Design of Wall-Destructive but Membrane-Compatible Solvents

Supporting information

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Materials and Methods

Materials

All structures and the abbreviation of ILs and ZIs used in this study are shown in Supplementary Fig. 1. $[C_2mim]OAc$ and $[C_2mim][EtOSO_3]$ was purchased from Iolitech GmbH and used after drying. $[C_2mim][MeSO_3]$ was purchased from Iolitech GmbH and used after passing aluminum oxide as dichloromethane solution and drying. [Ch]OAc[1], C_1imC_3S[2], C_1imC_4S[2] and $[C_2mim][(MeO)(H)PO_2]$[3] were synthesized as reported. OE_2imC_3C, C_1imC_3C, $[OE_2eim]OAc$, $[C_8mim]OAc$ were synthesized as shown below. Methanol, tetrahydrofuran (THF), diethyl ether, acetonitrile, acetic acid, dichloromethane and hexane were purchased from Kanto Chemical Co., Inc. and used as received. Imidazole, sodium hydride (in paraffin liquid) and ethyl 4-bromobutyrate were purchased from Tokyo Chemical Industry Co., Ltd. and used as

received. 1-Bromo-2-(2-methoxyethoxy)ethane was purchased from Nakalai tesque Inc. and used as received. 1-Methylimidazole was purchased from Acros Organics and used as received. Amberlite IRN 78A, alminum oxide and dimethyl sulfoxide were purchased from Sigma-Aldrich Co., Llc. and used as received. Commercial cellulase (Cellic® CTec2), a complex blend of cellulase and β-glucosidase, was obtained from Novozymes Japan, Ltd. A recombinant *E.coli* (KO11) was purchased from ATCC. Tryptone, NaCl, glucose and chloroamphenicol were purchased from Nacalai Tesque Inc. and used as received. Yeast extract was purchased from Becton, Dickinson and Company and used as received.

Synthesis of OE_2imC_3C

Under an argon atmosphere sodium hydride in paraffin liquid (15.7 g, 656 mmol as sodium hydride) was suspended in THF (50 mL). Imidazole (13.8 g, 202 mmol), which was dissolved in 50 mL THF, was added to the solution. The mixture was stirred at room temperature for 24 h. Then, 1-bromo-2-(2-methoxyethoxy)ethane (37.0 g, 202 mmol) was added to the solution. After stirring at 70 °C for 6 h, the resulted suspension was filtered under reduced pressure to remove white precipitation. The solvent was removed under reduced pressure. The product was purified

by distillation and a fraction was collected at 125 °C under reduced pressure of 1 mmHg to obtain an imidazole derivative, 1-(2-(2-methoxyethyl)ethyl)-1*H*-imidazole (OE_2im). OE_2im (25.5 g, 150 mmol) was washed with hexane several times to remove paraffin. After evaporation of water, OE_2im was dissolved in 250 mL acetonitrile, and then ethyl 4-bromobutyrate (29.3 g, 150 mmol) was added to the solution under argon atmosphere. The mixture was refluxed at 70 °C for 16 h. The resulting bromide salt was converted to zwitterion by passing an aqueous solution of the bromide salt through a column filled with anion exchange resin (Amberlite IRN 78A). After filtration, resulting liquid was dried under reduced pressure. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si) δ = 2.13-2.27 (4H, m, C*H*$_2$CO and C*H*$_2$CH$_2$CO), 3.37 (3H, s, C*H*$_3$O), 3.51-3.65 (4H, m, CH$_3$OC*H*$_2$C*H*$_2$), 3.86 (2H, t, *J* = 3.6 Hz, OC*H*$_2$CH$_2$N), 4.40 (2H, t, *J* = 6.7 Hz, NC*H*$_2$CH$_2$CH$_2$COO), 4.66 (2H, t, *J* = 3.7 Hz, OCH$_2$C*H*$_2$N), 7.29 and 7.49 (2H, t, *J* = both 1.6 Hz, NC*H*C*H*N), 11.00 (1H, s, NC*H*N). ^{13}C NMR (100 MHz; $CDCl_3$; Me_4Si) δ = 27.20 and 34.30 (NCH$_2$*C*H$_2$*C*H$_2$COO), 48.94 (OCH$_2$*C*H$_2$N), 49.47 (N*C*H$_2$CH$_2$CH$_2$COO), 58.65 (*C*H$_3$O), 69.19 (O*C*H$_2$CH$_2$N), 69.93 and 71.29 (O*C*H$_2$*C*H$_2$O), 121.22 and 122.58 (NCH*C*HN), 138.73 (N*C*HN), 176.63 (CH$_2$*C*OO). Elemental analysis: $OE_2imC_3C\cdot 2.5H_2O$ (Found: C, 48.0; H, 8.4; N, 9.3. Calc. for C12H25N2O6.5: C, 47.8; H, 8.4; N, 9.3%).

