Supplementary Information for

A droplet-to-digital (D2D) microfluidic device for single cell assays

Steve C.C. Shih,[1, 2] Philip C. Gach,[1, 2] Jess Sustarich, [1, 2] Blake A. Simmons,[1, 2] Paul D. Adams,[2] Seema Singh,[1, 2] and Anup K. Singh [1, 2 *]

[1] Sandia National Laboratories, 7011 East Ave, Livermore, CA, USA

[2] Joint Bioenergy Institute (JBEI), 5855 Hollis St. Emeryville, CA 94608, USA

* Corresponding Author
email: aksingh@sandia.gov
tel: (925) 294-1260
fax: 925-294-3020

Keywords: digital microfluidics, droplet microfluidics, high throughput screening, biofuel, ionic liquid

Growth rate sample calculation

To calculate the growth rate, we used equation (1) shown in the main text and we obtained the values from the exponential part of the curve (i.e. not the stationary region).

For example, here are two tables that show the values we used to calculate the growth rate for [C2mim] [Cl] at 37.5 mM concentration for the well-plate and the D2D device.

Table S1 – A table showing the values used to calculate the growth rate for [C2mim][Cl] at 37.5 mM in well-plates

Concentration	T_{final}-$T_{initial}$	O.D. at $T_{initial}$	O.D. at T_{final}	$\ln(T_{final}/T_{initial})$	Growth rate [h^{-1}]	Average G.R.	Standard deviation
37.5	4.71986111	0.06639999	0.16810001	0.9288622	0.19679863	**0.2038682**	**0.02505408**
	4.71986111	0.0527	0.162	1.12298092	0.23792669		
	4.71986111	0.07340001	0.17	0.83987443	0.17794473		
	4.71986111	0.06369999	0.16590001	0.95720083	0.20280275		

Table S2 – A table showing the values used to calculate the growth rate for [C2mim][Cl] at 37.5 mM in D2D device

Concentration	T_{final}-$T_{initial}$	# of cells at $T_{initial}$	# of cells at T_{final}	$\ln(T_{final}/T_{initial})$	Growth rate [h^{-1}]	Average G.R.	Standard deviation
37.5	6	2	6	1.098612	0.183102	**0.22005**	**0.04367**
	6	2	7	1.252763	0.208794		
	6	1	5	1.609438	0.26824		

