1} pyrolytic-glucose stream could directly be fermented.

GRAPHICAL ABSTRACT

Ionic liquids showed promising separation properties for pyrolytic sugar streams with high selectivity of aromatics over sugars and produced sugar was hydrolyzed and then fermented to ethanol.
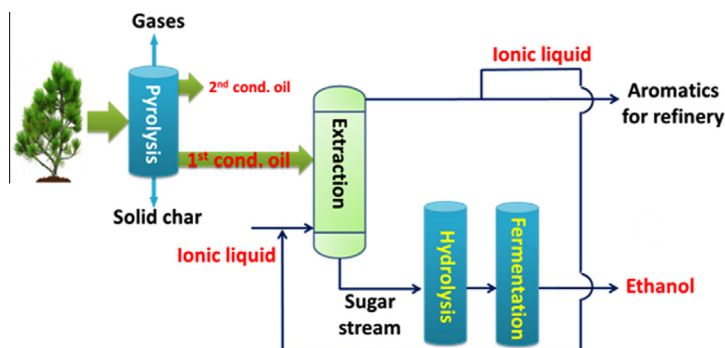
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ABSTRACT

Fermentative bioethanol production from pyrolytic sugars was improved via aromatics removal by liquid–liquid extraction. As solvents, the ionic liquid (IL) trihexyltetradecylphosphonium dicyanamide ($P_{666,14}[N(CN)_2]$) and ethyl acetate (EA) were compared. Two pyrolytic sugar solutions were created from acid-leached and untreated pinewood, with levoglucosan contents (most abundant sugar) of 29.0% and 8.3% (w/w), respectively. In a single stage extraction, 70% of the aromatics were effectively removed by $P_{666,14}[N(CN)_2]$ and 50% by EA, while no levoglucosan was extracted. The IL was regenerated by vacuum evaporation (100 mbar) at 220 °C, followed by extraction of aromatics from fresh pyrolytic sugar solutions. Regenerated IL extracted aromatics with similar extraction efficiency as the fresh IL, and the purified sugar fraction from pretreated pinewood was hydrolyzed to glucose and fermented to ethanol, yielding 0.46 g ethanol/(g glucose), close to the theoretical maximum yield.

1. Introduction

Lignocellulosic biomass, as a renewable feedstock, has become an alternative source for the production of chemicals and fuels (Bridgwater, 2004). Fast pyrolysis (heating biomass in absence of

oxygen to temperatures above 400 °C) is a promising technology to thermally depolymerize the polysaccharides and the lignin into a liquid product, named pyrolysis oil or bio-oil (Mohan et al., 2006).

Pyrolysis oil is a complex mixture containing hundreds of oxygenated organic compounds, mainly sugars and aromatics, and in addition water is present in significant amount. The exact composition of pyrolysis oil depends on the feedstock, process conditions and the recovery method. By applying fractional condensation, sugars and aromatics can be concentrated in one fraction, whereas the more volatile compounds such as glycolaldehyde and acetic acid are condensed in a second fraction (Westerhof et al., 2011). Pyrolytic sugars, especially levoglucosan, can be produced with high yields up tp 17% by pyrolysis of pretreated biomass (by acid leaching or infusing) (Carpenter et al., 2014; Kuzhiyil et al., 2012; Oudenhoven et al., 2013).

Pyrolytic sugars have potential to be transformed into valuable chemicals or fermented into bioethanol or lipids (Girisuta et al., 2006; Hu and Li, 2011; Lian et al., 2010b; van Putten et al., 2013). However, aromatics in the oil are inhibitory to most micro-organisms in fermentation process (Jarboe et al., 2011; Lian et al., 2010b). Hence, removal of these contaminants is necessary prior to fermentation. Next to sugars, the aromatics also can be valorized towards transport fuels or phenol formaldehyde resins (Kelley et al., 1997; Nguyen and Honnery, 2008).

One strategy to separate sugars and aromatics is by adding water to pyrolysis oil to obtain two fractions, a sugar-rich aqueous fraction and an aromatic-rich oil fraction (Bennett et al., 2009). However, after this split, the fermentability of the aqueous sugar fraction is still limited, due to the presence of a certain amount of inhibitors (Luque et al., 2014). These inhibitors need to be removed to enhance the fermentability of the aqueous pyrolytic sugar fractions.

