Supplementary Materials for

High-yield synthesis of glucooligosaccharides (GlOS) as potential prebiotics from glucose via non-enzymatic glycosylation

Ning Li[1], Zening Wang[1], Tianjiao Qu[1], Joseph Kraft[1], Jee-Hwan Oh[2], Jan Peter van Pijkeren[2], George Huber[3], and Xuejun Pan[1,*]

Corresponding Author

*Tel.: +1-608-2624951; Fax: +1-608-2621228; E-mail: xpan@wisc.edu

Supplementary Materials

Supplementary materials for this article are available, as follows:

Section S1 Batch addition of anhydrous LiBr to confine the released free water from glycosylation

Section S2 Choices of diluting solvents and anti-solvents for separation of GlOS from ALBTH

Fig. S1 Acid catalyzed side-reactions in ALBTH during glucose glycosylation.

Fig. S2 Formation of glucooligosaccharides (GlOS) and levoglucosan (LGA) in ALBTH at 70 °C as a function of reaction time.

Fig. S3 2D-HSQC NMR spectrum of the GlOS dissolved in D_2O from the acid catalyzed glycosylation in ALBTH at 110 °C for 10 min.

Fig. S4 HPAEC chromatograms showing the absence of cellobiose (B) in a GlOS sample (A).

Fig. S5 Comparison of the product distributions between the glucose glycosylation reactions in 4 wt% sulfuric acid (121 °C for 60 min) and in ALBTH (110 °C for 10 min).

Fig. S6 The ultra-high capacity of dissolving glucose in ALBTH with good fluidity.

Fig. S7 2D-HSQC NMR spectrum of the GlOS dissolved in D_2O from the acid catalyzed glycosylation reaction using the recovered ALBTH.

Fig. S8 Growth curves of *L. reuteri* (ATCC 6475), *L. rhamnonsus* GG, *L. casei* BFLM 218, *L. gasseri* ATCC 33323 with GlOS (9.5 g/L) + glucose (0.5 g/L) and minimal glucose (control, 0.5 g/L) as the carbon source.

Section S1. Batch addition of anhydrous LiBr to confine the released free water from glycosylation

In the progress of the glycosylation (dehydration) reaction, the released water can increase the water content in the system and drive the equilibrium opposite to the GlOS side, resulting in reduced GlOS yield. For example, approximately 22 g water is generated when 400 g glucose reacted in 171 g ALBTH to achieve 71.0% of GlOS yield. It thus increases the water contents from 38.4% to 45.4% and has negative effects on GlOS production. To address this issue, a strategy of adding anhydrous LiBr during the glycosylation reaction was tested with the aim to limit the free water released from the glycosylation reaction. The results in Table 1 clearly indicated that the addition of anhydrous LiBr (1.5 equivalent to water released, w/w) did elevate the yield of the oligosaccharides up to 74.7%, which was 3.6% higher than that in the control experiment without adding LiBr. This observation once again verified that a water deficient environment of the ALBTH system played a crucial role in GlOS production.

Section S2. Selection of diluting solvents and non-solvents for separation of GlOS from ALBTH

For practical consideration, efforts were made to afford the economical separation of GlOS from the reaction medium and the subsequent recycle/reuse of the LiBr solution. Unfortunately, it is challenging to separate the saccharides from the salt solution using solvent-solvent extraction approaches due to the strong inter-molecular interactions between ALBTH and saccharides. We noticed that GlOS and LiBr had distinct solubility in organic solvents. For example, LiBr can dissolve in acetone where GlOS are marginally soluble. Herein, a non-solvent crystallization approach was adopted to isolate GlOS and recover LiBr. In principle, a non-solvent should interrupt the interaction between the original solvent and solute, leading to an over-saturation and precipitation of the solute.[1] In this study, acetone was chosen as the non-solvent in virtue of the low dielectric constant (20.7) to over-saturate GlOS and the miscibility with ALBTH.[2, 3]

At high glucose concentration, glycosylation of glucose yielded a highly viscous syrup. Dispersing the syrup into acetone resulted in the formation of sugar gums in which a significant amount of LiBr was entrapped. It thus deteriorated the separation of GlOS and LiBr. It was found that diluting the syrup is critical for viscosity reduction prior to the non-solvent crystallization. Deionized water was firstly tested as a dilution solvent which effectively eliminated the formation of sugar gums. However, the defect was the reduced GlOS recovery since introducing water during dilution greatly elevated the solubility of GlOS in the non-solvent, as confirmed by the previous study.[4] Alternatively, the syrup was diluted with methanol (dielectric constant 32.6). In spite of its lower polarity than water, methanol was still capable of interrupting the interactions between ALBTH and the GlOS, and formed a transparent mixture

with reduced viscosity. When dropping the methanol-diluted syrup into acetone, regeneration of GlOS as white precipitates was achieved without aggregated gums.

