Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Purification and Concentration of Xylose and Glucose from Neutralized Bagasse Hydrolysates Using 3,5-Dimethylphenylboronic Acid and Modified Aliquat 336 as Coextractants

G. J. Griffin[a]
[a] School of Engineering, James Cook University, Townsville, Australia

To cite this Article Griffin, G. J.(2005) 'Purification and Concentration of Xylose and Glucose from Neutralized Bagasse Hydrolysates Using 3,5-Dimethylphenylboronic Acid and Modified Aliquat 336 as Coextractants', Separation Science and Technology, 40: 11, 2337 — 2351
To link to this Article: DOI: 10.1080/01496390500202522
URL: http://dx.doi.org/10.1080/01496390500202522

Separation Science and Technology, 40: 2337–2351, 2005

ISSN 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390500202522

Purification and Concentration of Xylose and Glucose from Neutralized Bagasse Hydrolysates Using 3,5-Dimethylphenylboronic Acid and Modified Aliquat 336 as Coextractants

G. J. Griffin
School of Engineering, James Cook University, Townsville, Australia

Abstract: Experimental trials of the extraction of xylose, glucose, and fructose from aqueous solutions were conducted using 3,5-dimethylphenylboronic acid (DMPBA) and modified Aliquat® 336 (MA) as combined extractants dissolved in Exxal®10 diluent. MA was produced by contacting an Aliquat 336/Exxal 10 solution with a concentrated caustic soda solution so that the quaternary ammonium ions of Aliquat 336 would ion pair with hydroxide ions. The DMPBA/MA/Exxal 10 organic solution containing equimolar amounts of MA and DMPBA was contacted with a neutral aqueous solution containing one of glucose, xylose, or fructose and the extraction isotherms were determined. The molar ratio of DMPBA:sugar in a fully loaded organic solution was 2:1. The use of the MA instead of Aliquat 336 enabled significant proportions of the sugars to be extracted from aqueous solutions over a wide pH range (~2–11). Loaded organic solutions were stripped using aqueous hydrochloric acid solutions. Complete recovery of the sugar was possible by ensuring sufficient acidity was available in the strip solution. Solutions of MA in Exxal 10 were also found to extract sugar, although to a lesser extent than when DMPBA was included. Extraction of xylose and glucose from solutions derived from the acid hydrolysis of bagasse was performed. By varying the volumetric ratio of strip to organic phases, strip solutions with xylose concentrations up to 4× that of the original hydrolysate were produced while reducing the concentration of the undesirable acid soluble lignin by up to

Received January 10, 2005, Accepted May 18, 2005
Address correspondence to G. J. Griffin, CSIRO MIT, Fire Sciences and Technology Laboratory, PO Box 56, Highett, Victoria 3190, Australia. Tel.: +61 3 92526116; Fax: +61 3 92526246; E-mail: greg.griffin@csiro.au

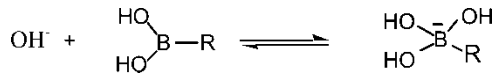
90%. Hence, this process has the potential to produce high concentration monosaccharide solutions suitable for direct fermentation.

Keywords: Solvent extraction, boronic acid, aliquat 336, xylose, glucose, fructose, acid soluble lignin, hydrolysate, purification

INTRODUCTION

The use of waste biomass material as a feedstock for the production of fuel ethanol has been the subject of considerable research over the past 20 yrs. It is recognized that the most economically attractive processes must involve the hydrolysis of the hemicellulose and cellulose components of the biomass into monosaccharides (primarily glucose and xylose) followed by fermentation (1, 2). However, despite the range of different methods available, none of the processes can avoid the production of toxic inhibitors (weak acids, furan derivatives, and phenolic compounds) during the hydrolysis step that limit the efficiency of the subsequent fermentation (3–5). To enhance the yield of ethanol, the hydrolysates may be detoxified by a variety of chemical, biological, or physical means. The most widely used detoxification process, overliming, although improving ethanol yield does not remove all inhibitors, especially the phenolic compounds derived from soluble lignins (3, 6, 7). Alternatively, the microorganisms responsible for fermentation may be adapted to the toxic environment (3) or special nutrients may be added to the hydrolysate to offset the toxic inhibition (3, 8). None of the processes developed have a reported yield of ethanol in excess of 85% of the theoretical maximum (2).

