Selective Extraction of Xylose from Acidic Hydrolysate—from Fundamentals to Process

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ABSTRACT: Xylose is a promising feedstock for the fuel and chemical industry. We propose here an integrated process to recover and purify xylose from an acidic hydrolysate stream, e.g., coming from pretreated lignocellulose. This process consists of selectively extracting the xylose as the diboronate ester and back-extracting it into a clean aqueous solution. We report 85% xylose extraction efficiency using toluene/phenyl boronic acid in a single stage. We show that the extraction procedure is compatible with acidic and basic xylose feed (from pH 1 to 12), does not need a phase transfer agent (such as Aliquat), is strongly selective for xylose, and proceeds best with aromatic solvents. We demonstrate the structure of the diboronate ester by means of 2D-NMR and show evidence for the intermediate formation of a monoboronate ester. The release of xylose from the diboronate ester is explored, and an integrated process for xylose purification is proposed and its boundaries are discussed.

KEYWORDS: Sugar, Pentose, Recovery, Extraction, Boronate ester, Boronic acid

INTRODUCTION

Xylose is the second most abundant sugar in nature, after glucose. Xylose is a valuable raw material in the sweetener, aroma, and flavoring industries, and it is a promising starting material for the production of chemicals and fuels, for example, by its conversion to furfural.1–6 Xylose is present in lignocellulosic biomass mainly as glucuronoxylan in hardwoods and as glucuronorabinoxylan in grasses and softwood.6 Average xylan contents are up to 5 w% in softwoods, 15 w% in hardwoods, and 20 w% in grasses and bagasse.6 Xylose syrup can be extracted from lignocellulosic biomass by means of various pretreatment or fractionation processes.6,7 Recovering xylose from its complex syrup typically employs time and/or energy-demanding techniques, such as water evaporation and crystallization, or requires costly and complex setups like chromatographic separation.8 Other approaches, such as liquid–liquid extraction, have been reported but remain challenging because of the high solubility of sugars in aqueous medium.3,9–11

One of the most common approaches for the liquid–liquid extraction of sugars from an aqueous medium proceeds by the formation of hydrophobic boronate esters of the saccharides.3,11–17 In principle, this can provide a concentrated sugar solution in organic solvent, which can be directly processed without any post-treatment.14 Xylose liquid–liquid extraction and sugar liquid–liquid extraction, in general, are commonly performed at basic pH with an equimolar concentration of a hydrophobic boronic acid (BA), for example, phenylboronic acid (PBA), and a phase transfer agent (e.g., the lipophilic quaternary ammonium salt Aliquat 336, aka Aliquat) to form a hydrophobic ion-pair complex.3,12–14,17–19 The need for a basic pH (pH > 9) has been rationalized by considering the optimal conditions for the formation of the tetrahedral, negatively charged form of the BA, which forms stable boronate ester bonds with sugars in water.3,11–15 This negatively charged ester can be then extracted into the organic phase through the interaction with the positively charged Aliquat.13,16 Because of the pH limitation, this process cannot be readily applied to acidic hydrolysates that are commonly obtained from biomass pretreatment.4–6

Here, we aim to extract xylose from an acidic hydrolysate as a neutral boronate ester, which obviates the use of a phase transfer agent. Earlier studies have reported the neutral diester of PBA and xylose.11 Here, we show the compatibility of this ester with the acidic xylose feed that is delivered by numerous pretreatment processes. We rationalize the observed pH independence of the extraction in the absence of Aliquat by...
discussing the coupled equilibria involved in this system. We demonstrate the high extraction selectivity for xylose over other sugars present in the xylose feed, investigate the recovery of xylose from the boronate ester by back-extraction, and use these findings to propose a conceptual process design for the purification of xylose.

