Utilising ionic liquids for the *in situ* swelling of Avicel towards enhanced enzymatic saccharification.

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Experimental method

Materials

Choline hydroxide (45 % solution in methanol), choline hydroxide (46 % solution in water), propionic acid (>99.5 %), hexanoic acid (>99.5 %), sodium acetate (anhydrous, 99 %), D-(+)-glucose (>99.5 %), 3,5-dinitrosalicylic acid (>98 %), sodium potassium tartrate (99 %), Avicel® PH-101 crystallinity ~75% and the enzyme, cellulase from *Aspergillus Niger* (~0.8 U/mg) were purchased from Sigma-Aldrich. Glacial acetic acid (99.7 %), citric acid (monohydrate, 99.5 %) and sodium hydroxide (98 %) were purchased from Chem-Supply. Choline acetate (98%) was purchased from IoLiTec Ionic Liquids Technologies GmbH. All chemicals were used as received without further purification.

Synthesis of cholinium ionic liquids

The cholinium ionic liquids were synthesised by neutralisation as previously described in the literature.[1-4] In brief, to a magnetically stirred choline hydroxide solution in an ice bath, an equimolar amount of carboxylic acid (glacial acetic acid, propionic acid or hexanoic acid) was slowly added drop-wise. The mixture was allowed to stir overnight at room temperature. The bulk solvent was then removed using a rotary evaporator at 40 °C and further dried under reduced pressured at 60 °C. The water content was analysed using a Karl-Fischer coulometer and determined to be <1 wt%.

Choline acetate
^{1}H NMR (270 MHz, DMSO-$_{d6}$), δ (ppm): 1.59 (3H, s, C$\underline{H}_3$), 3.13 (9H, s, 3 x C$\underline{H}_3$), 3.42 (2H, m, C$\underline{H}_2$), 3.81 (2H, m, C$\underline{H}_2$)

Choline propionate
^{1}H NMR (270 MHz, DMSO-$_{d6}$), δ (ppm): 0.88 (3H, t, C$\underline{H}_3$), 1.84 (2H, q, C$\underline{H}_2$CH$_3$), 3.14 (9H, s, 3 x NC$\underline{H}_3$), 3.43 (2H, m, NC$\underline{H}_2$), 3.83 (2H, m, C$\underline{H}_2$OH)

Choline hexanoate
^{1}H NMR (270 MHz, CDCl$_3$), δ (ppm): 0.78 (3H, t, C$\underline{H}_3$), 1.19 (4H, m, C$\underline{H}_2$C$\underline{H}_2$CH$_3$), 1.48 (2H, m, C$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 2.04 (2H, t, CO$_2$C$\underline{H}_2$), 3.27 (9H, s, 3 x NC$\underline{H}_3$), 3.60 (2H, m, NC$\underline{H}_2$), 3.99 (2H, m, C$\underline{H}_2$OH)

Enzyme hydrolysis reaction

Solutions were prepared by diluting the select IL with water to make concentrations of 100 %, 80 %, 60 %, 40 % and 20 % IL: water solutions (v/v if liquid and w/v if solid).
Avicel PH-101 (5 wt %) was added to each IL solution and incubated with magnetic stirring in a water bath (50 °C) for 1 hour to ensure homogeneous mixing. An enzyme solution (6 U/mL) was added to the mixture making the final enzyme concentration of 0.66 U/mL and further incubated for 20 minutes.
A similar setup as the above was adapted for the incubation temperature of 80 °C and was carried out using a heat block instead of a water bath. Incubation times varied from 20, 40 and 60 minutes. Samples were analysed via the DNS method and HPLC.

Glucose degradation reaction

[Cho][Pn] (2 g) was added to vial and stirred and heated at 80 °C. Glucose (20 wt%) and water (10 wt%) were then added to the IL. The reaction mixture was periodically sampled and quenched in an ice bath. Samples were analysed by HPLC.

Sample Analysis

DNS Method

The DNS reagent was prepared using 3, 5-dinitrosalicylic acid (1 g) and sodium potassium tartrate (3 g) in a sodium hydroxide solution (0.4 mM) to make a total volume of 100 mL. The DNS reagent were stored in a refrigerator in between experiments. Glucose standards were prepared from a glucose stock solution (500 µg/mL) to produce a calibration curve. Samples (0.5 mL) were diluted with water (2.5 mL) and DNS reagent (1 mL) was added. Prepared samples and standards were boiled in a water bath for 10 minutes. After allowing to cool to room temperature, spectra were taken between 400 nm to 700 nm using a Cary 5000 UV/VIS spectrophotometer where the data point was taken at 540 nm.