One potential method of improving the quality of a biomass hydrolysate is the use of reactive solvent extraction to separate the sugars from the hydrolysate into an immiscible phase and then recover the sugars into a ‘clean’ aqueous solution. Such schemes have been investigated for purifying other types of sugar solutions. These focus primarily on the separation of fructose from mixtures of sugar solutions using supported liquid membranes (SLMs) incorporating boronic acid/quaternary amine carriers (9–14). The mechanism by which extraction occurs in these processes is as follows (11). A lipophilic boronic acid (R-B(OH)$_2$ in Scheme 1) dissolved in an organic solvent is contacted with an immiscible aqueous solution containing dissolved sugars and buffered to a pH greater than the pKa of the boronic acid (i.e., generally a pH of $\sim$9 or greater). At the interface between the

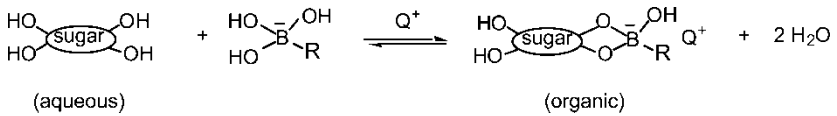


Scheme 1.

organic and aqueous phases the boronic acid ionizes to form a tetrahedral anion (Scheme 1) that then forms an anion complex with the vicinal diols of a sugar molecule (Scheme 2). (Note: there is another mode of complexation between the boronic acid and sugar molecule via the formation of a neutral, trigonal boronate ester, however, this is unfavorable in aqueous environments (15)). This anion complex is then dissolved within the organic solvent by forming an ion pair with a lipophilic quaternary ammonium cation (Q^+ in Scheme 2), such as trioctylmethylammonium ion. This process is reversible and the complex decomposes in acidic solution thus releasing the bound sugar.

Research data on the equilibrium constant for extraction of the sugars is limited. Data available indicates that, generally, the extraction of fructose is greater than that of glucose but not in all cases (16–19). Disaccharides are extracted to a negligible extent. The stoichiometry of the extraction process is also unclear, with researchers suggesting that the ratio of monoboronic acid to sugar molecule in the complex to be 2:1 or 1:1 (9, 18). There are no published papers on the equilibrium or stoichiometry for xylose extraction.

In a recent publication by Griffin and Shu (20), the extraction of xylose and glucose from buffered bagasse hydrolysates was reported. In the research reported in this paper, a modified version of the extraction process for purification and concentration of biomass hydrolysate is explored. In previous research the most common source of the quaternary amine used was trioctylmethylammonium chloride (TOMAC), either in its pure form or as the major component of an industrial grade extractant, Aliquat 336. Furthermore, the aqueous sugars solutions were buffered to maintain a high pH as the complexation of the sugars with boronic acids consumed hydroxide ions from the aqueous phase. In this research, Aliquat 336 (dissolved in Exxal 10 diluent) is contacted with a concentrated caustic soda solution so as to ion exchange the chloride ions with hydroxide ions and produce a trioctylmethylammonium hydroxide solution. It was hypothesized that this modification would remove the need to buffer the aqueous sugar solutions (thus improving the economics of any potential industrial process). This modified Aliquat 336 (MA), contained within Exxal 10, was mixed with 3,5-dimethylphenylboronic acid and the extraction of the monosaccharides glucose, xylose, and fructose from aqueous solution was measured. Stripping equilibrium of the loaded organic phase using hydrochloric acid was also determined. Trials were then conducted to extract xylose and glucose from solutions



Scheme 2.

derived from the dilute acid hydrolysis of sugar cane bagasse and to then strip the loaded organic solutions using an aqueous solution containing hydrochloric acid so as to both concentrate and purify the sugars.

METHODS

Sugar Solutions and Acid Hydrolysates of Bagasse

The sugars used were glucose (Sigma-Aldrich, Sydney, Australia—purity $>995\,g\,kg^{-1}$), fructose (Sigma-Aldrich, Sydney, Australia—purity $>990\,g\,kg^{-1}$), and xylose (Sigma-Aldrich, Sydney, Australia—purity $>990\,g\,kg^{-1}$) dissolved in distilled water.

The organic phase diluent was Exxal® 10 (ExxonMobil, Melbourne, Australia), an industrial solvent composed primarily of isodecyl alcohol (purity $>820\,g\,kg^{-1}$ isodecyl alcohol and $>990\,g\,kg^{-1}$ aliphatic alcohols).

The quaternary amine dissolved in the diluent was Aliquat® 336 (Sigma-Aldrich, Sydney, Australia), an industrial extractant composed predominantly of trioctylmethylammonium chloride. Trials were conducted to measure the ion exchange of chloride ions with hydroxide ions by mixing the organic phase (120 mM Aliquat 336 in Exxal 10) with aqueous NaOH solutions at varying organic phase to aqueous phase volumetric ratios and NaOH concentrations. The NaOH concentration in the aqueous phase, before and after mixing, was determined by titration with standardized HCl solutions. Concentration of hydroxide ions in the organic phase was determined by mass balance. The equilibrium isotherm for hydroxide extraction was determined; see Fig. 1. A line of best fit using a hyperbola equation was also computed and is shown in Fig. 1. From this isotherm it was determined that a 10 M NaOH aqueous solution, mixed in a 1:1 volumetric ratio with 120 mM Aliquat 336 in Exxal 10, would produce 100% exchange of chloride for hydroxide ions in the organic phase. Therefore, 120 mM Aliquat 336 in diluent was mixed with 10 M aqueous NaOH in a conical flask, allowed to separate, the organic phase was decanted, transferred to 1.5 mL microtubes and then centrifuged at 13,000 rpm in a Biofuge 13 centrifuge (Heraeus Sepatech, Germany) for 5 min to coalesce any entrained aqueous solution at the bottom of the microtube. The resulting upper layer of modified Aliquat 336 (MA), composed mainly of trioctylmethylammonium hydroxide in diluent, was then pippeted from the microtubes and collected.

