Production and Purification of Monosaccharides by Hydrolysis of Sugar Cane Bagasse in an Ionic Liquid Medium

T. R. Bandara, H. Jaelani, G. J. Griffin

Abstract—The conversion of lignocellulosic waste materials, such as sugar cane bagasse, to biofuels such as ethanol has attracted significant interest as a potential element for transforming transport fuel supplies to totally renewable sources. However, the refractory nature of the cellulosic structure of lignocellulosic materials has impeded progress on developing an economic process, whereby the cellulose component may be effectively broken down to glucose monosaccharides and then purified to allow downstream fermentation. Ionic liquid (IL) treatment of lignocellulosic biomass has been shown to disrupt the crystalline structure of cellulose thus potentially enabling the cellulose to be more readily hydrolysed to monosaccharides. Furthermore, conventional hydrolysis of lignocellulosic materials yields byproducts that are inhibitors for efficient fermentation of the monosaccharides. However, selective extraction of monosaccharides from an aqueous/IL phase into an organic phase utilizing a combination of boronic acids and quaternary amines has shown promise as a purification process. Hydrolysis of sugar cane bagasse immersed in an aqueous solution with IL (1-ethyl-3-methylimidazolium acetate) was conducted at different pH and temperature below 100 ºC. It was found that the use of a high concentration of hydrochloric acid to acidify the solution inhibited the hydrolysis of bagasse. At high pH (i.e. basic conditions), using sodium hydroxide, catalyst yields were reduced for total reducing sugars (TRS) due to the rapid degradation of the sugars formed. For purification trials, a supported liquid membrane (SLM) apparatus was constructed, whereby a synthetic solution containing xylose and glucose in an aqueous IL phase was transported across a membrane impregnated with phenyl boronic acid/Aliquat 336 to an aqueous phase. The transport rate of xylose was generally higher than that of glucose indicating that a SLM scheme may not only be useful for purifying sugars from undesirable toxic compounds, but also for fractionating sugars to improve fermentation efficiency.

Keywords—Biomass, bagasse, hydrolysis, monosaccharide, supported liquid membrane, purification.

I. INTRODUCTION

THE exploitation of agricultural lignocellulosic waste products (e.g. sugar cane bagasse, rice straw) is considered a key step to obtain a sustainable source of renewable fuels to supplant fossil fuels. Fuel ethanol has been readily adopted as a petrol substitute and can potentially be produced from agricultural waste by, generally, three processing steps [1]: pretreatment of the biomass, hydrolysis of the cellulose and hemicellulose into sugars (primarily the monosaccharides xylose and glucose), and fermentation of the sugars to ethanol.

To date, despite significant research effort over many years, an economically feasible process to produce ethanol from lignocellulosic waste has been proved elusive. Although the structure of such biomass is conducive to the degradation of hemicellulose to monosaccharides (mainly xylose) via simple, low-cost acid hydrolysis [2], the cellulose component, where most of the sugar content of the biomass resides, is highly resistant to degradation via acid hydrolysis due to the crystalline structure and stability of the cellulose fibrils [3]. Alternate methods to improve the extraction of monosaccharaides from cellulose, such a physical disruption using steam explosion or enzymatic treatment to selectively hydrolyse the cellulose chain, have improved sugar yield but at increased economic costs. Furthermore, whatever the method used to produce the sugars, it is almost inevitable that fermentation inhibitors, such as soluble lignin and furfurals, are also produced as side-products and must be removed [4].

IL treatment of biomass has shown promise to decrystallise, fractionate, and isolate cellulose [5]. It has been reported [6] that, under mild conditions, IL may be used to isolate cellulose from biomass as a precursor for hydrolysis to glucose. Enzymes are typically the favoured agent for hydrolysis with concomitant economical disadvantages [7]. Studies by Li et al. [8] and others [9], however, showed that bagasse cellulose may be digested directly using mineral acids (e.g. HCl) within certain IL liquids (e.g. 1-butyl-3-methylimidazolium chloride). Yields of TRS of 86 mg TRS/200 mg bagasse, or 68% of the potential TRS available, have been reported [8]. It is well known, however, that acid or base hydrolysis of lignocellulosic material produces undesirable byproducts (e.g. furfural, soluble lignin) [2] that must be separated; otherwise, they inhibit downstream fermentation processes. Purification of aqueous hydolysates by solvent extraction of monosaccharides using a mixture of boronic acids (e.g. phenyl boronic acid) and quaternary amines (e.g. trioctylmethyl ammonium chloride – TOMAC) contained in organic medium has been established [10], [11]. This has included the continuous extraction and stripping of sugars across a SLM [12]. Brennan et al. [13] produced hydrolysates of corn stover containing the IL 1-ethyl-3-methylimidazolium acetate ([EMIM]OAc) and then employed this solvent extraction process to extract and purify the monosaccharides. A