Different strategies have been developed to purify (or detoxify) the pyrolytic sugar streams, including overliming (Chi et al., 2013; Jarboe et al., 2011), activated carbon adsorption (Li et al., 2013), air stripping (Wang et al., 2012) and solvent extraction (Lian et al., 2010a; Luque et al., 2014). Several techniques for inhibitor removal from pyrolytic sugar fractions were compared by Wang et al. who found that air stripping and microbial digestion were not effective for inhibitor removal, while solvent extraction and activated carbon adsorption worked successfully (Wang et al., 2012). Although adsorption can be a strong technique with possibly high selectivity, applicability of the technique can have limitations due to the high cost associated either with the adsorbents and/or with the high costs of regenerating them (Lin and Juang, 2009).

Solvent extraction is an alternative method for inhibitor removal, and solvent capacities are typically higher than sorbent capacities, so that at high loading, extraction may be beneficial over adsorption. Most used solvents are organic solvents such as ethyl acetate (EA), butyl acetate and methyl isobutyl ketone (Fele Žilnik and Jazbinšek, 2012; Lian et al., 2010b; Won and Prausnitz, 1975), but for large scale applications the energy efficiency of the solvent recovery and the associated risks of utilization of large quantities of volatile organic compounds (VOCs) might be limiting.

Ionic liquids (ILs), considered as environmentally friendly solvents, have been applied in various fields of e.g. synthesis, separation and energy production (Meindersma et al., 2010; Rogers and Seddon, 2003; Welton, 1999; Zhang et al., 2014). Several researchers have successfully utilized ILs to remove aromatics from alkanes (Arce et al., 2007; Domanska et al., 2007; Jiao et al., 2015; Jongmans et al., 2011). Recently, this group has used ILs to effectively remove aromatics from artificial sugar solutions (Li et al., 2016). The IL trihexyltetradecylphosphonium dicyanamide ($P_{666,14}[N(CN)_2]$) exhibited higher selectivity for guaiacol than EA. Furthermore, the conceptual process design study showed that the IL-based process was five times less energy intensive than

the EA-based process (Li et al., 2016). Based on this study with a model mixture, it was decided to further study the potential of using $P_{666,14}[N(CN)_2]$ to extract aromatics from real aqueous pyrolytic sugars for production of fermentable sugar streams.

This study investigates the technical feasibility of liquid–liquid extraction with $P_{666,14}[N(CN)_2]$ to detoxify sugar-rich aqueous fractions of real pyrolysis oils. After detoxification also the fermentability of the purified sugar streams is investigated. The applied fermentation approach uses glucose obtained from hydrolysis of the levoglucosan in the sugar stream, however, in future, there may be options to work also directly with levoglucosan (Chi et al., 2013). The two studied pyrolytic sugar solutions were prepared from first condenser fractions of pyrolysis oils from acid leached pinewood and untreated pinewood, respectively. Fig. 1 shows the conceptual process scheme, including the pretreatment, pyrolysis, fractionation, solvent recovery and fermentation.

2. Materials and methods

2.1. Materials

Trihexyltetradecylphosphonium dicyanamide ($P_{666,14}[N(CN)_2]$) was supplied by Iolitec with a purity >95 wt% and used directly without purification. Levoglucosan (>98%) and cellobiosan (>98%) standards were obtained from Carbosynth. Guaiacol (99%), glucose (99%), acetic acid (99%), phenol (99%), furfural (99%), cresol (99%), vanillin (99%), tetrahydrofuran (THF, ⩾99.9%) and ethyl acetate (99.8%) were acquired from Sigma–Aldrich.