The boronic acid used was DMPBA (Frontier Scientific, Logan, USA—purity $>970\,g\,kg^{-1}$). This was dissolved in the MA/diluent solutions to the required concentration.

Acid hydrolysate solution was produced by diluting concentrated sulphuric acid (BDH, Kilsyth, Australia—purity $>980\,g\,kg^{-1}$) with distilled water to a concentration of 4% in a Pyrex beaker then adding bagasse

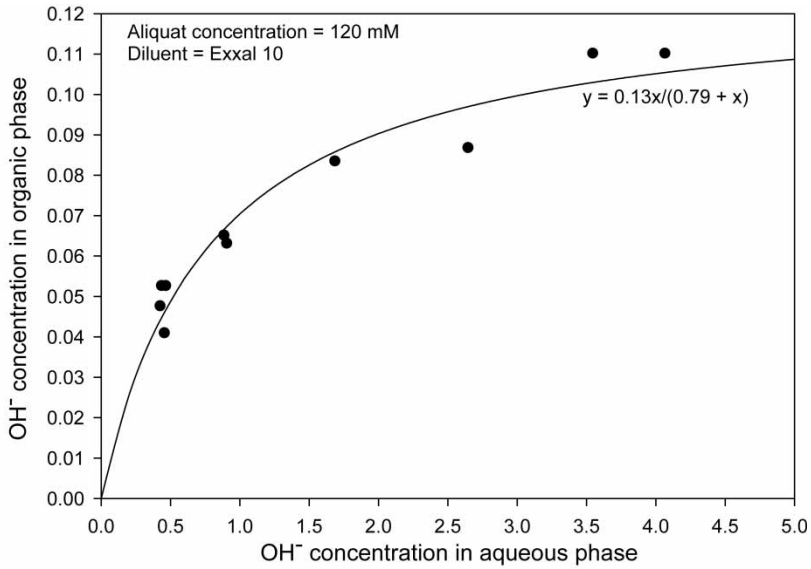


Figure 1. Extraction isotherm for OH^- distribution between aqueous phase and organic phase.

(Victoria Sugar Mill, Lucinda, Australia) in the weight ratio of 1 g bagasse: 20 g acid solution. The mixture was heated to 95°C and maintained at that temperature for 2 days in a high-temperature oil bath (Thermoline, Northgate, Australia). The resulting hydrolysate was filtered with Whatman® type 42 ashless filter paper (B&R Balston, England) then calcium hydroxide (BDH, Kilsyth, Australia—purity $>960\,g\,kg^{-1}$) was added to the filtrate until a pH of 6–7 was attained. The slurry was filtered with Millex® 0.22 μm filters (Millipore, USA) and the final filtrate contained $10.8 \pm 0.1\,g\,L^{-1}$ xylose, $1.04 \pm 0.01\,g\,L^{-1}$ glucose, and 46 ± 5 mg acid soluble lignin (ASL) g^{-1} xylose.

Aqueous strip solutions were produced by diluting concentrated hydrochloric acid (Ajax, Auburn, Australia—purity $>990\,g\,kg^{-1}$) with distilled water to the desired concentration.

Experimental Apparatus and Methods

Extraction experiments were conducted by pipetting a known volume of organic solution and sugar solution, or hydrolysate solution, into a 1.5 mL microtube. The tube was sealed and attached to a Thermolyne Maxi Mix II shaker (Lab Supply, Sydney, Australia) and then vigorously shaken for 30 min to attain equilibrium. The tubes was then transferred to a Biofuge 13

centrifuge and spun at 13,000 rpm for 5 min to separate the two phases; in all tests the formation of stable emulsions was not observed. A sample of the aqueous solution was then removed and analyzed to determine the amount of sugars transferred into the organic phase. Stripping experiments were conducted in the same manner, with known volumes of loaded organic solution and aqueous strip solution pipetted into a 1.5 mL microtube, shaking, centrifuging then sampling of the strip solution to determine the recovery of sugars from the loaded organic phase. All extraction and stripping experiments were performed at ambient temperature, 25°C.

The concentrations of glucose, fructose, and xylose in the aqueous phase were measured using high performance liquid chromatography (HPLC). The measured precision using this technique on standard solutions was $\pm 1\%$. A GBC HPLC system (GBC, Dandenong, Australia) was used, and consisted of a LC1150 pump, LC1650 autosampler, Alltech 330 column heater, and LC1240 refractive index (RI) detector. GBC Winchrom software, version 1.3, was used to control the HPLC apparatus. Separation of the analytes was performed using a Resex RPM Monosaccharide column with Carbo-Pb-2+ guard column (Phenomenex, Pennant Hills, Australia) in series. The column operation parameters are given in Table 1.