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recommendation of that work was to test the process using a SLM.

The purpose of the research reported here was to study the acid and base hydrolysis of bagasse when contained in an IL medium, and furthermore, to study the rate of transport of the monosaccharides glucose and xylose across a SLM. By conducting this study, an appreciation of the feasibility of employing such a method to large scale processing of lignocellulosic biomass to produce sugars suitable for fermentation may be achieved.

II. MATERIAL AND METHODS

A. Hydrolysis of Bagasse

Sugarcane bagasse was sourced from Racecourse Sugar Mill, Mackay, Australia. Bagasse samples were washed, then dried over night at 70 °C before being crushed and mechanically sieved by using 1~2 mm sieve mesh. Xylose (purity>99%) and [EMIM]OAc (assay>90%) were purchased from Sigma-Aldrich, Sydney, Australia. Dinitrosalicylic acid (DNS) reagents were prepared in house.

The use of the IL-[EMIM]OAc was made based on the literature review conducted on the use of ILs for dissolution of lignocellulosic material. [EMIM]OAc is commonly recommended and has been used by many researchers in their experiments for dissolving biomass.

Hydrolysis experiments were based on the methods reported in Li et al. [8]. A pre-weighed sample of sugarcane bagasse (0.4 g) was added to a preheated mixture of IL and water at 90 °C contained in a test tube, followed by an appropriate amount of concentrated HCl (36 wt.%) or NaOH (1 M). The total volume of all trials was 8 to 9 ml with a volumetric ratio of IL/water of 1:1. The test tube was placed in a constant temperature bath at 90 °C. The mixture was stirred, and the time of reaction began at the addition of the acid or base. At certain time intervals, samples were withdrawn (0.25 mL) and quenched in cold water and then neutralised with an appropriate amount of NaOH/HCl followed by a small addition of water to make up a total volume of 0.5 mL. The samples are stored in the fridge before proceeding to sugar analysis

Samples were mixed with 0.5 mL of DNS reagent and then heated in a hot water bath (80 °C) for 10 minutes. The samples were cooled to room temperature, centrifuged, and then diluted with deionised water in a ratio of 1:5 (0.5 mL sample: 2.5 mL water). The absorbance of the sugars was measured by using a UV-Vis spectrophotometer at 580 nm. A calibration curve based on absorbance for a xylose solution was used to calculate the TRS in the sample.

B. Transport of Sugars Across a SLM

An aqueous pH11 buffer solution were made by mixing NaHCO$_3$ (Sigma-Aldrich, Sydney, Australia – assay 99.7%) and NaOH (Sigma-Aldrich, Sydney, Australia – assay 97%) in Milli-Q water. The aqueous pH 7 buffer solutions were made by mixing KH$_2$PO$_4$ (Sigma-Aldrich, Sydney, Australia – assay 99.5%) and NaOH (Sigma-Aldrich, Sydney, Australia – assay

97%) in Milli-Q water.

For experiments that used aqueous solutions only, the departure phase composed of 0.25 M D Glucose (Sigma Aldrich, Sydney, Australia –assay 90%) and 0.25 M D xylose (Sigma Aldrich, Sydney, Australia) dissolved in a pH 11 buffer solution. For the experiments that uses IL/aqueous solutions, the departure phase was a mixture of equal volumes of IL [EMIM]OAc (Sigma Aldrich, Sydney, Australia – assay 90%) and 0.5 M D Glucose and 0.5 M D Xylose dissolved in a pH 11 buffer solution (buffer capacity adjusted accordingly) such that the resulting solution would have an equal concentration of D Glucose (0.25M) and D Xylose (0.25 M) and an equal amount of buffer components (NaOH and NaHCO$_3$) to those in the aqueous only departure phases. Sonication upto 30 minutes was necessary to completely dissolve the sugars.