The effect of dilution factor on the separation of GlOS from the ALBTH system was investigated. The resultant product syrup was diluted 2-15 folds with methanol and then dropped into acetone. It was found that the methanol dilution facilitated the separation and purification of the GlOS. As shown in Table S2, when the methanol dilution factor increased from 2 to 15, the distribution of LiBr and glucose in the precipitated GlOS fraction, decreased from 25.0% and 66.5% to 6.5% and 20.3%, respectively. Meanwhile, the yield of GlOS in the precipitate was relatively constant (less than 3% decrease with methanol dilution). It was confirmed that the methanol dilution of the glycosylation syrup followed by the non-solvent crystallization in acetone, could effectively isolation the GlOS from the ALBTH system.

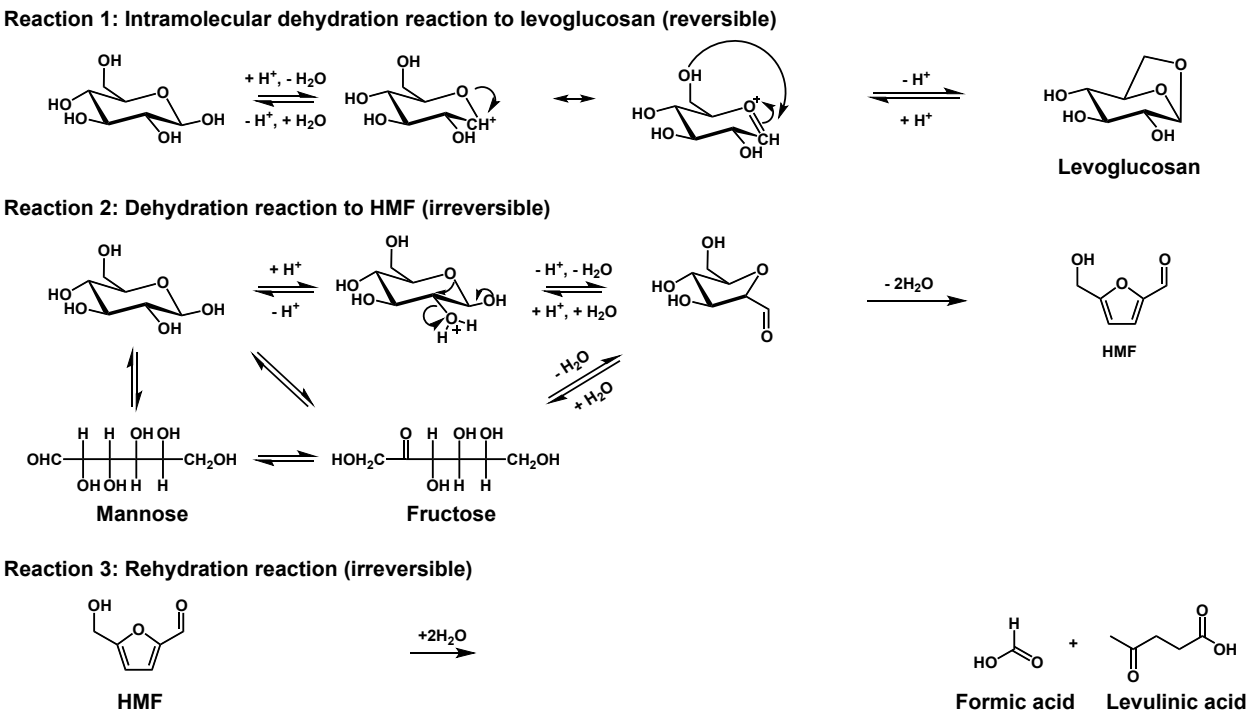

Fig. S1 Acid catalyzed side-reactions in ALBTH during glucose glycosylation.