For experiments involving bagasse hydrolysates, the concentration of acid soluble lignin (ASL) in the feed and strip phases was measured using a spectrophotometric method similar to that described by Ehrman (21). Samples of the aqueous phases were diluted (usually 200×) with 7% sulphuric acid, transferred to quartz cuvettes (1 cm path length) and absorbance measured over 190–210 nm wavelengths using a Hewlett Packard: HP 845x—UV Visible System. The peak absorbance measured (usually between 195–205 nm) was used to calculate the amount of ASL in the sample (see Ehrman (21)). For the feed hydrolysate, the samples were zeroed using 7wt% sulphuric acid. For strip solutions, significant absorbance was measured even if the organic phase had not been contacted with the hydrolysate solution beforehand. It was likely that this was due to small amounts of the DMPBA dissolving into the strip solution. Hence, to compensate for this effect, the strip solutions were zeroed using a strip solution that had been contacted with an unloaded organic solution and then diluted 200× with 7% sulphuric acid.

Table 1. HPLC column operation parameters

Maximum pressure	4.1 MPa
Temperature	85°C
Flow rate	0.6 mL/min^{-1}
Detection	Refractive index
Eluent	Double distilled water
Injection volume	20 μL

RESULTS AND DISCUSSION

Extraction of Sugars from Synthetic Solutions

Trials were conducted in which aqueous samples containing one of glucose, fructose, or xylose ($5-20\,gL^{-1}$) were mixed with organic solution (volumetric ratio of aqueous to organic phases = 1:1 to 3:1) composed of the diluent Exxal 10, MA (120 mM) and DMPBA (120 mM). The aqueous phases contained only distilled water and sugar. The concentration of sugar in the aqueous phase was determined by HPLC and the amount of sugar extracted into the organic phase was determined by mass balance. Figure 2 shows the extraction isotherm calculated for these trials, including lines of best fit using a simple hyperbola equation. Each trial was performed in triplicate; the data symbols on Fig. 2 represent the mean of the data measured. Variation was $\pm 5\%$. At lower aqueous sugar concentrations glucose was more favorably extracted than the other two sugars. As the aqueous concentration of sugars was increased the concentration of sugar in the organic phase increased monotonically to a limiting value that was one half of the extractant concentration (60 mM, or 9 gL^{-1} for xylose and $10.8\,gL^{-1}$ for glucose and fructose). This indicates the stoichiometry of the DMPBA/sugar complex in the organic phase was in the ratio of 2:1. It is likely that

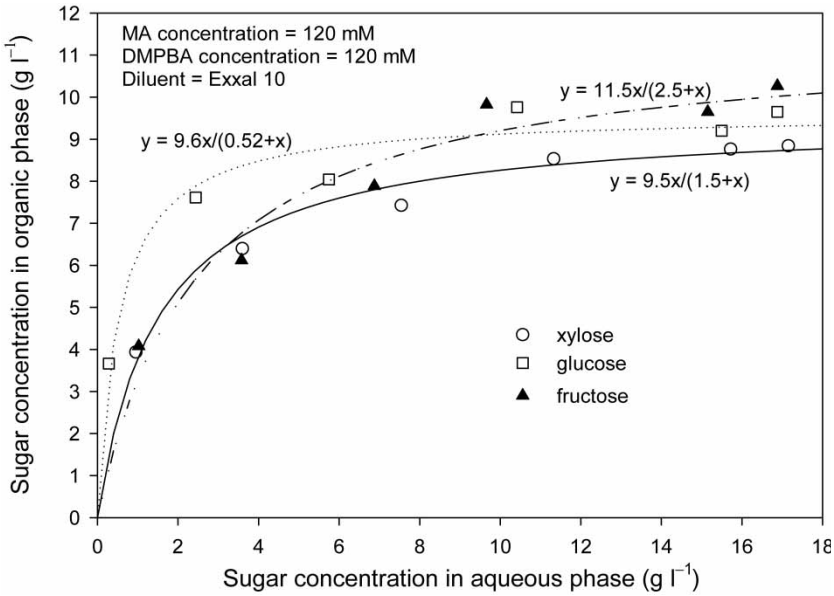


Figure 2. Extraction isotherm for sugar distribution between aqueous phase and organic phase.

a 1:1 DMPBA/sugar complex would not be soluble in an organic phase due to the presence of a significant number of hydroxyl functional groups present on the sugar moiety.

Some of the above trials were repeated using Aliquat 336 instead of MA. Under these conditions there was no measurable extraction of sugars into the organic phase.