2.2. Experimental methods

2.2.1. Preparation of pyrolysis oils

Two pyrolysis oils were studied in this work, generated from acid leached pinewood and untreated pinewood (lignocel 9, J Rettenmaier and Söhne) using a pyrolysis process with fractional condensation. Detailed information on the pretreatment, pyrolysis and fractional condensation methods can be found in a previous publication (Oudenhoven et al., 2013). For pyrolysis including pretreatment, the pinewood was leached with an artificial light pyrolysis fraction (rich in acetic acid) at 90 °C for 2 h, followed by rinsing and drying. The pretreated pinewood was pyrolyzed in a fluidized-bed reactor at 530 °C. Pyrolysis of untreated pinewood was done at 500 °C. For both oils the first condenser was operated at 80 °C (outgoing gas), and the second condenser was operated at −5 °C (outgoing gas). For the current study, only the first condenser oils are of interest, and to identify the two oils from the different processes, hereafter pyrolysis oil 1 (PO1) refers to the first condenser oil from acid leached pinewood and pyrolysis oil 2 (PO2) is the first condenser oil from untreated pinewood.

2.2.2. Production of aqueous sugar fractions by water addition

Both PO1 and PO2 were washed with water at a weight ratio of 1:2 in an ultrasonic bath for 12 h at 20 °C to obtain, an oil fraction and an aqueous fraction. Phase separation was enhanced by centrifugation for 5 min at 9000 rpm, after which the aqueous fraction was used in liquid–liquid extraction studies. Aqueous sugar fractions from PO1 and PO2 are further referred to as sugar fraction 1 (SF1) and sugar fraction 2 (SF2), respectively.

2.2.3. Liquid–liquid extraction procedure

Liquid–liquid extraction experiments were carried out in 50 mL centrifuge tubes, in which 15 g $P_{666,14}[N(CN)_2]$ or EA was added to 30 g SF1 or SF2. The mixtures were intensely mixed for 20 min at room temperature and then centrifuged for 10 min at 9000 rpm
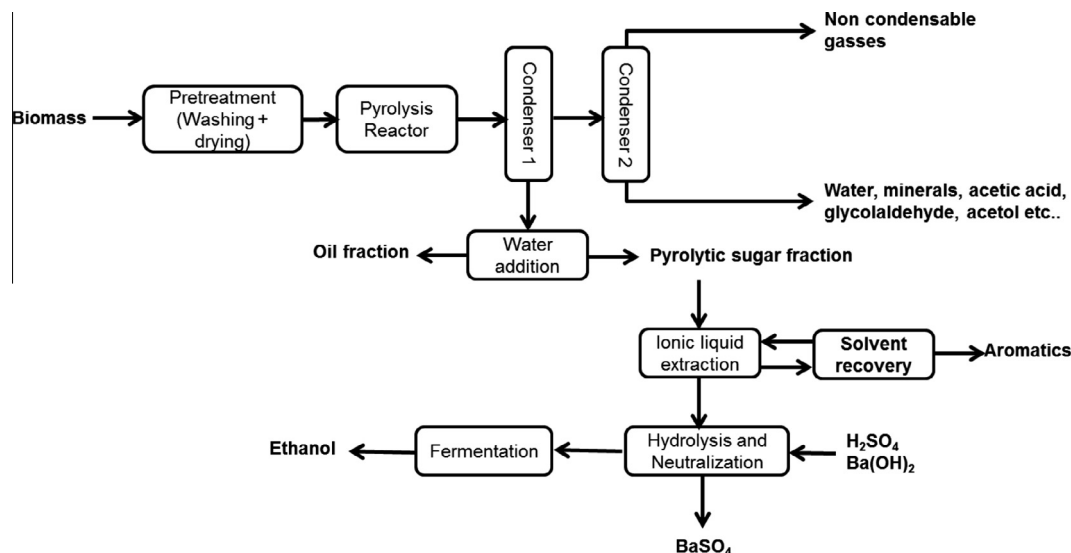
Fig. 1. Conceptual process scheme for pyrolysis process including downstream fractionation and fermentation operations.

to achieve phase separation. The raffinates were then analyzed by High Pressure Liquid Chromatography (HPLC) and Gel Permeation Chromatography (GPC). The thus obtained raffinate results are identified with the following codes:

RSF1-EA: raffinate after extraction of SF1 using EA.
RSF1-IL: raffinate after extraction of SF1 using IL.
RSF2-EA: raffinate after extraction of SF2 using EA.
RSF2-IL1: raffinate after extraction of SF2 using fresh IL.