The extractants phenyl boronic acid (PBA) (Sigma-Aldrich, Sydney, Australia – assay 95%) and Aliquat 336® (Sigma-Aldrich, Sydney, Australia – assay not given, assumed 100%) were mixed in the diluent 2-nitrophenyl octyl ether (Sigma-Aldrich, Sydney, Australia – assay 99%). Sonication up to 20 minutes was necessary to completely dissolve the extractants in the diluent.

The membrane support used was a polypropylene flat membrane supplied by Sterlitech Corporation WA USA. The mean pore size was 0.1 µm. Membrane preparation consisted of immersion of the support in an extractant–diluent mixture, and then placing the membrane under vacuum for 60-90 min, so that the extractant solution was impregnated into the membrane pores. Excess solution was then wiped from the membrane surface before subsequent usage.

1. SLM
2. glass half-cells
3. sample port
4. perspex stirrer with glass-encased metal bar
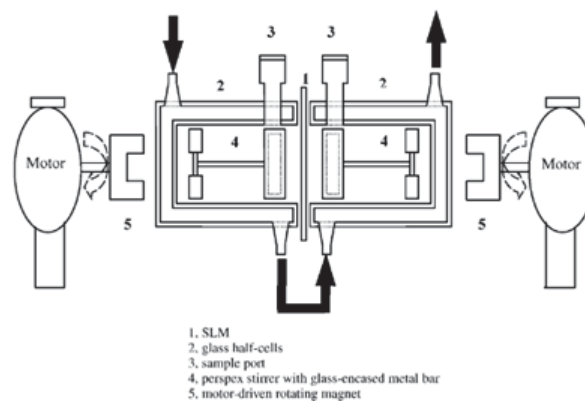5. motor-driven rotating magnet

Fig. 1 SLM apparatus [14]

A SLM apparatus was designed to be used in experiments, which was based on the SLM apparatus (Fig. 1) used in experiments performed by McMurray and Griffin [14]. The membrane was inserted between the two half-cells. Receiving phase was an aqueous pH 7 buffer solution (35 to 40 cm^3) and was poured into one of the half-cells (43.75 cm^3) through the sample port. An equal volume of the departure phase was poured into the other half cell. The contents in the departure phase were a synthetic solution containing a IL/pH11 aqueous buffer mixture at a volumetric ratio 1:1 or an aqueous pH 11

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buffer solution in which xylose and glucose were dissolved. Pouring of both half cells was performed simultaneously to avoid rupturing of the membrane especially when the departure phase contained the IL/aqueous mixtures. Each half-cell contained a magnetic stirrer and the stirring rate was maintained at 300 rpm. Duration of experiments varied from 150h to 200h depending on various process variables.

The concentrations of the sugars in the receiving phase were measured periodically. The concentrations were measured by using HPLC. A Shimadzu HPLC was used which consisted a refractive index (RI) detector (RID-10A). The column used was 300 x 7.8 mm Rezex RPM-Monosaccharide Pb+2 (8%) from Phenomenex, Australia. The column parameters are given in Table I.

TABLE I
COLUMN OPERATING CONDITIONS

Maximum pressure	1000 psi
Temperature	85 ˚C
Flow rate	0.6 ml min^{-1}
Detection	Refractive index
Mobile phase	Degassed Milli-Q water
Injection volume	10 µL