Trials were conducted to establish over what range of acidity or alkalinity sugar would be extracted from an aqueous phase into the organic phase. In previous studies on sugar extraction using boronic acids and quaternary amines the amines were ion paired with chloride ions so that, for significant levels of extraction to occur, the sugar solutions had to be buffered to a pH greater than ~9. In the trials of this study a modified Aliquat (MA) was used which ion pairs the quaternary amines with hydroxide ions. Aqueous xylose solutions containing $10\,gL^{-1}$ xylose and either NaOH (10^{-7} – 4×10^{-3}M) or HCl (10^{-7} – 0.5 M) were contacted with an organic solution (volumetric ratio of aqueous to organic phase of 1:1) and the percentage (%) extraction measured—see Fig. 3. (All trials were conducted in triplicate. The symbols on Fig. 3 represent the mean of the data and the error bars represent the standard deviation. Percentage extraction is defined as the percentage of sugar, on a mole or mass basis, initially present in the aqueous phase that is extracted into the organic phase.) Figure 3 shows that varying the alkalinity

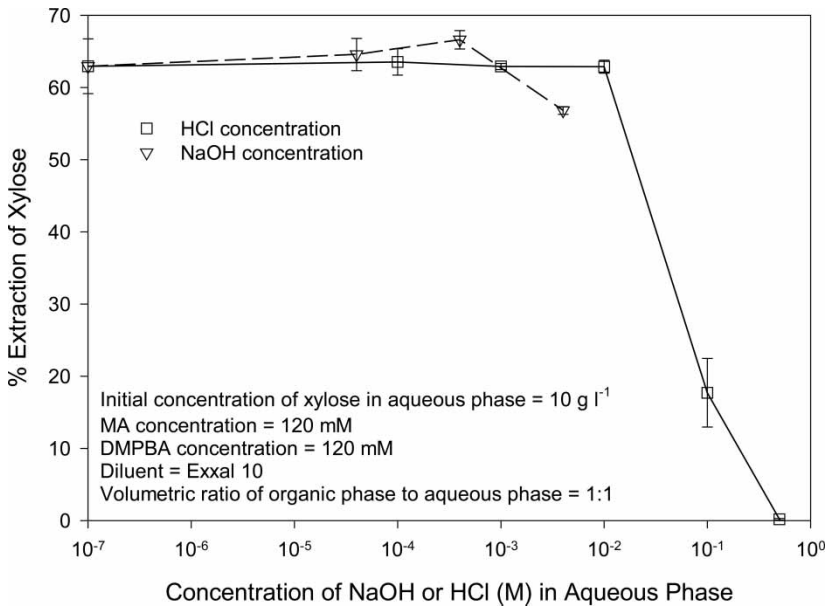


Figure 3. Effect of alkalinity and acidity on extraction of sugars into an organic phase.

or acidity of the xylose solution did not effect the % extraction of xylose except when the acid concentration was greater than 0.01 M. For acid concentrations greater than 0.01 M the % extraction decreased linearly with acid concentration. Over this range of acid concentration the acid reacts with the hydroxide ions in the organic phase, essentially consuming the hydroxide, neutralizing the aqueous phase and forcing the amines to ion pair with chloride ions. With less hydroxide available in the organic phase to ionize the DMPBA, extraction of sugars decreases in proportion to the reduction of hydroxide ions.

The effect of varying the relative concentrations of MA and DMPBA in the organic phase on the % extraction of glucose and xylose was tested and the results are shown in Figs. 4 and 5. It may be seen from these figures that no extraction is measured when only the diluent is present. The % extraction increases, as expected, as both extractant concentrations (in equimolar amounts) are increased. An excess of DMPBA (i.e., where the concentration of DMPBA is higher than the concentration of MA) does increase the % extraction above that which occurs when the molar ratio of the extractants are equal. Therefore it can be inferred that extraction of the sugars via the formation of the trigonal boronate ester was negligible. An excess of MA, however, did increase the % extraction of the sugars. Indeed, when only MA and no DMPBA was present as extractants there was still

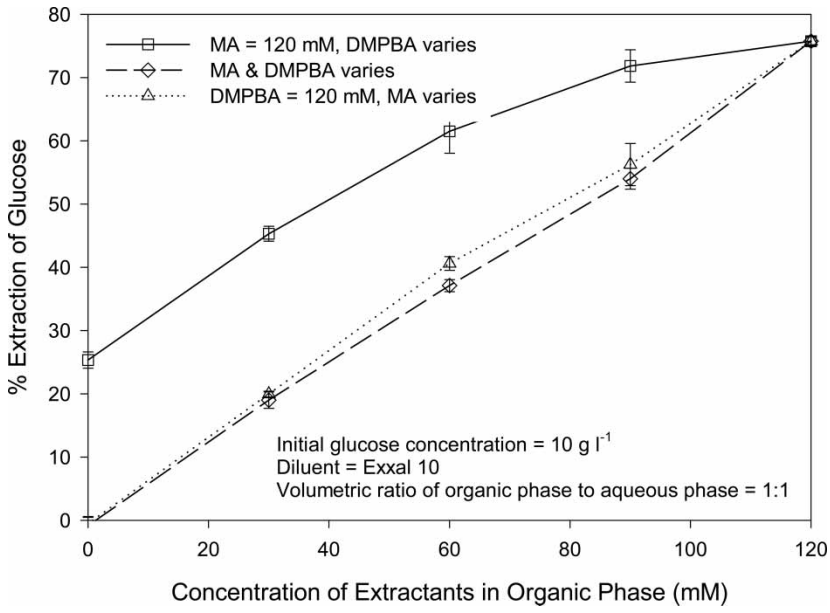


Figure 4. Effect of extractant concentration on extraction of glucose from an aqueous phase.

