Supporting Information

Solvent optimization for bacterial extracellular matrices: a solution for the insoluble

Thomas Seviour^{1,*}, Piyarat Weerachanchai^{2,3}, Jamie Hinks¹, Dan Roizman¹, Scott A. Rice^{1,4}, Linlu Bai³, Jong-Min Lee^{3,*}, Staffan Kjelleberg^{1,5}

¹ Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Nanyang

Technological University, SBS-01N-27, Singapore 637551. Email: twseviour@ntu.edu.sg; Fax: +65 6515-6751; Tel: +65 6592-7902.

² Nanyang Environment and Water Research Institute (NEWRI), Nanyang Technological University, Singapore.

³ School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637459. Email: jmlee@ntu.edu.sg; Fax: +65 6794-7553; Tel: +65 6513-8129.

⁴ School of Biological Sciences (SBS), Nanyang Technological University, Singapore 637551.

⁵ Centre for Marine BioInnovation and School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, 2052, Australia.

Table S1 Summary of solubilities of biofilms in all the organic solvents and ionic liquids screened.

	Р.	GAO-	DPAO	Glycogen			
	aeruginosa	enriched	granules				
	RSCV	granules					
Ionic liquids							
1-ethyl-3-methylimidazolium	High	High	High	High			

acetate (EMIM-Ac)				
1-ethyl-3-methylimidazolium diethyl phosphate (EMIM-DEP)	High	High	High	High
1-butyl-3-methylimidazolium chloride (BMIM-Cl)	High	High	High	High
1,3-dimethylimidazolium methylsulfate	Very Low	Very Low	Very Low	Not considered
1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide	Very Low	Very Low	Very Low	Not considered
Trihexyl(tetradecyl)phosphonium bis(trifluoromethylsulfonyl)imide	Very Low	Very Low	Very Low	Not considered
1-Butyl-3-methylimidazolium tetrafluoroborate	Very Low	Very Low	Very Low	Not considered
N-Butyl-1-methylpyrrolidinium dicyanamide	Very Low	Very Low	Very Low	Not considered
Trihexyl(tetradecyl)phosphonium tris(pentafluoroethyl) trifluorophosphate	Very Low	Very Low	Very Low	Not considered
1-Butyl-3-methylimidazolium tris(pentafluoroethyl)trifluoropho sphate	Very Low	Very Low	Very Low	Not considered
1-Butyl-1-methyl-pyrrolidinium tetracyanoborate	Very Low	Very Low	Very Low	Not considered
Organics				
Ethanolamine	Medium	Medium	Medium	High
Dimethyl sulfoxide	Very Low	Very Low	Very Low	High
Dimethylformamide	Very Low	Very Low	Very Low	High

Dimethylacetamide	Medium	Medium	Low	High
Diethylene glycol	Very Low	Very	Very	High
		Low	Low	
2-pyrollidone	Very Low	Very	Very	High
		Low	Low	
Allyl alcohol	Very Low	Very	Very	Very Low
		Low	Low	
Propylene Carbonate	Very Low	Very	Very	Very Low
		Low	Low	
1-butanol	Very Low	Very	Very	Very Low
		Low	Low	
2-butanol	Very Low	Very	Very	Very Low
		Low	Low	
2 propanol	Very Low	Very	Very	Very Low
		Low	Low	
Water	Very Low	Very	Very	High
		Low	Low	



Fig. S1 Representative images of *Pseudomonas aeruginosa* RSCV pellicle biofilms in phosphate buffer solution (PBS) pH 7 (10 mg dry solid/mL solvent) (A) PBS with NaOH to pH 10.0 (B), in Luria Bertani (LB) broth (C), ethanolamine (D), 1-butanol (E), ammonium bicarbonate buffer solution (50 mM), NaOH to pH 10.3 (F), N,N-dimethylacetamide (DMAc) (G), 1-ethyl-3methylimidazolium acetate (EMIM-Ac) (H) and 40:60 EMIM-Ac:DMAc (I) before and after solubilisation for 3 days at 60 °C.



Fig. S2 Representative images of Glycogen Accumulating Organism-enriched granular biofilms in phosphate buffer solution (PBS) pH 7 (10 mg dry solid/mL solvent) (A) PBS with NaOH to pH 10.0 (B), in Luria Bertani (LB) broth (C), ethanolamine (D), 1-butanol (E), ammonium bicarbonate buffer solution (50 mM), NaOH to pH 10.3 (F), N,N-dimethylacetamide (DMAc) (G), 1-ethyl-3-methylimidazolium acetate (EMIM-Ac) (H) and 40:60 EMIM-Ac:DMAc (I) before and after solubilisation for 3 days at 60 °C.



Fig. S3 Representative images of Denitrifying Polyphosphate Accumulating Organism-enriched granular biofilm in phosphate buffer solution (PBS) pH 7 (10 mg dry solid/mL solvent) (A) PBS with NaOH to pH 10.0 (B), in Luria Bertani (LB) broth (C), ethanolamine (D), 1-butanol (E), ammonium bicarbonate buffer solution (50 mM), NaOH to pH 10.3 (F), N,N-dimethylacetamide (DMAc) (G), 1-ethyl-3-methylimidazolium acetate (EMIM-Ac) (H) and 40:60 EMIM-Ac:DMAc (I) before and after solubilisation for 3 days at 60 °C.



Fig. S4 MW distributions of all three biofilms in N,N-dimethylacetamide only (i.e. with no ionic liquid present) showing the complete absence of peaks corresponding to high MW compounds (i.e. $> 2x10^5$ Da).