2.2.4. IL recovery

IL recovery was investigated using the IL after aromatics extraction from SF2. In these experiments, 15 g extract were stirred and heated at 220 °C and 100 mbar in a 100 mL flask for 1 h while bubbling the flask with N_2 to avoid condensation in the neck of the flask. $P_{666,14}[N(CN)_2]$ was thus recovered three times, followed by reuse as solvent in liquid–liquid extractions to extract aromatics from aliquots of fresh SF2. The obtained raffinates from the multiple extractions of SF2 are given the following identification codes:

RSF2-IL2: raffinate after extraction of SF2 using IL for the second time after recovery.
RSF2-IL3: raffinate after extraction of SF2 using IL for the third time after recovery.
RSF2-IL4: raffinate after extraction of SF2 using IL for the fourth time after recovery.

2.2.5. Acid hydrolysis and fermentation

Acid hydrolysis of levoglucosan to glucose was performed by adding 5 mL aliquots of the raffinate RSF1-IL to microwave vials (VWR, Canada), followed by the addition of H_2SO_4 (final concentration of 0.5 mol/L) and hydrolysis in an autoclave for 20 min at 121 °C. The resulting hydrolysates were neutralized by adding solid $Ba(OH)_2$ to reach a final pH of 6.5. Following neutralization, samples were transferred to 15 mL centrifuge tubes and solids were precipitated via centrifugation at 3500 rpm for 20 min. Supernatant was recovered and filtered with a microfilter (0.20 μm) and transferred to a new sterile 15 mL centrifuge tube.

Hydrolysates were diluted with demineralized water to a final glucose concentration of 40 g/L. 10 g/L solid yeast extract (BD, USA) and 20 g/L peptone (BD, USA) were added to prepare YPG (yeast, peptone and glucose) media. Once prepared, the media were filtered and sterilized. Then this YPG media was blended in

different fractions with model YPG which contains the same concentrations of yeast, peptone and glucose with the former YPG media, but was prepared with laboratory grade glucose (Alfa Aesar, USA).

Microtiter plates were filled with 180 μL of each blend, and inoculated with 20 μL of active seed culture of *Saccharomyces ceevisiae* DSM 1334 (Braunschweig, Germany). The seed culture was in mid-exponential growth phase with an average DCW of 1.3 ± 0.07 g/L. After inoculation, plates were sealed with a sterile PCR film (VWR, Canada). The film was punctured using a sterile 16 g needle (BD, USA). Incubation was performed at 30 °C and 80 rpm using a Micro Titer plate reader (Tecan, Austria). Growth was monitored by measuring optical density at 600 nm every 10 min for 24 h. Anaerobic conditions (Nitrogen environment) were guaranteed using a gas control unit connected to the microplate reader. Ethanol and glucose concentrations were monitored using HPLC at the end of the incubation.

2.3. Analytical methods

The SF1, SF2 and raffinates were analyzed with HPLC, for which an Agilent 1200 system equipped with Hi-Plex-H column was operated at 60 °C. Two detectors were applied, a Refractive Index Detector (RID, relative standard deviation from 5 measurements: 1.2%) and a Variable Wavelength Detector (UV, operated at 285 nm with relative standard deviation from five measurements: 0.2%). 5 mM sulfuric acid was used as mobile phase at a flow rate of 0.6 mL/min. Ethanol and glucose concentrations at the end of the incubation were also monitored using HPLC, using mobile phase 0.5 mM H_2SO_4 at 0.7 mL/min, keeping the RID detector at 55 °C and the Hi-Plex-H column at 60 °C.

The SF1, SF2 and all raffinates were also studied with Gel Permeation Chromatography (GPC) using a system from Agilent Technologies 1200. Samples were dissolved in THF and filtered over a microfilter (0.20 μm). For the measurement, 20 μL of sample was injected to a system composed by three columns placed in series (7.5 × 300 mm, particle size 3 μm), and UV detectors operated at 254 nm were applied. A highly crosslinked polystyrene–divinylbenzene copolymer gel was used as column packing (Varian, PLgelMIXED-bed E). The chromatography was performed during 40 min at 40 °C and with 1 mL/min of THF as eluent. The calibra-

tion to correlate elution time and molecular weight was performed using polystyrene of 162–29,510 g/mol as standard.