RESULTS AND DISCUSSION

To develop an extraction process to isolate xylose from an acidic hydrolysate, several elements need to be taken into account. Xylose needs to be extracted selectively from a mixture that contains multiple sugars, and the extraction should be achievable at acidic pH to avoid costly pH changes. The organic solvent used to extract the xylose into the organic phase should favor extraction but also allow back-extraction into water. We will show here that such a process can be designed around a hydrophobic and charge-neutral boronate diester of xylose (Figure 1). The efficiency and selectivity of the extraction will be discussed, as well as the solvent dependence and sugar selectivity. The limitations of the method to achieve a full process are explored as well.

Xylose Extraction without Phase Transfer Agent.

Earlier studies have reported the possibility to extract xylose into toluene at neutral pH by the formation of a hydrophobic, charge-neutral sugar-boronate diester. This procedure did not require the assistance of Aliquat as a phase transfer agent. We explored the possibility to stretch these conditions to the typical acidic pH encountered in hydrolysates, by using an acidic (pH = 1) aqueous xylose solution (350 mM, 5.2 w%) and an equivalent volume of toluene/PBA solution with various PBA concentrations, in the absence of Aliquat (Figure 2). After reaching equilibrium, the two phases were analyzed by $^1$H NMR spectroscopy to determine the concentration of xylose in the aqueous phase and of the boronate ester in the organic phase. When working at a PBA–xylose ratio of 2:1, approximately 85% of the xylose is extracted into the organic phase. At PBA/xylose ratios > 4, nearly 100% is extracted.

Figure 2a indicates an inflection point at a PBA/xylose ratio of 2:1. Comparison with literature data and further experimental analysis using 2D NMR, FTIR, and MS (Figure S1) confirmed the extracted species to be the (neutral) boronate diester of xylose and two units of PBA (PBA$_2$X).

The efficiency of the extraction appeared to be pH-independent between pH 1 and 12 (Figure 2b). In this pH range, the nature of the xylose boronate diester remained unchanged (Figure S2). This wide pH range in which the extraction functions supports the role of a neutral hydrophobic species in the extraction equilibrium. Under acidic conditions and in the absence of Aliquat, the extraction is promoted by the strong hydrophobic nature of the PBA units in the diester in the charge-neutral trigonal state. This implies that the affinity of the PBA units for the organic phase is the driving force for the extraction into the organic phase.

Equilibria Involved in the Extraction of Xylose.

The extraction process takes time, and transient turbidity was observed during the experiments. Both literature indications and control experiments (Figure S3) suggest that, in all cases, equilibrium between the organic and aqueous phases is reached after a maximum of 1–2 h at room temperature. In all cases, the extraction process can be divided into three distinct stages. At the early stages of the process, the biphasic system is composed of two clear solutions. When the mixing starts, the upper toluene phase becomes turbid within the first 30 min. This turbidity disappears after 1.5–2 h to leave again a clear biphasic system (Figure S4). Thereafter, the biphasic extraction system remained stable for some 500 h (Figure S3). When operated at higher temperature, the process still showed the transient development of a turbid toluene phase, though the extraction reached completion with two clear phases within a shorter time, e.g., approximately 20 min at 70 °C (Figure S5).

The analysis of the two phases at the end of the extraction (2:1 PBA/xylose, pH 1) showed free xylose in the aqueous phase (approximately 15%) and PBA$_2$X in the toluene phase (approximately 85%), with no monoester intermediate (PBA$_X$) being detected. However, analysis (both with $^1$H NMR and MS) of the turbid toluene phase, obtained by stopping the extraction at an early stage, strongly suggests the formation of a PBA$_X$ boronate monoester intermediate (Figures S6 and S7).

The presence of a putative intermediate monoester (PBA$_X$) suggests a two-step extraction process (Figure 3a): the first molecule of PBA binds xylose at the water/toluene interface and forms a monoester, which resides as an amphiphile at the interface between the two phases, causing turbidity, probably due to the formation of an emulsion. It eventually binds to a second unit of PBA to form the diester PBA$_2$X which is well soluble in toluene.