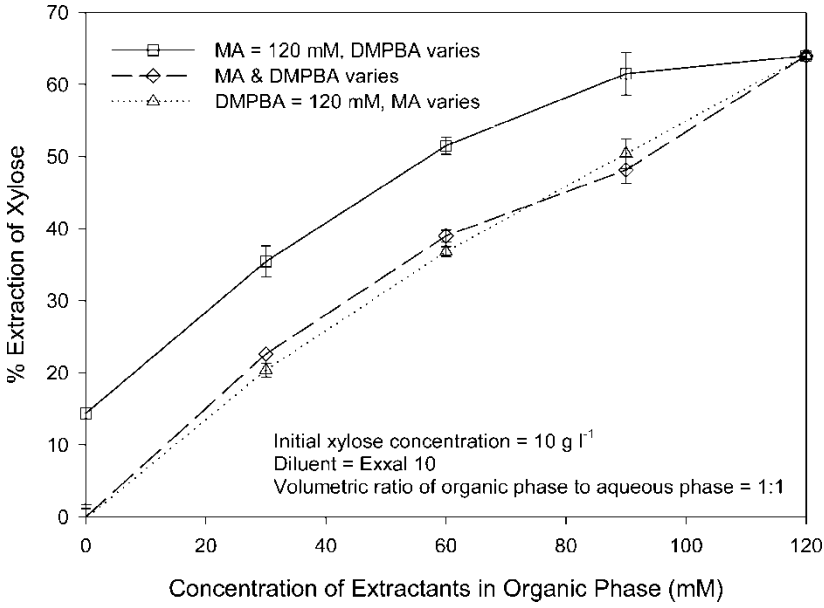


Figure 5. Effect of extractant concentration on extraction of xylose from an aqueous phase

significant extraction of the sugars, presumably via the hydroxide ions in the organic phase ionizing the sugar molecules to such an extent that the sugar anions could ion pair with the quaternary amines. It is interesting to note that the % extraction of glucose was higher than that of xylose despite the glucose molecules having a greater proportion of hydroxyl functional groups.

Stripping experiments were conducted to determine the effect of strip acid concentration on the recovery of sugars from a loaded organic stream into the aqueous strip solution—see Fig. 6. The loaded organic solutions were produced by contacting the organic phase (120 mM of MA and DMPBA in Exxal 10) with an aqueous phase containing 10 gL^{-1} of xylose or glucose (volumetric ratio of organic phase to aqueous phase was 1:2). The resulting loaded organic solution contained 7.1 gL^{-1} of glucose or 6.1 gL^{-1} of xylose. These were then stripped using an aqueous stream containing HCl (volumetric ratio of organic phase to aqueous phase was 1:1) and the percentage (%) recovery calculated. (The % recovery is defined as the percentage of sugar in the organic phase transferred to the strip solution.) It can be seen that the % recovery is linearly dependent on the concentration of acid in the strip solution with complete recovery occurring when the concentration of acid is greater than 0.12 M, i.e. when all the hydroxide present in the organic phase is neutralised.

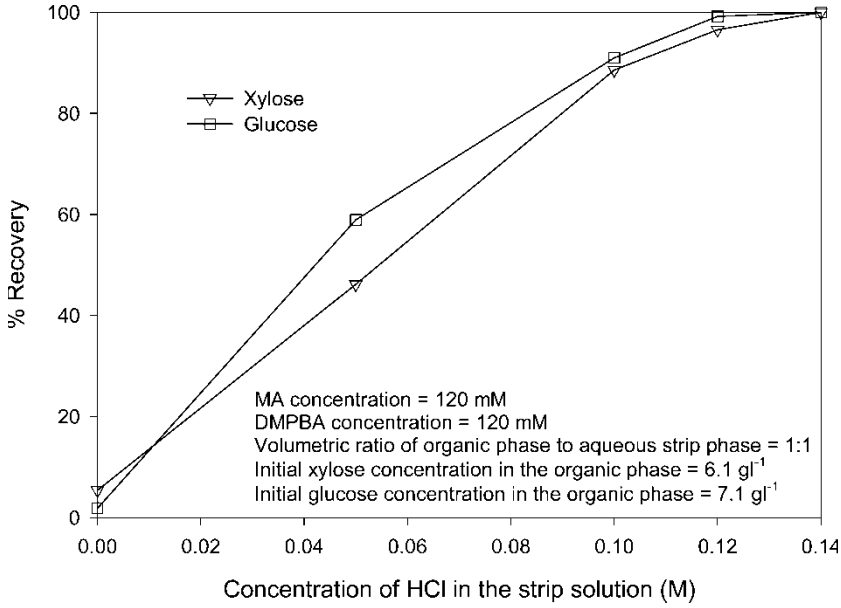


Figure 6. Effect of strip acid concentration on recovery of sugars from an organic phase.