Levoglucosan in the pyrolysis oils, sugar fractions and raffinates was quantified using an Agilent 7890A gas chromatograph with a Varian CP9154 column and coupled with an Agilent 5975C mass spectrometer (GC/MS). The samples were diluted ten times with acetone and filtered with a microfilter (0.20 μm). 1 μL of sample was injected into the injection port set at 250 °C, with a split ratio of 20:1. The column was operated in a constant flow mode using 2 mL/min of helium as a carrier gas. Identification of levoglucosan was based on retention time and matching the mass spectrum recorded with those in the spectral library (NIST/EPA/NIH Mass Spectral Library, Version 2.0f, FairCom Corporation).

Water contents of sugar fractions and raffinates were determined with relative standard deviations from triplicate measurements of <1.5% by Karl Fisher titration (titrant: hydranal composite 5, Metrohm 787 KFTitrino). A solution of methanol and dichloromethane (3:1, volumetric ratio) was used as solvent.

3. Results and discussion

3.1. Pyrolytic sugar fractions production

The pyrolytic sugar fractions SF1 and SF2 were created by adding two mass equivalents of water to the first condenser fractions of the pyrolysis oils PO1 and PO2. In this procedure, biphasic systems are created to wash out the sugars, while the remaining viscous oil fraction consists primarily of lignin-derived aromatic oligomers and some leached water (Bennett et al., 2009). The amount of washed out matter was strongly dependent on the applied pyrolysis method, i.e. 69.3 (±1.5) wt% of PO1 and 49.3 (±0.3) wt% of PO2 ended up in the aqueous fractions SF1 and SF2, respectively. This marked difference is due to the reduced catalytic activity in pyrolysis of pretreated wood, leading to a higher sugar fraction, as was also observed by other researchers (Dalluge et al., 2014; Oudenhoven et al., 2015). The amount of levoglucosan in the pyrolysis oils and the sugar fractions could be determined with GC/MS analysis (see Table 1), and it was found that the levoglucosan concentration increased significantly from 8.3 (±0.5) wt% in PO2 to 29.0 (±0.5) wt% in PO1 where acid leached pinewood was used. The levoglucosan in SF1 was thus much more concentrated than in SF2. After a single wash, 96.1 (±0.7) wt% of the levoglucosan was transferred from PO1 to SF1, and 96.1 (±0.2) wt% from PO2 to SF2. Thus, with a single wash the majority of the sugars is washed from the pyrolysis oils.

Due to the complex compositions of pyrolysis oils, it is difficult to identify and quantify all the individual components, so the lumped sugars and aromatics are analyzed in this work. The total amount of sugars are roughly estimated from HPLC-RID chromatograms. As reference, a known mixture was also analyzed with HPLC, containing glucose, cellobiosan, levoglucosan, acetic acid, phenol, guaiacol, furfural, cresol and vanillin. It was found that the various sugars have very similar response factors, i.e. levoglucosan (1.38 * 10⁸), glucose (1.43 * 10⁸), cellobiosan (1.42 * 10⁸) and cellobiose (1.49 * 10⁸). Fig. S1(a) and (b) shows that in the known mixture most sugars have retention times less than 15 min,

whereas for aromatics the retention times exhibit longer than 20 min. Assuming that the compounds with retention times between 7.0 and 14.5 min are all sugars, and because of the similar response factors, the total amounts of sugars in SF1 (21.8 wt%) and in SF2 (13.0 wt%) were obtained by estimation based on the response factor of levoglucosan. According to the water content in these fractions shown in Table 1, the rest compounds, mostly phenolics and aromatics, are approximately 4.5 wt% and 7.5 wt% in SF1 and SF2 respectively.

Molecular weight distributions (MWD) of the aromatics present in SF1 and SF2 were recorded using GPC-UV at 254 nm. Since most aromatics can be detected at 254 nm whereas carbohydrates and most organic acids are transparent, it is assumed that the measured UV signals correspond to the UV absorption of aromatics. This analysis thus provides further insight in the composition of the sugar fractions. Fig. S2(a) and (b) shows that the fraction of large molecules (molecular weight > 1000 g/mol) is negligible in both sugar fractions. Furthermore, the peaks around 108 g/mol are assigned to be mono-aromatics and the ones around 182 g/mol to aromatic dimers. By comparison of the GPC-UV results from both sugar fractions, it can be concluded that the SF2 from untreated pinewood contains a higher amount of aromatics than SF1 from pretreated pinewood.