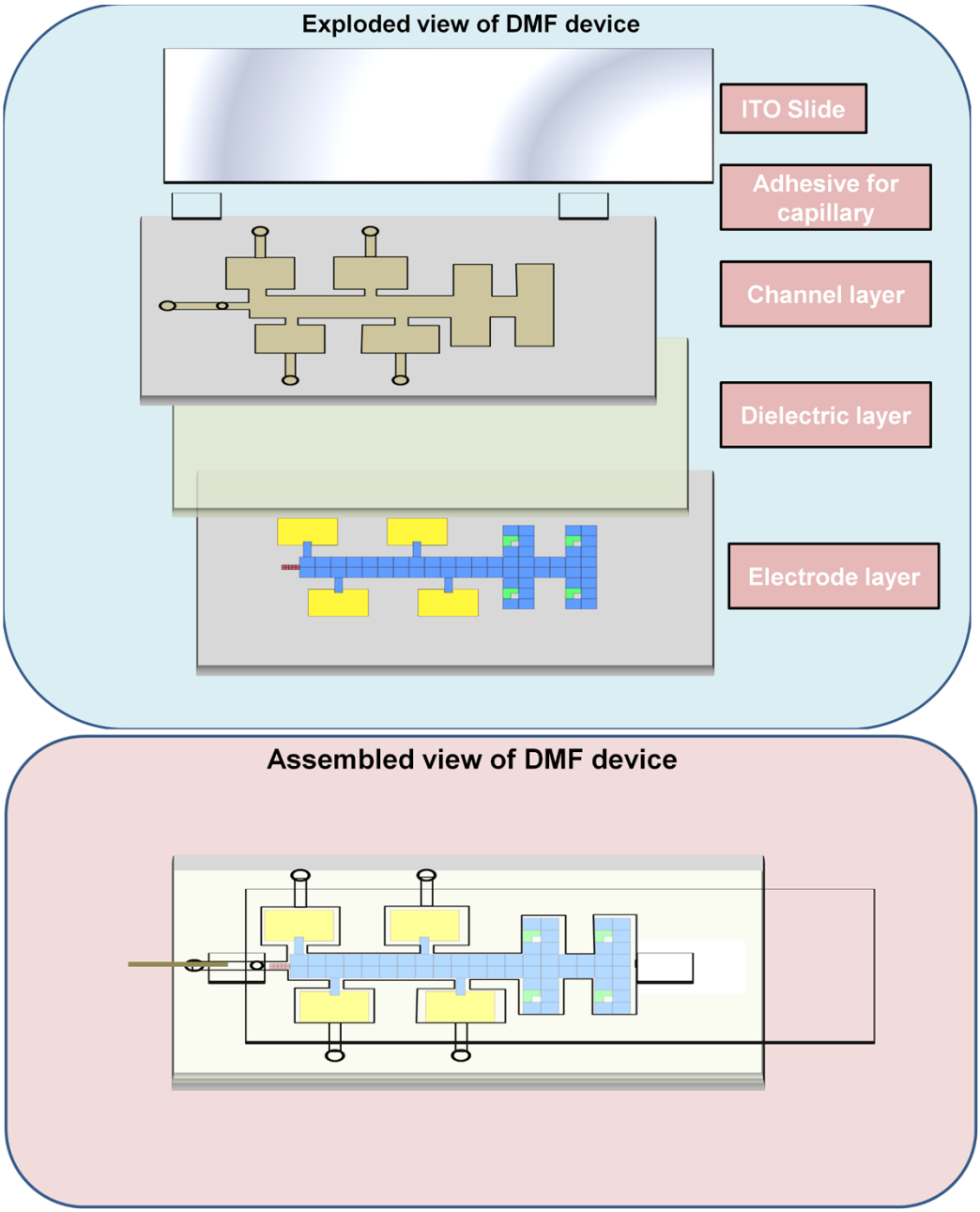

Figure S1: Exploded view of the digital microfluidic device. The two-plate DMF device consists of the following layers: electrode, dielectric, channel, adhesive, and ITO. The channel layer shows the device outline in brown which is a ~140 μm channel trench. The adhesive is a ~40 μm spacer (measured using a caliper) and therefore giving a total gap height of 180 μm. The assembled view of the device shows the mated capillary from the droplet microfluidic device.