Aliquat has been shown to be ineffective at nonalkaline pH. To verify this experimentally, solutions of xylose (350 mM) at different pH values were brought into contact with an equal volume of a solution of PBA in toluene (700 mM, 8.5 w%) to which Aliquat was added as the chloride salt (700 mM, 28 w%). When the organic phase containing Aliquat was brought into contact with the aqueous phase at pH = 7, the Cl$^-$ anion migrated to the aqueous phase, as evidenced by dropwise addition of an aqueous solution of AgNO$_3$ and the visualization of the precipitation of AgCl. In the meantime, the...
pH dropped from 7 to 4. However, the PBA resided mainly in the organic phase, as shown by NMR (Figure S8). When lowering the Aliquat concentration in toluene from 700 mM to 200 mM, after reaching equilibrium, consistent xylose extraction can be detected, even though the efficiency is lowered (Figure S9).

This behavior can be rationalized assuming that the deprotonated PBA anion is exchanged with Cl\(^-\) and scavenged from the aqueous phase by Aliquat. At basic pH, the water-soluble boronate anion reacts with xylose to form a boronate monoester anion in the aqueous phase, and this is then extracted to the toluene phase with the assistance of Aliquat (Figure S10). At neutral/acidic pH (when pH < pK\(_a\)), however, no water-soluble boronate anion forms in the aqueous phase (Figure 3b), which renders the Aliquat phase transfer inoperable.\(^{13,16}\) PBA resides predominantly in the organic phase as neutral species, and the Aliquat exchanges its chloride anion with an OH\(^-\), which is bound by PBA, with concomitant acidification of the water phase. This causes the PBA to be inaccessible for extraction of the xylose (Figure 3b). In accordance, when a lower amount of Aliquat is used, only a comparable fraction of PBA is inactivated (Figure S9).

Although more work would be needed to assess all equilibria, the following rationale is proposed. At basic pH, all boronates are already quaternary and charged; also in the two options (free and sugar-bound boronate), the sugar-bound one then goes, apparently, preferentially into the organic phase (but only as the monoester; apparently a diester-bis-anion is too polar and/or gives too much internal charge repulsion in the organic solvent). The preference for binding OH\(^-\) to the boron center does not play a role in this case in predicting this preference, as the high pH sets both in this quaternary state. In neutral/acidic medium, in contrast, the coupled sugar binding/OH-coordination dictates the species populations; apparently the speciation lies less on the side of the sugar-bound complex than on the side of the PBA.

**Effect of Organic Solvent Type on the Extraction Efficiency.** We performed the extraction with different organic solvents to study the effect of the solvent type on the extraction efficiency. Xylose in water, at pH 1, was contacted with an equal volume of an organic solvent with PBA (PBA/xylose = 2:1), and the fractions of diester that partitioned into the organic phase upon a single extraction step were analyzed by \(^1\)H NMR. Aromatic, nonaromatic, and one mixture were tested, and the results are shown in Figure 4, in which the
extractions. Among the aromatic solvents, the presence of an electron-donating group (i.e., anisole) reduces it. Among the nonaromatic solvents, partial mixing and emulsification with the aqueous phase (e.g., 1-octanol) are detrimental for the extraction. Using a combination of an aromatic solvent with a nonaromatic one (1:1 toluene–γ-valerolactone (GVL); red point in Figure 4) suppresses the extraction efficiency compared to pure toluene. From this set of experiments, it can be concluded that π–π stacking plays an important role in the partitioning of PBA₂X into the organic phase. Moreover, these results confirm that the affinity of the PBA units for the organic phase, at acidic conditions, is the driving force for the sugar extraction.

Selectivity for Xylose Extraction from Pure Solutions and a Model Hydrolysate. The high structural variability of sugars strongly affects their ability to bind to boronic acids. To investigate the effect of the sugar structure on the extraction efficiency, different common sugars (i.e., glucose, fructose, arabinose, galactose, sucrose) were studied using the same extraction protocol (Figure 5). The selection of the monosaccharides in this analysis is based on their abundances in lignocellulosic hydrolysates. Specifically, these monosaccharides are found, at different relative w%, as building blocks of polysaccharides in hemicelluloses.