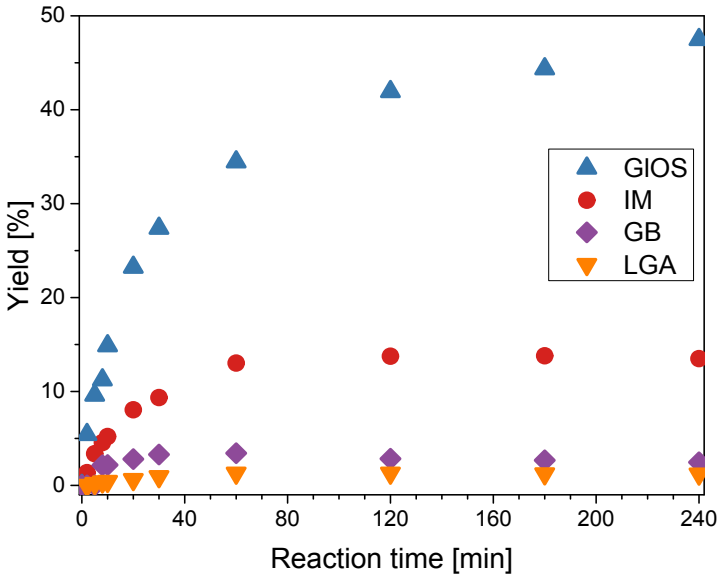

Fig. S2 Formation of glucooligosaccharides (GlOS) and levoglucosan (LGA) in ALBTH at 70 °C as a function of reaction time. The batch reaction was conducted using 19% (w/w) initial glucose concentration in 60% LiBr with 40 mM HCl. GlOS, IM, GB, and LGA denote total glucooligosaccharides (DP ≥ 2), isomaltose, gentiobiose, and levoglucosan, respectively.

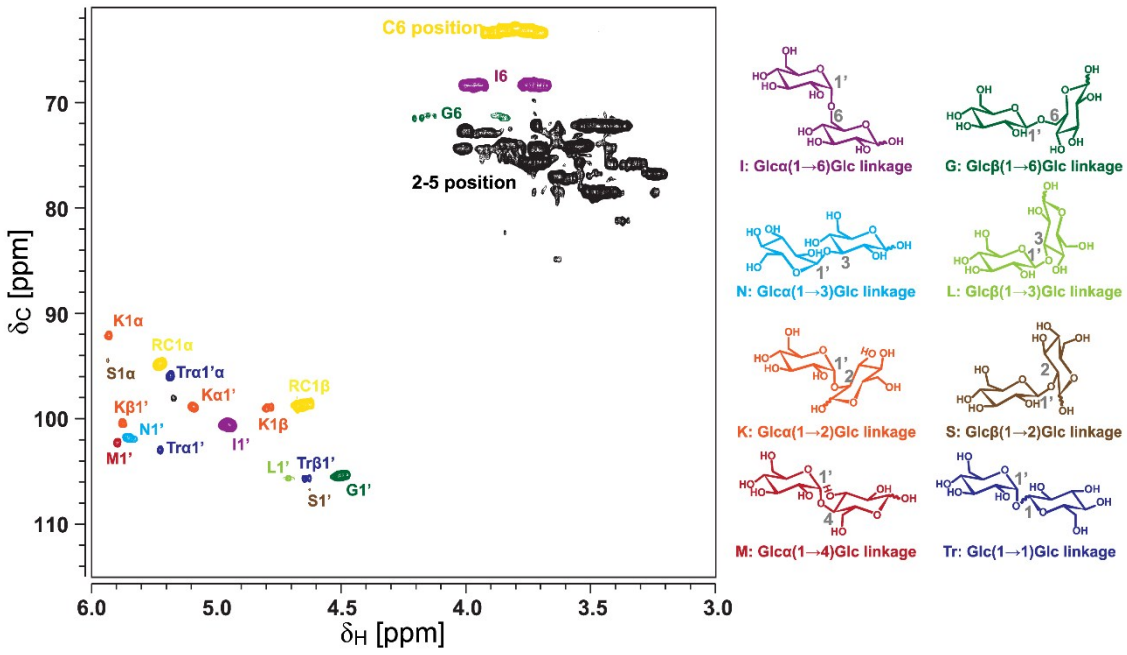

Fig. S3 2D-HSQC NMR spectrum of the GlOS dissolved in D_2O from the acid catalyzed glycosylation in ALBTH at 110 °C for 10 min. DSS was used as a chemical shift reference.