SF1 is thus clearly the preferred sugar fraction to examine the fermentability after extraction of the aromatics, whereas SF2 with its higher aromatics content is well suited to examine more closely the recyclability of the IL after extraction.

3.2. Extraction of aromatics from SF1 and SF2

In liquid–liquid extraction experiments using either $P_{666,14}[N(CN)_2]$ or EA, the extent of the extraction was measured using GPC and HPLC analyses, as described in the experimental section.

For analysis of the sugar distributions, the first 20 min retention in the HPLC-RID chromatograms is considered, whereas for the aromatics the RID-signal from 20–120 min is considered. Fig. S1 (a) and (b) represent SF1 and its raffinates after extraction, and Fig. S1(c) and (d) represent SF2 and its raffinates after extraction. The split in the results before and after 20 min was made to allow a change in the scale on the y-axis. It follows from Fig. S1(a) that the sugar signals from SF1 overlap with the signals of the raffinates, implying that the amount of sugars did not change, i.e. the sugars were not extracted. More specifically for levoglucosan, this negligible extractability was confirmed with GC/MS (see Table 2). Therefore, it was concluded that the levoglucosan and other sugars are hardly extracted from SF1 with either IL or EA. Similarly for SF2, it can be seen in Table 3 and Fig. S1(c), that sugars are not extracted by either the IL or EA. Thus, levoglucosan was collected in the raffinates to be subsequently hydrolyzed and fermented.

The aromatics extraction efficiency was interpreted using the chromatograms in Fig. S1(b) and (d). In these figures, the signal intensities for all raffinates are lower than those for the original sugar fractions SF1 and SF2. This shows that both the IL and EA extract aromatics. The amount of extracted aromatics was quantified by normalizing the total area of all HPLC-UV peaks for the raffinates with those of SF1 and SF2, respectively (in Fig. 2). Using the IL as solvent, for both SF1 and SF2, significant and comparable

Table 1
The compositions of sugar fractions SF1 and SF2.

	SF1	SF2
Levoglucosan (wt%)	10.0 (±0.6)	3.4 (±0.2)
Total sugars (wt%)	21.8	13.0
Water (wt%)	73.7 (±0.7)	79.5 (±0.5)
The rest compounds (wt%)	4.5	7.5

Table 2
Levoglucosan and water concentrations in SF1 and its raffinates (RSF1-EA, RSF1-IL) after extraction with IL or EA.

Fraction	Levoglucosan content (wt%)	Water content (wt%)
SF1	10.0	73.7
RSF1-EA	10.5	68.7
RSF1-IL	12.1	78.2

Table 3
Levoglucosan and water concentrations in SF2 and its raffinates after extraction with IL or EA.

Fraction	Levoglucosan content (wt%)	Water content (wt%)
SF2	3.4	78.8
RSF2-EA	3.3	75.7
RSF2-IL1	3.4	82.9
RSF2-IL2	3.6	83.7
RSF2-IL3	3.4	84.7
RSF2-IL4	3.4	84.4

reductions in peak area of 72% and 70%, respectively, were observed. When EA was applied instead, the reduction in peak area was only 48% for SF1, and 56% for SF2. That the relative reduction in aromatics using EA was more for SF2 than for SF1 could be due to the higher aromatics content in this sugar fraction. In order to get a more complete understanding of the aromatics extraction, the raffinates were also analyzed using GPC-UV.

The MWD of aromatics before and after extraction are presented in Fig. S2(a) for SF1, and in Fig. S2(b) for SF2. In these figures it can be seen that the decrease in the area of the UV spectra for all raffinates happens for both the IL and EA over the entire weight range. There is thus no visible preference of either the IL or EA with regard to molecular size of the solutes that are extracted. Furthermore, the integrated results in Fig. 2 show a good resemblance with the HPLC results, and indicate once more that $P_{666,14}[N(CN)_2]$ extracts more aromatics than EA and the behavior is similar for both sugar fractions SF1 and SF2.

These results of single stage extractions, at a solvent to feed ratio of only 0.5, show an aromatics removal of over 70% for the IL, which is a clear indication that achieving very high extraction yields should be straight forward when multistage contacting is applied.