III. RESULTS AND DISCUSSION

A. Hydrolysis of bagasse

Fig. 2 shows the TRS yields from the hydrolysis experiments conducted in a IL/water medium in which HCl or NaOH was added to act as a catalyst. These results were all conducted at 90 ºC over a 50h period although only the first 10 hours (600 minutes) are shown here. The calculated yield of TRS is based on the potential maximum yield of TRS as reported in Li et al. [8]. It is immediately apparent that when bagasse is dissolved in the IL/water mixture with no catalysts addition, there is a significant level of hydrolysis of the bagasse which reaches a maximum of 8%. This may be due to the presence of natural acid catalyst within the bagasse - autohydrolysis of bagasse in water media has been reported due to the release of acetic acid and other organic acids from the bagasse material [15]. The addition of 3 wt.% HCl shows an increase in the rate of hydrolysis over the first 180 minutes but no overall increase in the ultimate yield of TRS. When a

significantly higher concentration of HCl is added, the TRS yield drops to nearly zero %. This may indicate an inhibition of the hydrolysis of the bagasse or, more likely, the rapid degradation of the TRS produced by hydrolysis. The addition of NaOH to the solution shows the most rapid rate of TRS yield, again to a maximum of about 6% in 5 minutes, followed by a steady degradation in the concentration. Note that for all the other results, the yield of sugars was invariant after 10h.

It must also be noted that the yield of TRS was significantly less than expected when compared to similar conditions in an aqueous medium with HCL catalyst. Fig. 3 shows a comparison between the TRS yield for an aqueous medium containing 3 wt.% HCl compared to that for the IL/water solution. It was observed that the IL/water solution dissolved the bagasse to a significant extent, yet the yield, when compared to the water only medium, was significantly less, indicating that the IL employed presented an inhibitory effect on the hydrolysis process.

B. Transport of TRS across a SLM

Fig. 4 shows the concentration of the TRS xylose and glucose in the receiving phase over 200h. Interestingly, over time, the rate of transport of xylose is enhanced for a IL/aqueous departure phase compared to that for a water departure phase. For glucose, this effect is reversed, with consistently lower transport rates for the IL/water departure phase. Note also that the rate of transport is significantly greater for xylose than glucose. Brennan et al. [13] reported that extraction of glucose and xylose from IL/water media using these extractants favours glucose extraction over xylose, thus the superior transport rate of xylose indicates that the lower molecular weight and size of the xylose/Aliquatt336/PBA moiety confers significantly higher diffusion rates across the SLM. This effect is reinforced by the results shown in Fig. 5, where transport rates using combined Aliquat 336 and PBA with Aliquat 336 only are provided. The addition of PBA with Aliquat 336 as an extractant is known to increase the extraction of monosaccharides into an organic solvent phase [10] but, as shown in the figure, the transport using Aliquat 336 only is significantly higher due to the improved diffusion rates of the Aliquat 336/monosaccharide species.
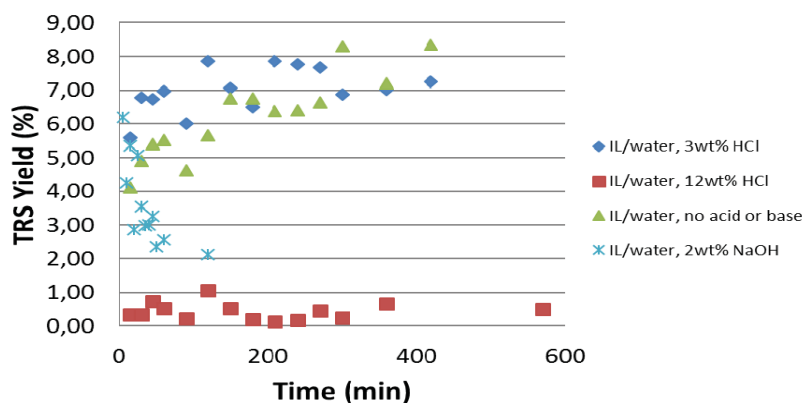

Fig. 2 TRS yields from hydrolysis of bagasse in an IL/water medium using NaOH or HCl catalyst