Xylose presents two pairs of OH groups which both occur in an equatorial position, favorable for binding PBA, as discussed above. Fructose and glucose also present two pairs of OH groups eligible for binding PBA but have an additional OH group that is left unbound. This free OH group is hydrophilic and thus reduces the affinity of these diesters for the apolar organic phase. Arabinose, galactose, and sucrose present only one pair of equatorial OH groups and can only bind one PBA molecule, leaving two, three, or six OH groups unbound, respectively, leading to decreasing extraction efficiencies. More information on these structures is available in the SI (Figure S11). Interestingly, the extraction selectivity for xylose is maintained at temperatures as high as 90 °C (Figure S12).

The extraction selectivity reported here significantly deviates from the one reported in the literature at basic pH in the presence of a phase transfer agent. Most sugars present one pair of equatorial OH groups that can bind PBA and form an anionic sugar boronate ester at high pH. The negative charge and the proximity of the bulky Aliquat further hinders the binding of a second boronate anion to an equatorial OH pair that would eventually remain. Yet, the ion pairing with Aliquat overcomes the hydrophilicity of the esters and makes them partition into the organic phase without regard to the number of free OH groups. Hence, no significant sugar selectivity was observed under such conditions.

To further demonstrate the value of the extraction selectivity at low pH, we attempted to extract xylose from a mixture of sugars that is supposed to represent a hydrolysate obtained by dilute acid pretreatment of lignocellulose. A model pretreatment hydrolysate, representative for a xylose-rich hydrolysate obtained from hardwood and/or agricultural residues at mild conditions of temperature and acidity, was prepared by mixing 52% xylose, 35% arabinose, 6% galactose, and 3% glucose, and dissolving this mixture in water at 5 w% total sugar concentration. Acidification to pH = 3 was achieved by using acetic acid, a common coproduct of pretreatment.

Upon application of the extraction protocol (see above, using acetic acid and a 2:1 PBA/sugar molar ratio), the aqueous phase appeared to retain most of the initial arabinose and galactose but no xylose anymore (Figure S13). In contrast, the toluene phase contained >95% of xylose in the form of PBA₂X. Xylose could be extracted to high percentages because arabinose and galactose did not bind it, and the extraction was effectively operated at a PBA/xylose ratio of around 4:1, which was shown to lead to >95% xylose extraction (Figure 2a). The limited amount of glucose present in the model hydrolysate and the competition with a more suitable sugar (i.e., xylose) led to no detectable extraction of glucose.

Integrated Process to Isolate Xylose. So far, the discussion has been limited to the extraction of xylose from the hydrolysate. For industrial application, however, we also
need to investigate the recovery of the xylose from the extracted boronate diester and of the extraction solvent.

An obvious approach consists of back-extracting the xylose with an acidic aqueous solution. Accordingly, an integrated extraction process would contact the xylose-containing hydrolysate with a solution of solvent and phenylboronic acid (PBA), usually in counter-current operation, to form the xylose diboronate ester and entrain it upward with the solvent phase, while the resulting xylose-lean hydrolysate is withdrawn at the bottom of the column. The solvent/ester solution is subsequently contacted with an acidic aqueous phase to hydrolyze the ester and back-extract the resulting xylose into the water phase. Such a scheme is typically practiced using two extraction columns in a series as shown in Figure 6a. However, both steps could be integrated into a single extraction column with a lower section performing the extraction step and an upper part performing the back-extraction (Figure 6b). Such a column would comprise a side-feed for the hydrolysate, a side-draw for the aqueous xylose product stream, and a solvent/PBA recycle from top to bottom. Overall, the process would ideally be carried out at elevated temperature, e.g., 60−90 °C, to proceed swiftly.