Extraction of Sugars from Bagasse Hydrolysates

Trials were conducted to determine the recovery of sugars from an organic phase that had been loaded by contacting with a neutralized hydrolysate solution. The organic phase was initially mixed with hydrolysate (volumetric ratio of organic to aqueous phase = 2:1) that had been neutralized (pH = 6 – 7) with calcium hydroxide. The resultant loaded organic phase contained $0.42 \pm 0.04\,\mathrm{gl}^{-1}$ glucose and $4.8 \pm 0.1\ \mathrm{gl}^{-1}$ xylose (this is close to the extraction expected for synthetic sugar solutions). The organic phase was then mixed with an equal volume of an aqueous HCl solution (strip solution) that stripped the sugar from the organic phase. The effect of the initial HCl concentration of the stripping solution and the volumetric ratio of strip phase to organic phase on the % recovery is shown in Table 2. In trials 1–4 the initial HCl concentration was set at that calculated to neutralize all hydroxide present in the organic phase (and, therefore, theoretically recover all sugars into the strip solution). It can be seen in these trials that an increase in volumetric ratio of organic phase to strip phase has little effect on sugar recovery and the sugar concentration in the strip solution can be considerably enhanced over the concentration of sugars within the organic phase and the original hydrolysate. At the highest volumetric ratio the sugar concentration in the strip solution was 4× that of the original

Table 2. Recovery and concentration of sugars into a strip solution

Trial no.	Volumetric ratio (aqueous phase: organic phase)	Initial HCl concentration in strip solution (M)	Recovery glucose (%)	Recovery xylose (%)	Final glucose concentration in strip solution ($g\ L^{-1}$)	Final xylose concentration in strip solution ($g\ L^{-1}$)	Final ASL concentration in strip solution ($mg\ g^{-1}$ xylose)
1	1:1	0.12	87	91	0.45	4.4	18
2	1:3	0.36	89	93	1.4	13.4	4.8
3	1:5	0.6	80	92	2.1	22.1	5.1
4	1:10	1.2	82	82	4.3	40.4	8.1
5	1:1	0.13	90	94	0.46	4.5	1.5
6	1:3	0.40	80	94	1.2	13.5	3.1
7	1:5	0.66	86	94	2.3	22.9	8.8
8	1:10	1.3	79	83	4.1	40.7	4.0

hydrolysate and 8× that of the organic solution. However, there was some reduction in % recovery at the highest volumetric ratio of organic phase to aqueous phase. Not all the sugars were recovered, % recovery ranged from 83–91% for xylose and 80–89% for glucose. To improve recovery, trials were repeated using an acid concentration approximately 10% in excess of that theoretically required to release all sugars—see trials 5–8 on Table 2. There was a very slight improvement in recovery of xylose but it appears that not all sugar could be recovered. This may be due to degradation of sugars in the process for reasons that are unclear.

It should be noted that the monosaccharide arabinose was also present in the bagasse hydrolysate at concentrations similar to that of glucose. There was no attempt to quantify the extraction or recovery of this sugar although it was obvious from the analyses that significant amounts of this sugar were extracted and recovered into the strip solution.

The extraction and stripping processes appear to have been highly selective toward the transport of sugars as evidenced by the low amounts of acid soluble lignin (ASL) measured in the final strip solutions (see last column of Table 2). The concentration of ASL in the strip solution was reduced by up to 96% of the ASL present in the hydrolysate (on a g ASL g^{-1} xylose basis). Coupled with the very high sugar concentrations achieved this solvent extraction process may provide a means for producing a suitable substrate for direct fermentation.

CONCLUSIONS

Experimental trials of the solvent extraction of sugars from synthetic, aqueous sugar solutions consisting of xylose, glucose, and fructose showed that DMPBA/MA extracted these monosaccharides to form complexes with a molar ratio of sugar to DMPBA of 1:2. Significant levels of extraction were possible over a wide pH range without the need to buffer the aqueous solutions. The use of excess DMPBA (i.e., greater molar concentrations than MA) did not increase sugar extraction. Excess amounts of MA did increase % extraction and MA alone could act as an extractant, although this was not as effective. Complete recovery of the sugars from the organic phase could be achieved if sufficient acid was provided in the strip solution.

The use of equimolar amount of DMPBA and MA in an Exxal 10 diluent provided efficient extraction of xylose and glucose from bagasse hydrolysate solutions. By stripping the loaded organic phase with an aqueous HCl solution both a very high recovery of sugar and high sugar concentration in the strip solution could be obtained. The process is highly selective to monosaccharides as the concentration of the undesirable phenolic impurity, ASL, in the final strip solution was up to 96% less than that of the original hydrolysate. The resultant sugar solution from the strip process would appear to be suitable for direct fermentation processes. The quaternary ions in the stripped

organic phase may be regenerated as MA by contacting with concentrated hydroxide solution for further sugar extraction.