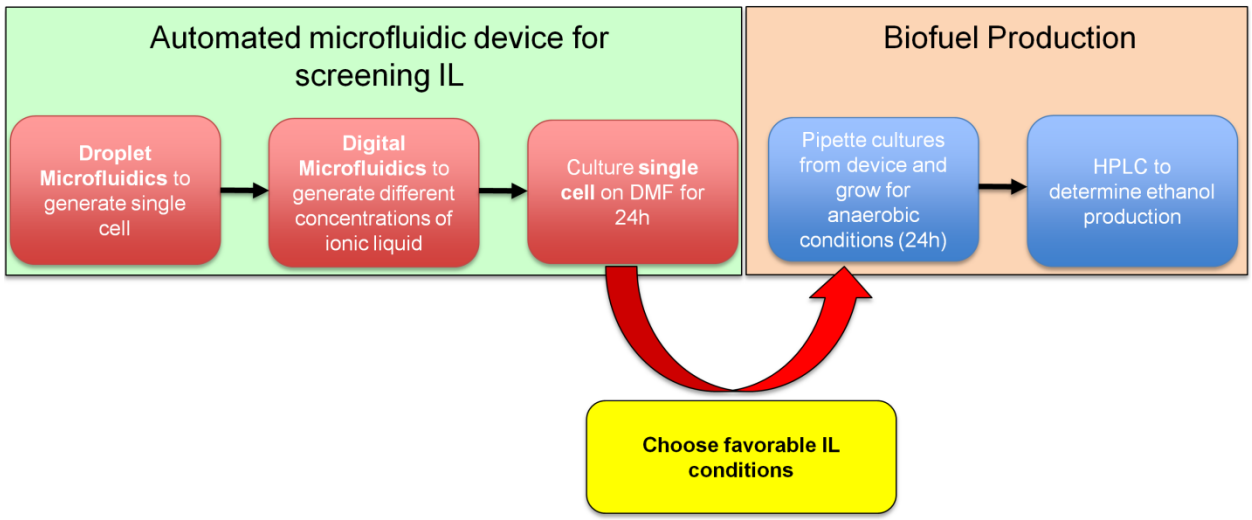

Figure S2: Workflow from microfluidic screening of the effects of ionic liquid to ethanol production. An automated D2D microfluidic device is used to encapsulate and culture a single cell and screen the impact of ionic liquid on cell growth (shown in green area). Next, the cultures can be grown in anaerobic condition to determine the effects of ionic liquid on ethanol production (shown in orange).

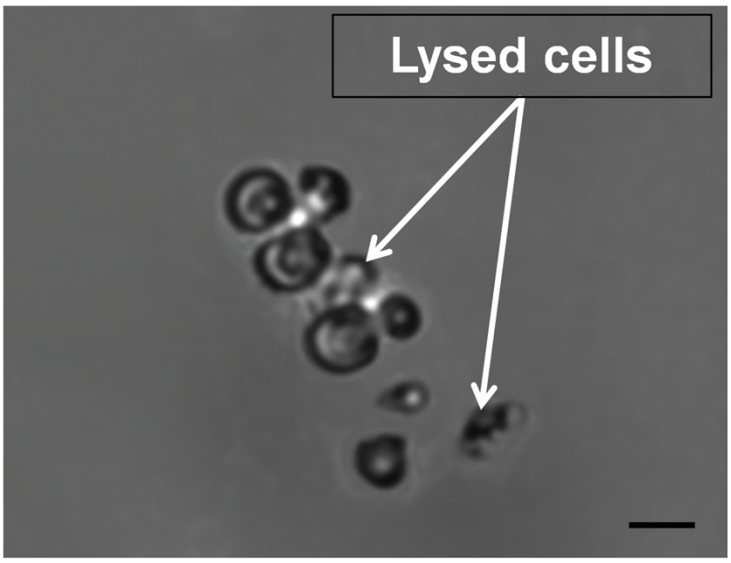

Figure S3 – Image of lysed yeast cells in 150 mM [C2mim] [OAc]. The type and concentration of this IL is toxic to the yeast cell and therefore starts to inhibit growth. Picture was taken at 24 h. Scale bar is 10 μm.