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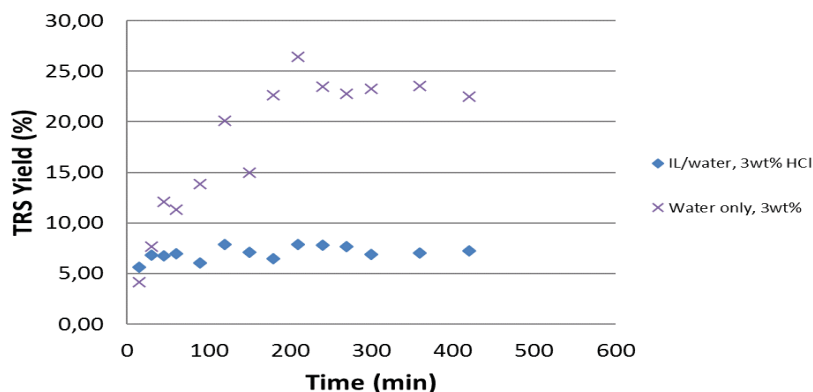International Journal of Biotechnology and Bioengineering
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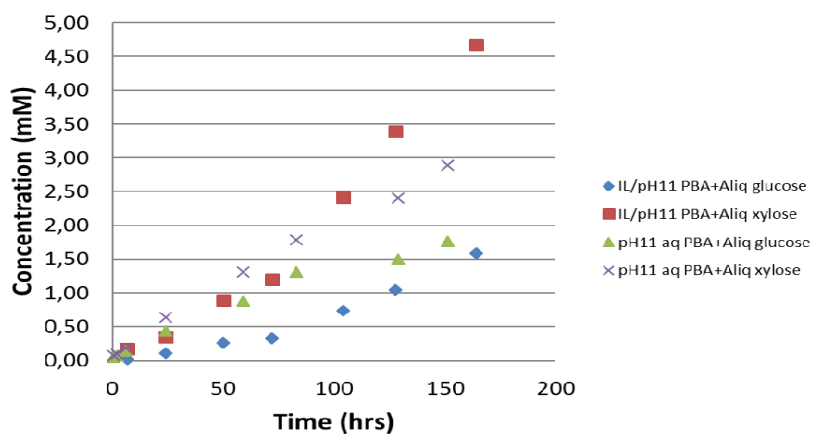Fig. 3 TRS yields from hydrolysis of bagasse in an IL/water or water medium using HCl catalyst

Fig. 4 Transport of xylose and glucose to a receiving phase using PBA/Aliquat 336 as extractants
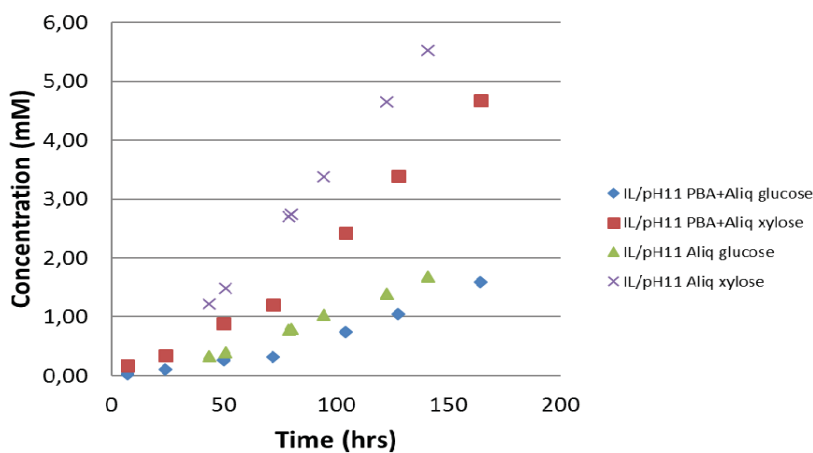
Fig. 5 Transport of xylose and glucose to a receiving phase using PBA/Aliquat 336 or Aliquat 336 only extractants

IV. CONCLUSION

Trials were conducted to measure the hydrolysis of bagasse to TRS in a IL/water medium with the addition of HCl or NaOH catalyst. It was found that, although the addition of catalyst increased the rate of TRS formation, the total yield of TRS was less than the case where a water only media was used. Therefore, the use of the IL [EMIM]OAc conferred no advantage for direct hydrolysis of the bagasse. The transport of synthetic sugar solutions across a SLM using a boronic acid/quaternary amine in an organic media was also trialed. The monosaccharide xylose was found to be transported significantly more rapidly than glucose. This may allow the different monosaccharides to be separated. The addition of the PBA extractant gave no advantage over using Aliquat 336 itself.

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