Synthesis of C_1imC_3C

1-Methylimidazole (22.2 g: 270 mmol) and ethyl 4-bromobutyrate (53.7 g: 270 mmol) was added to 20 mL of acetonitrile, and stirred at 50 ^oC for 5h. The resulting solution was dried under reduced pressure. The solid was washed with excess amount of diethylether for three times. The resulting solid was dried under reduced pressure. The bromide salt was converted to zwitterion by passing an aqueous solution of the bromide salt through a column filled with anion exchange resin. After filtration, resulting liquid was dried under reduced pressure. ^1H NMR (400 MHz; DMSO-d_6; Me_4Si) δ = 1.77 (2H, t, J = 6.4 Hz, CH_2CO), 1.86 (2H, J = 7.3 Hz, quin, CH_2CH_2CO), 3.82 (3H, s, CH_3N), 4.13 (2H, t, J = 6.8 Hz, NCH_2CH_2), 7.66 and 7.76 (2H, t, J = both 1.6 Hz, NC*H*C*H*N), 9.50 (1H, s, NC*H*N). ^{13}C NMR (100 MHz; DMSO-d_6; Me_4Si) δ = 27.80 (*C*H_2CH_2CO), 35.36 (*C*H_2CO), 36.10 (*C*H_3N), 49.61 (NCH_2*C*H_2), 122.91 and 123.89 (N*C*H*C*HN), 137.700 (N*C*HN), 174.02 (CH_2*C*OO).

Synthesis of $[OE_2eim]OAc$

1-Bromo-2-(2-methoxyethoxy)ethane (25.6 g, 140 mmol) was dissolved into 50 mL of diethyl ether and washed with 25 mL of water for two times to remove stabilizer. After

evaporation of diethylether, molecular sieves were added to remove water. The resulting liquid and 1-ethylimidazole (13.5 g, 140 mmol) was added to 50 mL of THF, and stirred at 80 ^{o}C for 4h. The resulting solution showed two phases; the IL phase and the THF phase. The IL phase was dissolved into 10 mL of methanol and dropped into excess amount of diethyl ether. This procedure was repeated four times. After drying under reduced pressure, the bromide anion was converted to hydroxide anion by passing an aqueous solution of the bromide salt through a column filled with anion exchange resin. After filtration, resulting liquid was neutralized by a small excess amount of acetic acid and dried under reduced pressure. To remove the excess acetic acid, the resulting solution was diluted with dichloromethane/methanol solution and passed through a column filled with aluminium oxide. The resulting solution was dried under reduced pressure. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si) δ = 1.58 (3H, t, J = 7.4 Hz, CH_3CH_2N), 1.98 (3H, s, CH_3CO), 3.37 (3H, s, CH_3O), 3.51-3.67 (4H, m, CH$_3$OCH_2CH_2), 3.86 (2H, t, J = 4.0 Hz, OCH_2CH$_2$N), 4.36 (2H, quin, J = 7.4 Hz, CH$_3$CH_2N), 4.62 (2H, t, J = 5.4 Hz, OCH$_2$CH_2N), 7.34 and 7.55 (2H, t, J = both 1.6 Hz, NCHCHN), 11.23 (1H, s, NCHN). ^{13}C NMR (100 MHz; $CDCl_3$; Me_4Si) δ = 15.29 (NCH$_2$CH_3), 25.28 (CH_3COO), 44.67 (NCH_2CH$_3$),

49.06 ($OCH_2\mathit{C}H_2N$), 58.90 ($\mathit{C}H_3O$), 69.22 ($O\mathit{C}H_2CH_2N$), 70.01 and 71.36 ($O\mathit{C}H_2\mathit{C}H_2O$), 120.31 and 122.96 (N*C*H*C*HN), 139.16 (N*C*HN), 177.79 ($CH_3\mathit{C}OO$).