3.3. Recycling of $P_{666,14}[N(CN)_2]$

For economically feasible processing, it is key that the IL is recyclable, which may be done by evaporating the extracted aromatics. Because SF2 contains more aromatics than SF1, IL recovery was studied for this sugar fraction. It is esteemed that if recovery works for SF2, it will also work for SF1. Extraction with $P_{666,14}[N(CN)_2]$ followed by regeneration was repeated three times, and thus four extraction cycles were studied in total.
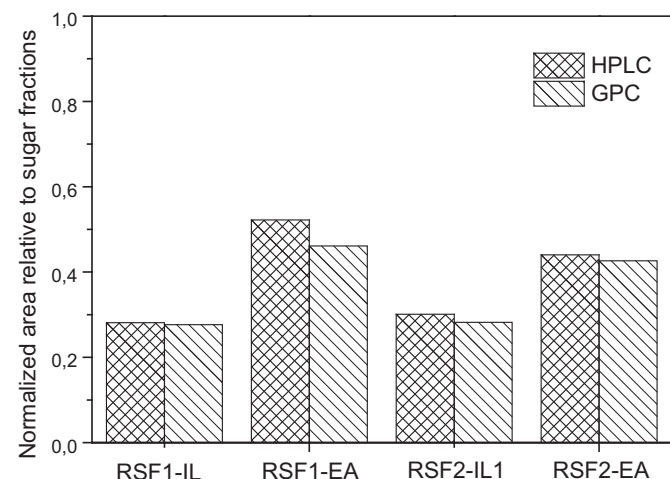
The aromatics extraction efficiency of reused IL was evaluated with the analyses of HPLC and GPC. In Fig. 3 the integrated area of HPLC-UV signals normalized to SF2 is displayed. It can be seen that for all four raffinates the integrated aromatics signal is approximately 30%, showing no deterioration of the extraction capacity. From the overlapping GPC signals of the raffinates RSF2-IL1 to RSF2-IL4 in Fig. S3 it becomes clear that the molecular weight distribution of aromatics in the raffinates is similar after all extraction cycles, i.e. the extraction performance is stable for recycled IL, confirming the HPLC-results displayed in Fig. 3. The stable performance confirms the high thermal stability of the phosphonium IL (Fraser and MacFarlane, 2009), as well as the minimal leaching of the IL to the raffinate, similar to earlier studies with a simplified feed (Li et al., 2016). Thus, vacuum evaporation of aromatic solutes originating from aqueous pyrolytic sugar solutions is an effective method for IL recovery. Not only the aromatics content was analyzed after the extractions, but also the levoglucosan content (see Table 3). The levoglucosan content remained constant in all extractions, validating the use of the recycled IL for selective removal of aromatics from sugar fractions.

3.4. Fermentation

The suitability of the pyrolytic sugar from SF1 as a fermentation substrate after detoxification by extraction with IL was investigated. The data in Fig. 2 shows substantial removal of aromatic compounds, however, the combined effect of the complex mixture, including possible negative effects of any leached solvent is difficult to predict, hence experimental determination is preferred (Wood et al., 2015). Most yeast cannot directly convert levoglucosan which was therefore hydrolyzed to glucose and subsequently fermented to ethanol.

Parallel experiments were conducted with an initial glucose concentration of $40\,g\,L^{-1}$, using mixtures of pure glucose and pyrolytic-glucose (glucose derived from RSF1-IL). The fraction of pyrolytic-glucose (Xp) was varied from 0 to 1 in order to assess the inhibitory effect of residual aromatics or other inhibitory compounds. The respective growth curves are shown in Fig. 4. It can be seen that growth rate and final biomass concentration (dry cell weight (DCW)) decreased with an increased fraction of pyrolytic-glucose as the carbon source. However, the pure pyrolytic-glucose stream (Xp = 1) could directly be fermented at initial concentrations of $40\,g\,L^{-1}$, and an ethanol yield of $Y_{ethanol/glucose}$ of $0.46\,g\,g^{-1}$ was achieved, which is close to the theoretical maximum ($0.51\,g\,g^{-1}$) and similar to values obtained with pure glucose