Back-extraction experiments from toluene/PBA were executed and appeared to be challenging: >10 extraction stages were required to recover >80% of the xylose (Figures S14 and S15). The extraction efficiency of the toluene/BPA system, 85% in a single stage, turned out to hinder the back-extraction. In contrast, the use of a less efficient extraction mixture such as n-heptane/BPA (45% in a single stage, see Figure 3) allowed a much easier back-extraction of xylose, as illustrated in Figure S16. For an efficient process, we need, therefore, to consider the combined efficiencies of solvent extraction and back-extraction.

An analysis that is detailed in the SI revealed that the maximum overall efficiency did not really depend on the partition coefficient $K$ observed in single-stage extraction as long as the solvent/feed ratio (S/F) was adjusted to reach, but not exceed, 50% extraction in a single stage. Hence, the process is free to use a large variety of extraction solvents and can be optimized on other performance parameters than extraction efficiency.

Recovery and concentration are not the sole critical factors for the economic viability of such a process. Losses of solvent and PBA in both aqueous streams, i.e., in the xylose-lean hydrolysate and in the xylose-rich product stream, also need to be minimized. Loss of toluene should not expect to be very critical owing to its low solubility in water of 0.05 w% (at 25 °C). Loss of PBA could be more substantial, since it dissolves in water up to 1 w% (at 25 °C). Analysis of the aqueous phase after extraction revealed indeed the presence of 0.9 w% PBA. Appropriate measures should be considered to mitigate these losses.

While achieving a respectable yield, such an extraction/back-extraction scheme still delivers a product stream that is slightly more diluted than the feed stream. This drawback can be improved upon inserting a solvent reconcentration step, e.g., by partial evaporation, before the back-extraction (Figure 6c). In the case modeled in Figure 7, the evaporation of 50% of the solvent delivers the 82% recovery at 8.2% concentration, i.e., 64% higher than present in the feed stream (more information in the SI). This would obviously increase the energy consumption of the process, but much less than upon evaporation of a comparable volume of water to concentrate the hydrolysate or the final xylose aqueous product stream, for the heat of evaporation of hydrocarbon solvents is less than a fifth of that of water, namely 0.3 kJ/mL vs 2.2 kJ/mL. Economic considerations could be used to define the optimum solvent reconcentration level.

Gori et al.11 have proposed another approach to recover the xylose from a PBA$_X$/solvent stream. It involves the transesterification of PBA$_X$ with another diol (e.g., ethylene glycol) to produce a PBA-glycol ester and to precipitate xylose from the solution. The xylose can then be advantageously used as crystalline xylose, be redissolved in water, or be transferred to a...
polar organic solvent for further processing. Once the crystalline xylose is recovered, the resulting PBA-glycol ester (PBA-EG) can then be hydrolyzed with an acidic aqueous solution to form a decantable PBA phase for recycling. However, the resulting acidic aqueous glycol solution needs further workup, e.g., by water evaporation, to allow recycling of the glycol for the next run. All these steps eventually make the xylose recovery quite complicated. This approach seems particularly elegant when one targets crystalline xylose or when one wishes to transfer the xylose into a nonaqueous solvent. It is, however, fairly complex for transferring xylose back to water. A deeper process modeling study would be needed to compare it to the method proposed here in terms of energy demand and equipment cost and component losses.

CONCLUSIONS

We propose here an integrated process to recover and purify xylose from an acidic hydrolysate stream, e.g., coming from steam expulsion or dilute acid pretreatment of lignocellulose. This process consists of selectively extracting the xylose as the boronate diester in the absence of a polar organic solvent. It is, however, fairly complex for transferring xylose back to water. A deeper process modeling study would be needed to compare it to the method proposed here in terms of energy demand and equipment cost and component losses.