Synthesis of [C_8mim]OAc

1-Methyl-3-octylimidazolium chloride was purchased from Sigma-Aldrich Co., Llc. and the chloride anion was converted to hydroxide anion by passing an aqueous solution of the chloride salt through a column filled with anion exchange resin. After filtration, resulting liquid was neutralized by a small excess amount of acetic acid and dried under reduced pressure. To remove the excess acetic acid, the resulting solution was diluted with dichloromethane and then passed through a column filled with aluminium oxide. The resulting solution was dried under reduced pressure. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si) δ = 0.87 (3H, t, J = 7.0 Hz, $C\mathit{H}_3(CH_2)_7N$), 1.2–1.4 (10H, m, $CH_3CH_2(C\mathit{H}_2)_5CH_2N$), 1.88 (2H, m, $CH_3C\mathit{H}_2(CH_2)_6N$), 2.01 (3H, t, $C\mathit{H}_3COO$), 4.09 (3H, s, $C\mathit{H}_3N$), 4.29 (2H, t, J = 6.8 Hz, $CH_3(CH_2)_6C\mathit{H}_2N$), 7.27 and 7.37 (2H, t, J = both 1.8 Hz, NC*H*C*H*N), 11.42 (1H, s, NC*H*N). ^{13}C NMR (100 MHz; $CDCl_3$; Me_4Si) δ = 14.02($CH_2\mathit{C}H_3$), 22.52, 26.20, 28.92, 28.97 and 31.61 ($NCH_2(\mathit{C}H_2)_5CH_2CH_3$), 25.58

(CH_2CH_3), 30.28 (CH_3COO), 36.21 (NCH_3), 49.84 (NCH_2), 121.18 and 122.84 (NCHCHN), 140.45 (NCHN), 178.04 (CH_3COO).

Differential scanning calorimetry (DSC) measurement

DSC (DSC-60A Plus; Shimadzu Co.) was measured to investigate melting point and glass transition temperature with heating/cooling rate of ±10 °C/min. The lowest temperature in the measurement was –100 °C.

Dissolution of cellulose, hemicellulose and lignin

Cellulose (Avicel PH-101) and lignin (lignin, alkali) were purchased from Sigma-Aldrich Co., Llc. and used as received. Hemicellulose (xylan) was purchased Tokyo Chemical Industry Co., Ltd. and purified by dissolution into dimethylsulfoxide (80 ^oC) followed by filtration before use. Each compound (1 wt%) was added to 250 mg of OE_2imC_3C under stirring at 100 or 120 °C. When the compounds dissolved, we added another 1 wt% of the compounds, and the procedure was repeated until they did not dissolve. The dissolution was confirmed by visual observation.

Measurement of Kamlet-Taft parameters of OE_2imC_3C

Measurement of the Kamlet-Taft parameters of a series of ILs and OE_2imC_3C was carried out as follows. The solvatochromic dyes, 2,6-dichloro-4-(2,4,6-triphenyl- 1-pyridinio)phenolate (Reichardt's dye #33, from Fluka), 4-nitroaniline (from Tokyo Chemical Industries Co., Ltd) and *N*,*N*-diethyl-4-nitroaniline (from Kanto Chemical Co., Inc.) were used as received. The dyes were added to 0.25 g of ILs and OE_2imC_3C as concentrated methanol solutions. The methanol was then carefully removed by vacuum drying. These IL solutions were placed into quartz cells with 0.1 mm light-path length. From the wavelength at the maximum absorption (λ_{max}) determined, the α, β and π^* values were calculated by use of the following equations:

$$\nu\,(\text{dye}) = 1/(\lambda_{\max(\text{dye})}10^{-4})$$

$$E_T(30) = 0.9986\,(28\,592/\lambda_{\max\ (\text{Reichardt's dye \#33})}) - 8.6878$$

$$\pi^* = 0.314(27.52 - \nu_{(N,N\text{-diethyl-4-nitroaniline})})$$

$$\alpha = 0.0649E_T(30) - 2.03 - 0.72\pi^*$$

$$\beta = (1.035\,\nu_{(N,N\text{-diethyl-4-nitroaniline})} + 2.64 - \nu_{(4\text{-nitroaniline})})/2.80$$

Since [Ch]OAc is solid at room temperature, Kamlet-Taft parameters of [Ch]OAc were measured at room temperature as supercooled state after heating at 80 °C.