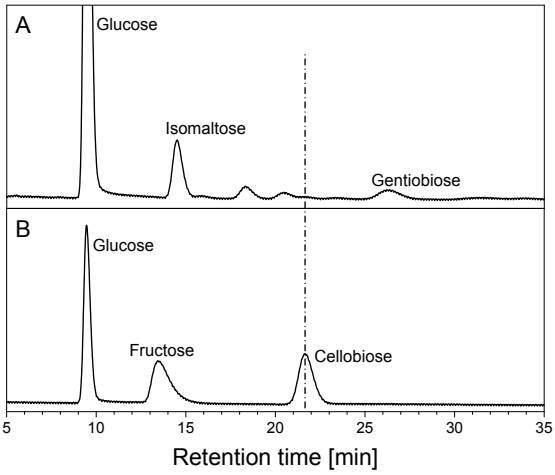

Fig. S4 HPAEC chromatograms showing the absence of cellobiose (B) in a GlOS sample (A).

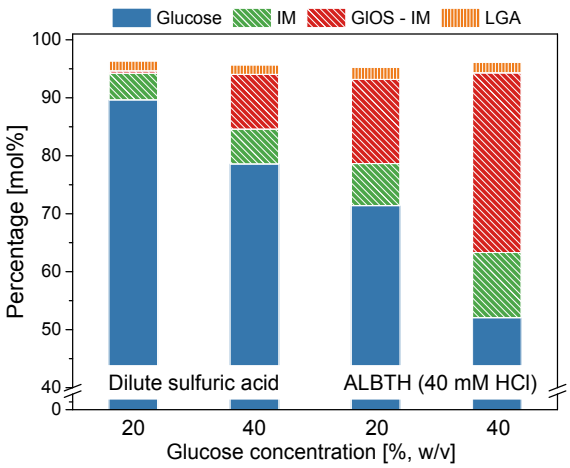

Fig. S5 Comparison of the product distributions between the glucose glycosylation reactions in 4 wt% sulfuric acid (121 °C for 60 min) and in ALBTH (110 °C for 10 min).

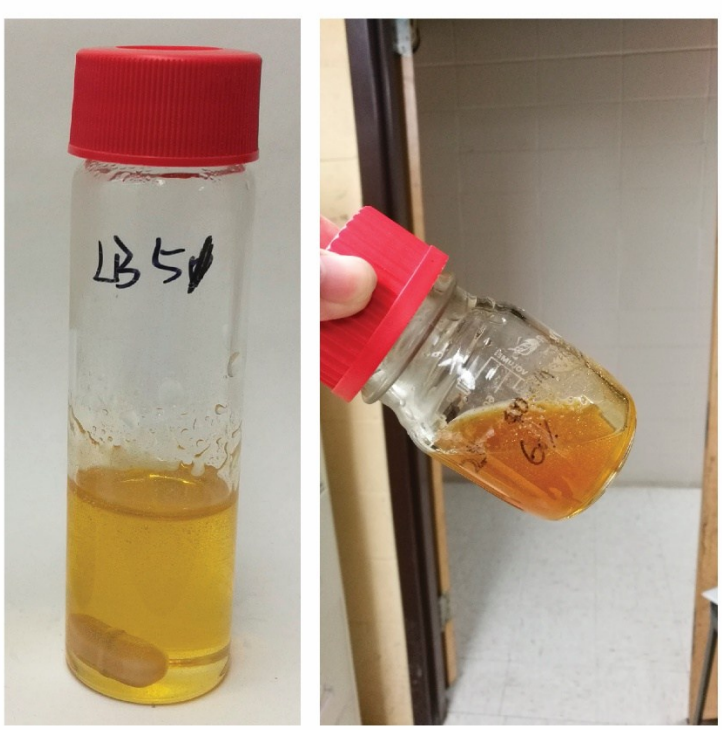

Fig. S6 The ultra-high capacity of dissolving glucose in ALBTH with good fluidity.