Fig. 2. Normalized area of peaks recorded with HPLC-UV and GPC-UV for raffinates relative to the area of the original sugar fractions SF1 and SF2.
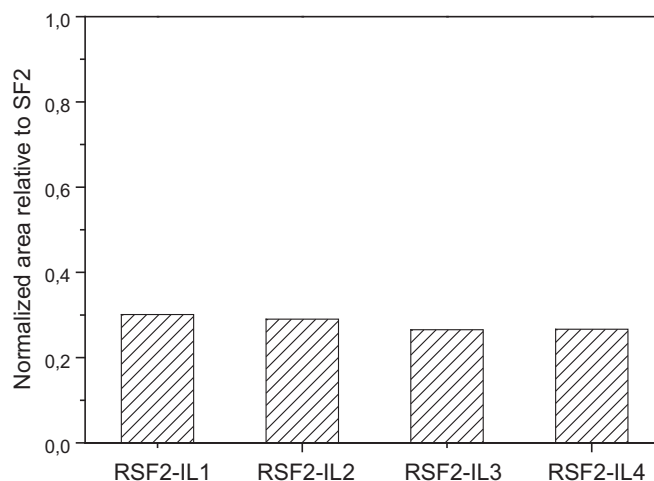

Fig. 3. Normalized area of peaks for raffinates of SF2 detected by HPLC-UV.

under the employed conditions. No significant difference in the ethanol yields was observed between pure glucose, pyrolytic glucose, or the tested blends (0 < Xp < 1). However, the growth rate was reduced, as clearly shown in Fig. 4, indicating that the presence of residual aromatics would still have a negative impact on ethanol fermentation. This limitation might be addressed through simple adaption of the strains or active strain development. The pyrolytic sugar fractions without any extraction could only be fermented up to Xp = 0.2, as reported in detail elsewhere (Luque et al., 2014), thus highlighting the importance of detoxification steps. The results therefore show that pyrolysis in combination with ionic liquid mediated upgrading can be used to produce fermentable sugars from biomass.

4. Conclusions

Solvent extraction with ionic liquids can be used effectively to separate aromatics from pyrolytic sugar rich streams. In a single extraction stage approximately 70% of aromatics can be removed by IL $P_{666,14}[N(CN)_2]$, and only 50% by EA. The IL was regenerated three times by vacuum evaporation, and the recycled IL showed similar extraction performance as fresh IL. The sugar stream can further be fermented to ethanol in a close to the theoretical maximum yield, indicating the toxic molecules were extracted effectively. Thus, solvent extraction with $P_{666,14}[N(CN)_2]$ is an effective detoxification method for obtaining fermentable sugars from pyrolysis oil.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.05.035.

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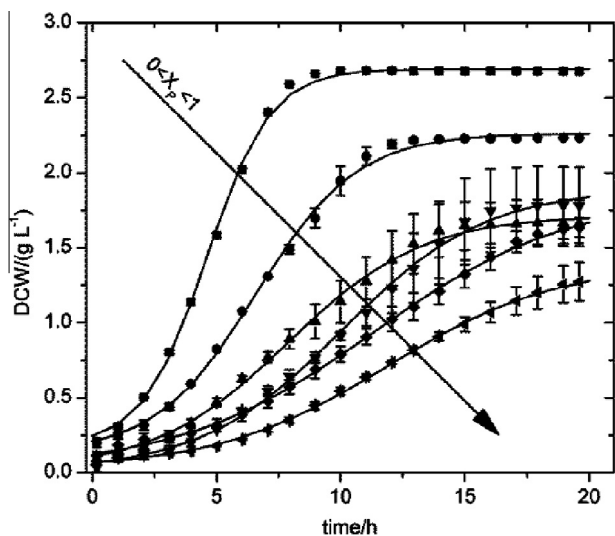**Fig. 4.** Growth curves of S. cerevisiae in 40 g/L glucose with an increasing fraction of pyrolysis derived glucose (Xp = 0-■, Xp = 0.2-●; Xp = 0.4-▲, Xp = 0.6-▼, Xp = 0.8-◆, Xp = 1-◄). The error bars represent the standard deviation of 6 replicates and the solid line a model fit based on Baranyi and Roberts as described elsewhere (Wood et al., 2015).

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