Soluble sugars per mL were calculated using a calibration curve derived from the standards. Relative soluble sugars were calculated using the equation as follows:

$$Relative\ soluble\ sugars = \frac{Soluble\ sugars\ of\ Samples\ (mg/mL)}{Soluble\ sugars\ of\ NaOAc\ buffer\ (mg/mL)}$$

Negative controls, in the absence of enzyme where measured no soluble sugars where detected.

HPLC

In the one-pot hydrolysis, the samples were diluted (x10 dilution) during sampling (150 µL) and centrifuged in eppendorf tubes (1.5 mL) for 30 minutes before HPLC analysis. Samples collected to study glucose degradation were similarly diluted (x10 dilution). Samples were micro-filtered using nylon filters (0.45 µm) and transferred into HPLC sample vials. The samples were analysed using an Agilent 1260 Infinity HPLC system equipped with a refractive index detector, fitted with ion-exclusion, Aminex HPX-87H column (300 x 7.8 mm, 10 mM H_2SO_4, 0.6 mL/min, 55 °C and 40 minute acquisition time) equipped with a guard cartridge (cation H^+). The glucose standards for calibration were prepared at 0.1, 1.0, 2.0, 3.0 and 4.0 mg/mL and all samples were run with an internal standard of citric acid present.

POM

Avicel in the choline IL's were observed using a Nikon i80 polarised optical microscope (POM) using x10 and x20 magnification. Avicel (5 wt%) in the IL was heated and stirred at 80 °C for [Cho][Pn] and [Cho][Hex] and 90 °C for [Cho][OAc] (due to the melting point of the IL) for 1 hour. After 1 hour, a sample of the mixture was removed, placed on a glass slide and observed using the POM and an image captured.

FT-IR

The infrared spectra were obtained using a Bruker LUMOS FT-IR microscope. The infrared spectra were recorded using attenuated total reflectance mode (ATR) in the range of 4000-600 cm^{-1}. The scans were averaged from 128 scans at a resolution of 4 cm^{-1}. Spectra were then baseline corrected and normalised to the 1022 cm^{-1} peak.

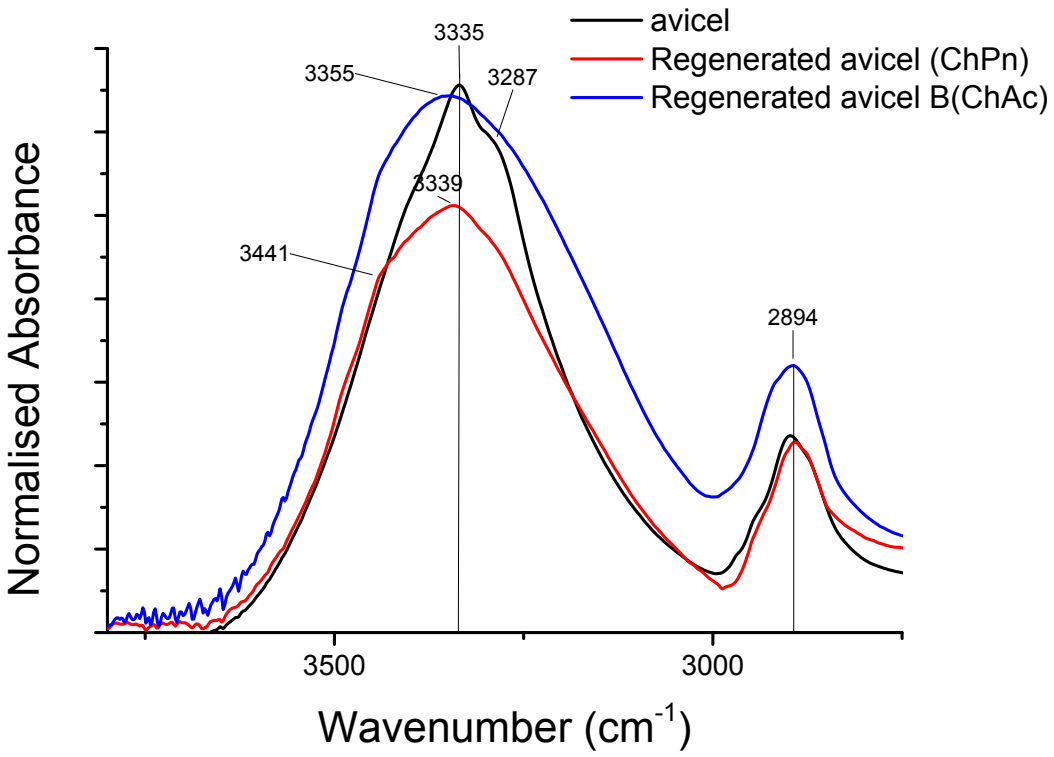

Fig.S.1 Enlargement of the hydrogen bonded –OH (3335 cm^{-1}) and –CH (2894 cm^{-1}) regions, comparing pre-treated Avicel with native Avicel.