Assay of inhibition by ZIs and ILs toward growth of *E. coli* KO11

ZI or IL solutions (2 mL) at various concentration were prepared by dilution with media (10 g/L of tryptone, 5 g/L of NaCl and 5 g/L of yeast extract). *E. coli* KO11, which can convert glucose to ethanol, were precultured aerobically at 37 °C in the test tube containing the pure medium free from ZIs and ILs. The cells were obtained by centrifugation and transferred into the medium/IL or ZI mixtures as to be an initial optical density at 600 nm (OD_{600}) of 0.1. The broth was incubated at 37 °C for 24 h using a reciprocal shaker at 160 rpm, and the OD_{600} of solutions was measured. The response to the ILs and ZIs was evaluated based on the relative growth, which is defined as the percentage of the OD_{600} at 24 h of the medium/IL or ZI mixtures relative to that of the pure medium. The half maximal effective concentration based on growth (EC_{50}) was determined as the IL or ZI concentration at which the relative growth was reduced to 50%. LiCl content of LiCl/DMAc is 8 wt%.

Assay of inhibition by ZIs and ILs toward fermentation by *E. coli* KO11

ZI or IL solutions (10mL, 0.5 mol/L) were prepared by dilution with the media (10 g/L of tryptone, 5 g/L of NaCl, 5 g/L yeast extract, 50 g/L of glucose and 100 mg/L chloroamphenicol). *E. coli* KO11 was precultured aerobically at 37 °C in the test tube containing the pure medium free from ZIs and ILs. The precultured *E. coli* KO11 was transferred into the solutions as to be OD_{600} of 1.0. The broth was incubated at 37 °C for 48 h with stirring. Ethanol concentration of the samples was measured by high performance liquid chromatography (HPLC). LiCl/DMAc solution (0.5 mol/L) denotes 0.5 mol/L of DMAc with 8 wt% LiCl relative to DMAc.

Analysis of glucose, ethanol and other metabolites

The compounds were analysed with HPLC. The HPLC system was as follows: a refractive index detector (Shimadzu Co.), a ICSep ION-300 column (Tokyo Chemical Industry Co. Ltd.). Sulfuric acid aqueous solution (5 mmol/L) was used as the mobile phase. The injected volume of the sample was 10 μL, and the column was heated at 85 °C. The flow rate of 0.4 mL/min was applied.

Ethanol production from biomass via starch-like process: dissolution, hydrolysis and fermentation in one-pot system

Bagasse (128 mg) was added to 1.28 g of OE_2imC_3C and stirred at 120 °C for 8 h. After dilution of the resulting solution with 200 mmol/L acetate buffer (pH: 5.0) as to be 0.5 mol/L, 50 μL of cellulase cocktail was added into the solution. The solution was stirred at 50 °C for 48 h to hydrolyse. Neutrients (0.1 g of tryptone, 0.05 g of NaCl, 0.05 g yeast extract and 1 mg chloroamphenicol) without water and the precultured *E. coli* KO11 were added to the resulting solution (initial OD_{600}:1.0). Fermentation was conducted at 37 °C for 48 h, and the concentration of ethanol of the solution was analysed with HPLC.

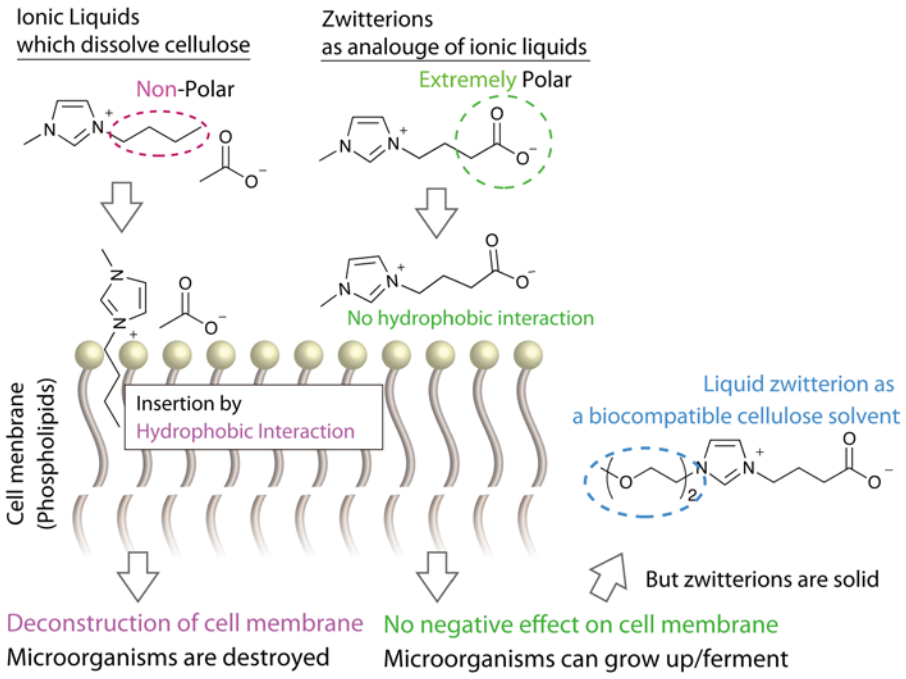

Figure S1. Mechanism of toxicity of ionic liquid (left) and our non-toxic strategy (middle and right).