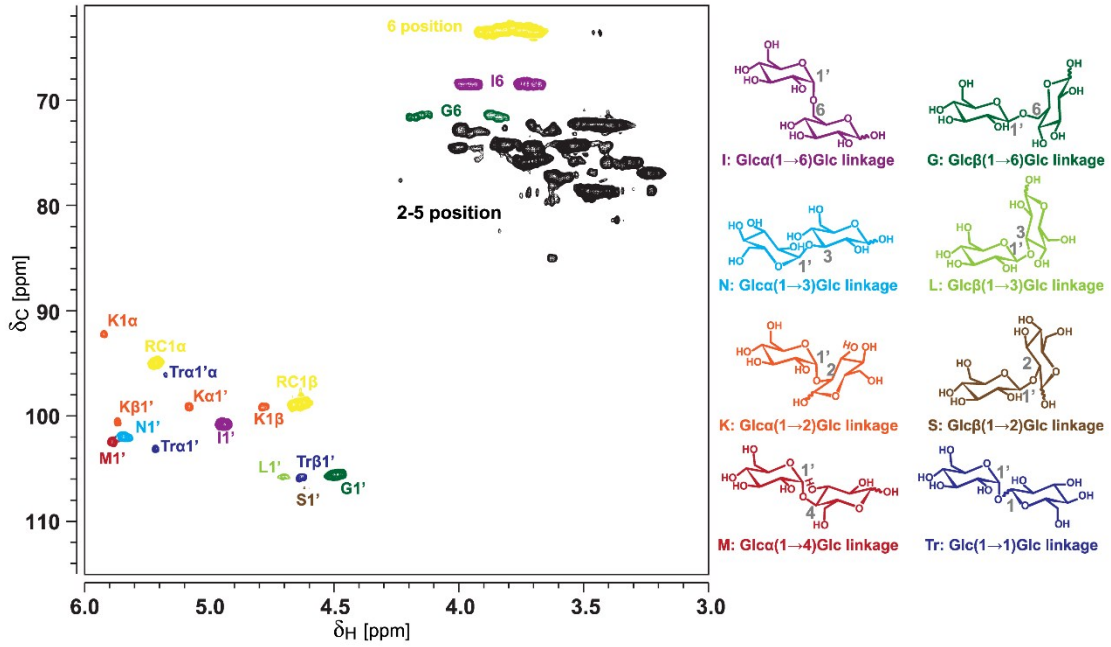

Fig. S7 2D-HSQC NMR spectrum of the GlOS dissolved in D_2O from the acid catalyzed glycosylation reaction using the recovered ALBTH. DSS was used as a chemical shift reference. The GlOS were synthesized at 70 °C for 120 min.

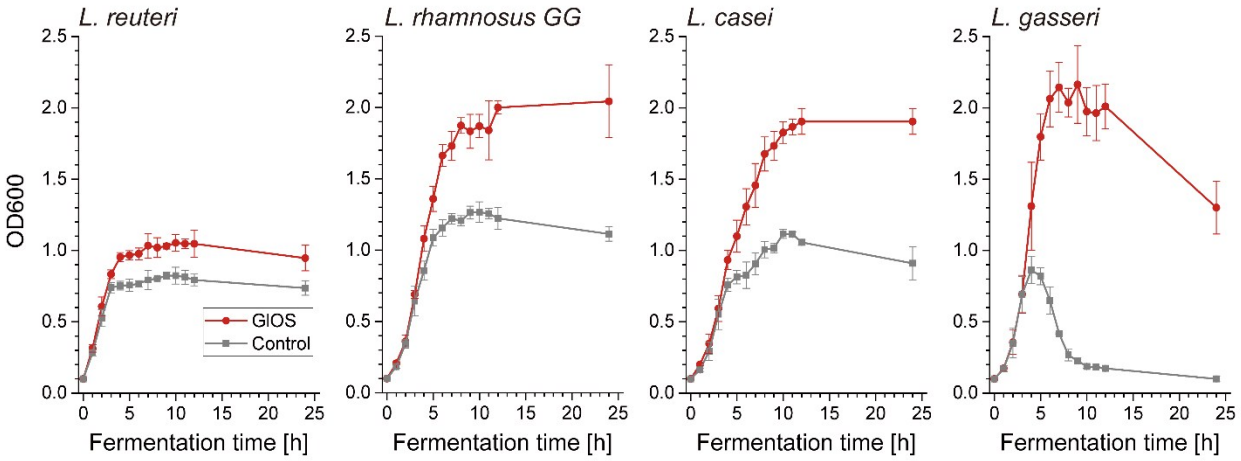

Fig. S8 Growth curves of *L. reuteri* (ATCC 6475), *L. rhamnonsus* GG, *L. casei* BFLM 218, *L. gasseri* ATCC 33323 with GlOS (9.5 g/L) + glucose (0.5 g/L) and minimal glucose (control, 0.5 g/L) as the carbon source.