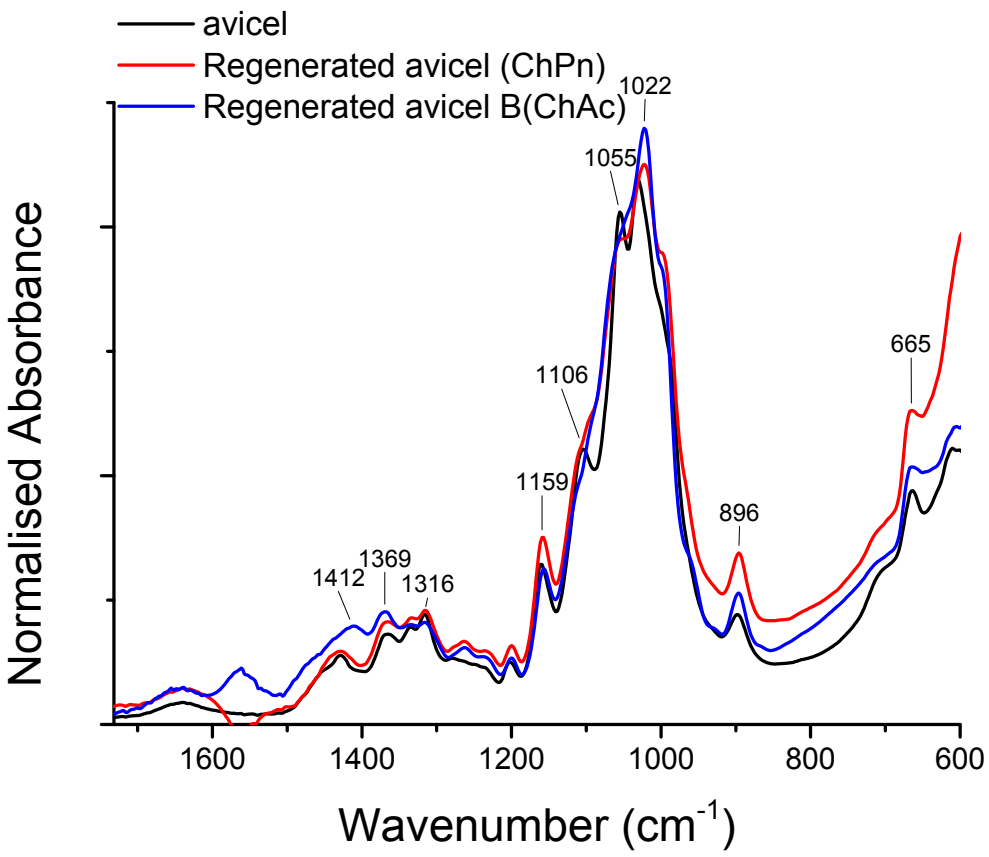

Fig.S.2 Enlargement at the lower wavenumber regions, comparing pre-treated Avicel with native Avicel.

REFERENCES

1. Y. Fukaya, Y. Iizuka, K. Sekikawa and H. Ohno, *Green Chemistry*, 2007, **9**, 1155-1157.
2. O. Winther-Jensen, R. Vijayaraghavan, J. Sun, B. Winther-Jensen and D. R. MacFarlane, *Chemical Communications*, 2009, 3041-3043.
3. M. Petkovic, J. L. Ferguson, H. Q. N. Gunaratne, R. Ferreira, M. C. Leitao, K. R. Seddon, L. P. N. Rebelo and C. S. Pereira, *Green Chemistry*, 2010, **12**, 643-649.
4. N. Muhammad, M. I. Hossain, Z. Man, M. El-Harbawi, M. A. Bustam, Y. A. Noaman, N. B. Mohamed Alitheen, M. K. Ng, G. Hefter and C.-Y. Yin, *Journal of Chemical & Engineering Data*, 2012, **57**, 2191-2196.