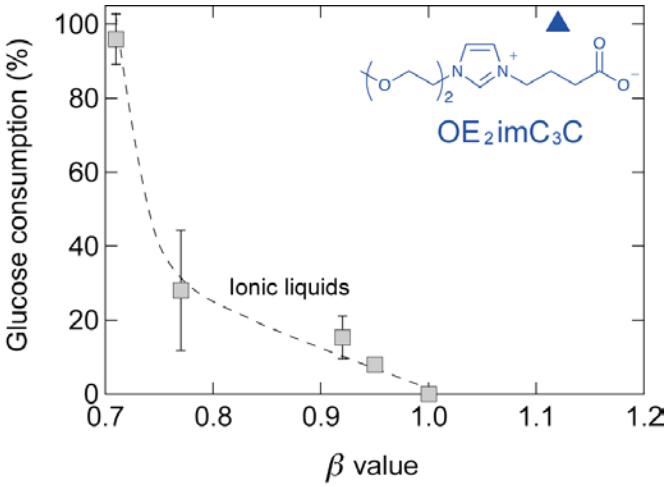

Figure S2. Relation between β values of ILs and OE_2imC_3C and glucose consumption by *E. coli* KO11 in 0.5 mol/L IL or OE_2imC_3C solutions after 48h of fermentation. β values of $[C_2mim]OAc$, and $[C_2mim][(MeO)(H)PO_2]$ are from the literature[4].

Table S1. EC_{50}, concentration of ethanol produced by *E. coli* KO11 in 0.5 mol/L IL or ZI or organic solvent solutions, and cellulose dissolution ability of the solvents.

	IL or ZI	EC_{50} (g/L)	Ethanol concentration (g/L)	Cellulose solubility
$[C_2mim]OAc$	IL	9	1.0	Soluble
$[C_2mim][(MeO)(H)PO_2]$	IL	19	0.0	Soluble
$[C_2mim][MeSO_3]$	IL	12	3.2	Insoluble
$[C_2mim][EtOSO_3]$	IL	12	18.3	Insoluble
$[OE_2eim]OAc$	IL	7	0.3	Soluble
$[C_8mim]OAc$	IL	<0.01	–[b]	Soluble
[Ch]OAc	IL	70	3.0	Insoluble
OE_2imC_3C	ZI	158	19.4	Soluble
C_1imC_3C	ZI	141	20.4	–[d]
C_1imC_3S	ZI	>200	21.1	–[d]
C_1imC_4S	ZI	>200	20.4	–[d]
LiCl/dimethylacetamide[a]	–	28	3.8	Soluble
Dimethyl sulfoxide	–	91	–[b]	Insoluble
Ethanol	–	17	–[b]	Insoluble
–	–	–	20.3[c]	–

[a]Content of LiCl is 8 wt%.

[b]Not measured.

[c]Ethanol concentration produced via fermentation in pure medium.

[d]Not determined because they are solid below 100 °C.

Table S2. Yield of metabolites as by-products after 48h of fermentation by *E. coli* KO11 in 0.5 mol/L IL solutions.

Ionic Liquid	Yield / consumed glucose (mol%)			
	Acetic Acid	Formic Acid	Lactic Acid	Succinic Acid
$[C_2mim][MeO(H)PO_3]$	–[a]	–[a]	–[a]	–[a]
$[C_2mim]OAc$	–[b]	–[a]	–[a]	0.10
$[C_2mim][MeSO_3]$	0.25	–[a]	–[a]	0.14
$[C_2mim][EtOSO_3]$	0.29	0.01	–[a]	0.05
[Ch]OAc	–[b]	–[a]	–[a]	0.30
–	0.39	–[a]	–[a]	0.05

[a]Not detected in the chromatograms.

[b]Not calculated because the signal of acetic acid overlapped with the large signal of acetate anion of ILs.

Reference

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