EXPERIMENTAL SECTION

Chemicals. Reagents with the following purity were purchased from Sigma-Aldrich: D-xylose (99%) (99% atom D), D- (+)-glucose (99%), D- (+)-fructose (99%), D- (+)-arabinose (99%), D- (+)-galactose (99%), D- (+)-sucrose (99.5%), D3O (99% atom D), toluene-d8 (99% atom D), DMSO-d6 (99% atom D), dioxane (99.8%), 3-(trimethylsilyl)propionic-2,3,3-d4 acid sodium salt (TMSp, 98% atom D), Aliquat 336, 3-valerolactone (99%), nitrobenzene (99%), anisole (99%), methylisobutylketone (99%), 1-octanol (99%), n-heptane (99%), and 2-methylnaphthalene (98%) were purchased from Alfa Aesar.

Methods and Equipment. All chemicals are commercially available and were used without further purification. 1H NMR spectra were recorded on a 400 MHz Bruker spectrometer in a 1:1 H2O/D2O mixture with TMSp as the internal standard in case of the aqueous phases or in a 1:1 mixture of toluene and toluene-d8 with dioxane as the internal standard in case of the organic phases. These mixtures are composed by equal volumes (250 μL) of the sample and the deuterated solvent, containing the standard. The 1H NMR characterization of PBAX and PBA-X was performed in DMSO-d6 to ensure the possibility to directly compare all the esters with xylose. IR spectra were recorded on an FT-IR spectrometer (Thermo Scientific Nicolet 6700) with a diamond ATR accessory (Thermo Optec-Smart Orbit). MS spectrometry was carried out with an ESI-TOF spectrometer (Waters Micromass LCT) with an automatic injection device (Harvard Apparatus, Pump 11 Elite). In all cases, stirring was performed with a magnetic stirrer at 1000 rpm at room temperature.

PBA-Mediated Extractions. Equal volumes of an aqueous sugar solution and a solution of PBA in organic solvent were put into contact and stirred at room temperature for a maximum time of 2 h. Solutions of D-xylose (350 mM) in water (pH = 1, 7, and 10) and PBA and Aliquat 336 in toluene (both at equimolar conditions and in excess of PBA) were used to study the effect of Aliquat 336 on the extraction (only in this case the total time was prolonged to 24 h).

Solutions of D-xylose (350 mM) in water (pH varying from 1 to 9) and a PBA solution in toluene (700 mM) were used to study the effect of pH. A solution of D-xylose (350 mM) in water (pH = 1 from H2SO4) and PBA solutions in toluene at various concentrations were used to study the effect of different xylose-PBA ratios. A solution of D-xylose (350 mM) in water (pH = 1 from H2SO4) and PBA solutions (700 mM) in various organic solvents were used to explore the effect of the organic solvent on the extraction. Solutions of various sugars (350 mM) in water (pH = 1 from H2SO4) and a PBA solution in toluene (700 mM) were used to analyze the behavior of different sugars in the extraction process. In all cases, both phases were analyzed by 1H NMR spectroscopy as described above.

Isolation and Analysis of PBAX and PBA-X. In the case of PBAX, a solution of D-xylose (350 mM) in water (5 mL; pH = 1 from H2SO4) and a PBA solution in toluene (5 mL; 700 mM) were mixed for 2 h at room temperature. In the case of PBAX, a solution of D-xylose (350 mM) in water (5 mL; pH = 1 from H2SO4) and a PBA solution in toluene (5 mL; 350 mM) were mixed for 30 min. After the two phases were removed with a continuous stream of dry N2. The solid samples obtained were characterized with 1H NMR spectroscopy (in DMSO-d6) and MS spectrometry.

Xylose Extraction from Complex Matrix. A mixture of different sugars, based on the composition of hemi-cellulose derived from rice husk, in water (pH = 3 from acetic acid) and a solution of PBA in toluene (700 mM) were mixed for a total time of 2 h. The two phases were analyzed with 1H NMR spectroscopy as described above.
**ASSOCIATED CONTENT**

- Supporting Information
  The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.1c00167.
  Additional characterization of different boronate esters and additional experimental results and data (PDF)

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**DEDICATION**

Dedicated to Herman van Bekkum and Joop A. Peters. In memory of Herman van Bekkum.

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