Additional research must be carried out to optimize the conditions for extraction and stripping and to test for phase separation characteristics in this process. It will also be necessary to test whether leakage of the diluents or extractants into the solutions would act as inhibitors of any downstream fermentation process. Of particular concern is the presence of DMPBA in the aqueous strip phase that has the potential to act as a more potent inhibitor than ASL. Alternatively, more lipophilic boronic acid compounds that are less soluble in aqueous solution may be more suitable for use as an extractant.

ACKNOWLEDGMENTS

The author gratefully acknowledges Frontier Scientific Ltd. and Exxon Mobil Chemical Australia for the donation of DMPBA and Exxal 10 used in this project.

REFERENCES

1. Sun, Y. and Cheng, J. (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83: 1–11.
2. von Sivers, M. and Zacchi, G. (1996) Ethanol from lignocellulosics: a review of the economy. *Bioresource technology*, 56: 131–140.
3. Olsson, L. and Hahn-Hägerdal, B. (1996) Fermentation of lignocellulosic hydrolysate for ethanol production. *Enzyme and Microbial Technology*, 18: 312–331.
4. Palmqvist, E. and Hahn-Hägerdal, B. (2000) Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. *Bioresource Technology*, 74: 25–33.
5. Palmqvist, E. and Hahn-Hägerdal, B. (2000) Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. *Bioresource Technology*, 74: 17–24.
6. Nigam, J.N. (2002) Bioconversion of water-hyacinth (*Eichhornia crassipes*) hemicellulose acid hydrolysate to motor fuel ethanol by xylose-fermenting yeast. *Journal of Biotechnology*, 97: 107–116.
7. Ward, O.P. and Singh, A. (2002) Bioethanol technology: developments and perspectives. *Advances in Biotechnology*, 51: 53–80.
8. Barber, A.R., Hansson, H., and Pamment, N.B. (2000) Acetaldehyde stimulation of the growth of *Saccharomyces cerevisiae* in the presence of inhibitors found in lignocellulosic-to-ethanol fermentations. *Journal of Industrial Microbiology & Biotechnology*, 25: 104–108.
9. Striegler, S. (2003) Selective carbohydrate recognition by synthetic receptors in aqueous solution. *Current Organic Chemistry*, 7: 81–102.
10. Paugam, M.-F., Riggs, J.A., and Smith, B.D. (1996) High fructose syrup production using fructose-selective liquid membranes. *Chemical Communications*, 22: 2539–2540.
11. Smith, B.D. (1996) Liquid membrane transport using boronic acid carriers. *Supramolecular Chemistry*, 7: 55–60.

12. Altamore, T.M., Barrett, E.S., Duggan, P.J., Sherburn, M.S., and Szydzik, M.L. (2002) Cavitand boronic acids mediate highly selective fructose transport. *Organic Letters*, 4 (20): 3489–3491.
13. Di Luccio, M., Smith, B.D., Kida, T., Alves, T.L.M., and Borges, C.P. (2002) Evaluation of flat sheet and hollow fiber supported liquid membranes for fructose pertraction from a mixture of sugars. *Desalination*, 148: 213–220.
14. Duggan, P.J. (2004) Fructose-permeable liquid membranes containing boronic acid carriers. *Australian Journal of Chemistry*, 57: 291–299.
15. Morin, G.T., Paugam, M.-F., Hughes, M.P., and Smith, B.D. (1994) Boronic acids mediate glycoside transport through a liquid membrane via reversible formation of trigonal boronate esters. *Journal of Organic Chemistry*, 59: 2724–2728.
16. Shinkai, S., Tsukagoshi, K., Shikawa, Y., and Kunitake, T. (1991) Molecular recognition of mono- and di-saccharides by phenylboronic acids in solvent extraction and as a monolayer. *Journal of the Chemical Society, Chemical Communications*, 1039–1041.
17. Gardiner, S.J., Smith, B.D., Duggan, P.J., Karpa, M.J., and Griffin, G.J. (1999) Selective fructose transport through supported liquid membranes containing diboronic acid or conjugated monoboronic acid-quaternary ammonium carriers. *Tetrahedron*, 55: 2857–2864.
18. Karpa, M.J., Duggan, P.J., Griffin, G.J., and Freudigmann, S.J. (1997) Competitive transport of reducing sugars through a lipophilic membrane facilitated by aryl boron acids. *Tetrahedron*, 53 (10): 3669–3678.
19. Yan, J., Springsteen, G., Deeter, S., and Wang, B. (2004) The relationship among pK_a, pH, and binding constants in interactions between boronic acids and diols—it is not as simple as it appears. *Tetrahedron*, 60: 11205–11209.
20. Griffin, G.J. and Shu, L. (2004) Solvent extraction and purification of sugars from hemicellulose hydrolysates using boronic acid carriers. *Journal of Chemical Technology and Biotechnology*, 79: 505–511.
21. Erhman, T. (1996) Determination of acid soluble lignin in biomass. Chemical Analysis and Testing Task. Renewable Energy Laboratory, US Department of Energy Laboratory Analytical Procedure, report no. LAP-004, National Renewable Energy Laboratory, US Department of Energy.