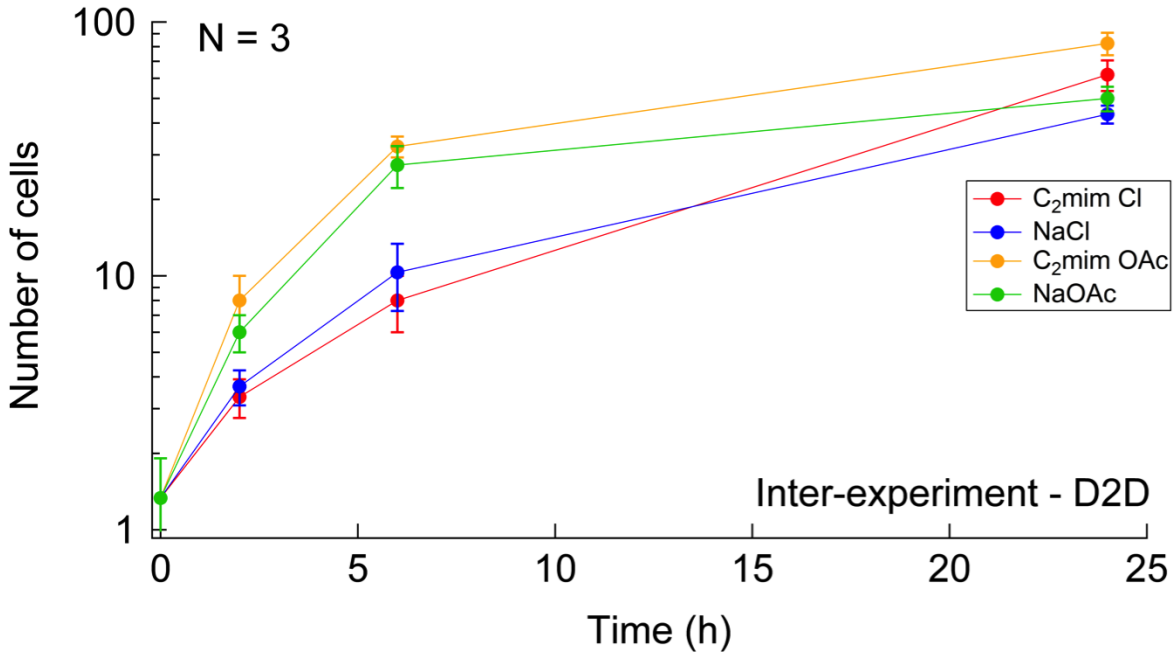

Figure S4 – Growth curves for yeast cultured in 0 mM IL/salt in the D2D microfluidic device. The D2D microfluidic experiments were evaluated in triplicate (i.e. intra-experiments) and error bars represents one standard deviation. The different growth curves for each IL/salt show the inter-experiments for 0 mM concentration. Statistical differences were found at the 2 h and 6 h time points ($p < 0.05$).

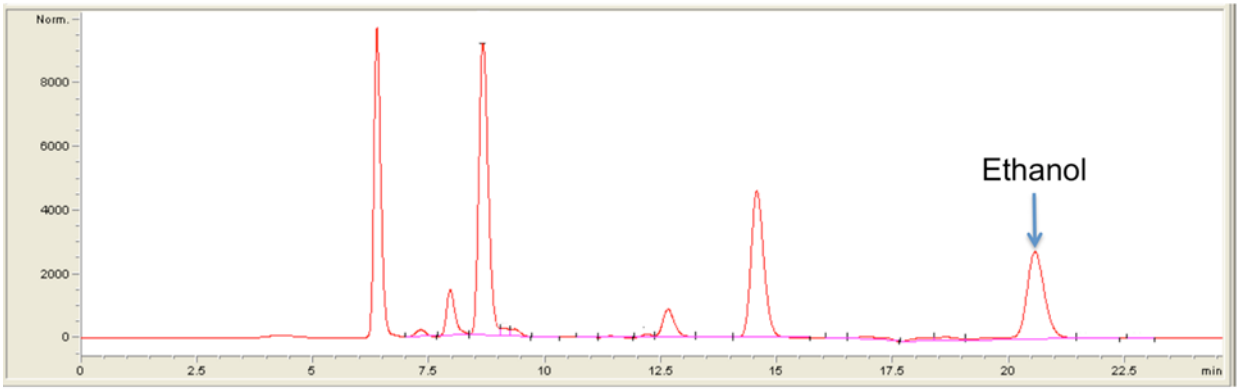

Figure S5 – HPLC chromatogram shown for a yeast sample. Ethanol is well separated (occurring ~ 21 min) from all the other separated sugars and byproducts found in the anaerobic yeast sample. A sample run typically requires 25 min to complete.

Table S3: Growth rates for yeast cultures grown in IL using the well-plate or D2D device

	Concentration of IL (mM)							
	0		37.5		75		150	
Ionic Liquid	W.P	D2D	W.P	D2D	W.P	D2D	W.P	D2D
NaCl	0.262±0.04	**0.345±0.12**	0.253±0.04	**0.379±0.05**	0.244±0.05	**0.288±0.02**	0.233±0.08	**0.211±0.05**
NaOAc	0.228±0.04	**0.510±0.08**	0.268±0.05	**0.444±0.07**	0.241±0.08	**0.379±0.08**	0.095±0.06	**0.227±0.10**
[C_2mim] [OAc]	0.239±0.03	**0.540±0.06**	0.117±0.01	**0.445±0.05**	0.049±0.02	**0.354±0.08**	0.017±0.03	**0.09±0.03**
[C_2mim] [Cl]	0.208±0.02	**0.304±0.08**	0.204±0.03	**0.220±0.04**	0.218±0.03	**0.250±0.06**	0.168±0.05	**0.173±0.10**

W.P – Well plate; D2D – Droplet-to-digital microfluidics; Growth rate units: [h^{-1}]