Table S1 Regio- and stereo-selectivity of the GlOS synthesized in ALBTH

Glycosylic linkage	70 °C				110 °C			
	D1=10 s		D1=1 s		D1=10 s		D1=1 s	
	Cont. (%)	α/β ratio	Cont. (%)	α/β ratio	Cont. (%)	α/β ratio	Cont. (%)	α/β ratio
1→6	69.1	2.5	69.3	2.6	69.8	3.1	68.0	3.0
1→4	4.9	>10	4.5	>10	3.0	>10	3.4	>10
1→3	13.7	4.2	12.5	4.3	13.5	2.1	12.9	2.5
1→2	8.8	11.3	8.8	9.1	9.7	10.0	10.9	7.7
1→1	3.6	1.3	1.9	1.4	4.0	2.6	4.8	2.2

Table S2 Separation of GlOS and LiBr by dilution in methanol and crystallization in acetone

		Methanol dilution		
		2×	5×	15×
After reaction	LiBr (g)	5.13	5.13	5.13
	Glucose (g)	2.18	2.22	2.17
	GlOS (g)	2.77	2.73	2.78
	IM (g)	0.68	0.65	0.69
	GB (g)	0.21	0.20	0.19
Anti-solvent precipitates	LiBr (g)	1.28	0.78	0.33
	Glucose (g)	1.45	1.07	0.44
	GlOS (g)	2.47	2.43	2.40
	IM (g)	0.58	0.52	0.43
	GB (g)	0.20	0.15	0.13
Recovered LiBr hydrate	LiBr (g)	3.70	3.98	4.62
	Glucose (g)	0.51	0.75	1.30
	GlOS (g)	0.30	0.30	0.38
	IM (g)	0.02	0.06	0.17
	GB (g)	0.01	0.02	0.13
	Methanol (g)	0.08	0.01	0.05
	Acetone (g)	0.02	0.00	0.24

Note: GlOS – total oligosaccharides; IM – isomaltose; GB – gentiobiose. Reaction condition: The glycosylation reaction was conducted at 70 °C in ALBTH with 40 mM HCl for 2 h using 37% (w/v) initial glucose concentration.

Table S3 Consumption of glucose and GlOS by probiotics and the resultant SCFA production after 24 h anaerobic incubation at 37 °C

	Substrate consumption (%)				SCFA conc[a] (g/L)				
	Glucose	GlOS			LcA	FA	AA	PA	BA
		TOS[b]	IM	GB					
L.buchneri	100.0	25.5	90.7	0.0	1.2	<0.1	1.1	<0.1	<0.1
L. reuteri	99.5	12.6	22.5	100.0	0.5	<0.1	0.6	<0.1	<0.1
L. rhamnosus GG	100.0	27.2	13.9	99.0	0.3	0.6	1.5	<0.1	<0.1
L.casei	98.2	20.9	21.1	95.5	1.0	0.2	1.3	<0.1	<0.1
L. gasseri	99.4	26.1	15.4	98.1	2.1	0.3	1.4	<0.1	<0.1
B. bifidum	100.0	12.7	12.1	2.4	2.2	<0.1	<0.1	<0.1	<0.1
B. animalis	10.9	40.6	42.6	47.5	0.9	0.3	1.4	<0.1	<0.1
B. animalis[c]	69.4	42.7	79.9	74.1	1.6	0.3	2.3	<0.1	<0.1

Note: (a) Production of SCFA by fermentation using GlOS, LcA, FA, AA, PA, and BA denote lactic, formic, acetic, propionic, and butyric acid, respectively, (b) TOS denotes total oligosaccharides, (c) Incubation duration: 48 h

References

1. W. Liu, Y. Hou, W. Wu, S. Ren, Y. Jing and B. Zhang, Solubility of glucose in ionic liquid+ antisolvent mixtures, *Industrial & Engineering Chemistry Research*, 2011, **50**, 6952-6956.
2. D. Zasadowski, J. Yang, H. Edlund and M. Norgren, Antisolvent precipitation of water-soluble hemicelluloses from TMP process water, *Carbohydrate Polymers*, 2014, **113**, 411-419.
3. M. C. Gray, A. O. Converse and C. E. Wyman, in *Biotechnology for Fuels and Chemicals*, Springer, 2003, pp. 179-193.
4. L. A. Alves, J. B. Almeida e Silva and M. Giulietti, Solubility of D-glucose in water and ethanol/water mixtures, *Journal of Chemical & Engineering Data*, 2007, **52**, 2166-2170.