Supplementary Material for Pick and Fish, "Optimisation of Drinking Water Biofilm Cell Detachment and Sample Homogenisation Methods for Rapid Quantification via Flow Cytometry"

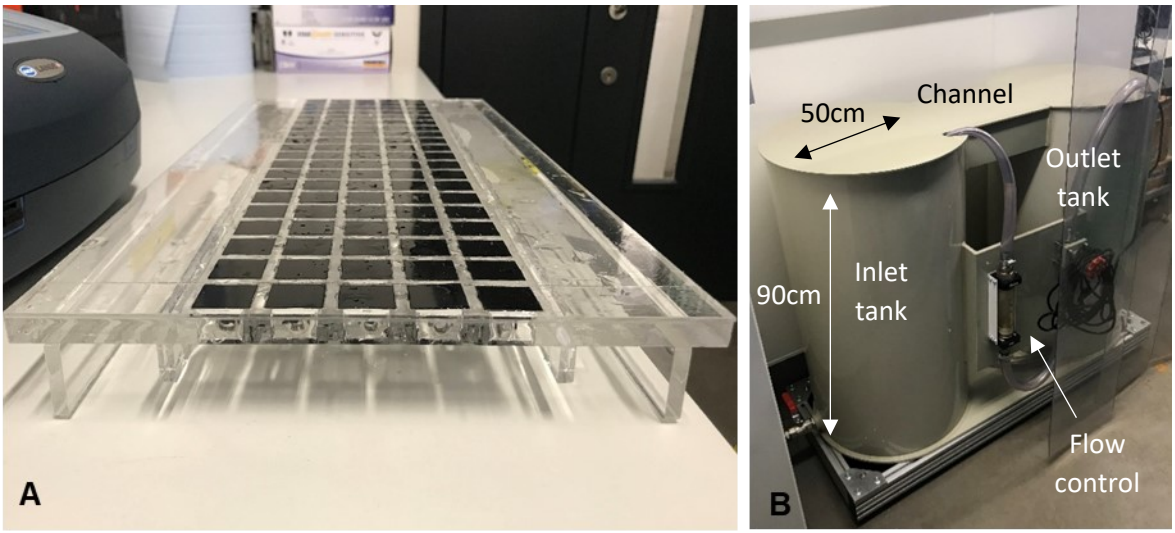

Figure S1. Unpressurised bio-reactor designed and constructed at the University of Sheffield for drinking water biofilm growth. A. High-density polyethylene (HDPE) coupons (2cm x 2cm) in a holding grid, facilitating drinking water biofilm growth and sampling. Grid was inserted into the channel of the reactor shown in B: two cylindrical tanks, one connected to the inlet tap, one fitted with an overflow drain. Inlet rate was set to provide a hydraulic retention time of 24 hours in the tank. A submersible pump (Swell UK, Cheshire) was installed in the outlet tank, allowing water to be re-circulated around the tank at a steady state flow of 4 L/min. The entire system had a volume of 0.180 m^3.

Table S1: Bulk-water quality supplying the coupons or tube sections during each biofilm removal or biofilm homogenisation experiment. Mean and standard deviation of bulk-water parameters collected weekly (in triplicate) are presented (3 months n=36; 6 months n=72, 9 months n=108).

Experiment (Figures in manuscript)	Biofilm Removal			Biofilm Homogenisation:	
	Brushing Optimisation (Fig 2)	Brushing Optimisation (Fig 3)	Brushing vs sonicating water bath (Fig 4)	Vortex vs. sonicating needle (Fig 5 &6)	Vortex vs sonicating needle (Fig 7)
Biofilm Age	6 months	9 months	3 months	3 months	6 months
Biofilm sampling surface	BMD*	HDPE¥ coupon	BMD*	BMD*	BMD*
Water Quality Parameter	**Mean (St. Dev)**	**Mean (St. Dev)**	**Mean (St. Dev)**	**Mean (St. Dev)**	**Mean (St. Dev)**
Total Chlorine (mg/l)	0.54 (0.04)	0.50 (0.07	0.58 (0.02)	0.57 (0.05)	0.45 (0.06)
Free Chlorine (mg/l)	0.41 (0.07)	0.41 (0.07)	0.49 (0.04)	0.45 (0.06)	0.57 (0.050
Temperature (°C)	12.83 (84)	13.61 (0.80)	12.64 (0.42)	14.70 (0.79)	12.90 (1.54)
pH	6.95 (0.11)	6.89 (0.14)	6.99 (0.09)	6.77 (0.11)	6.95 (0.09)
ORP (millivolts)	482 (17.42)	484 (15.79)	481 (4.79)	494 (11.53)	471 (11.97)
Turbidity (NTU)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)

*BMD = Biofilm Monitoring Device; ¥ HDPE = High Density Polyethylene

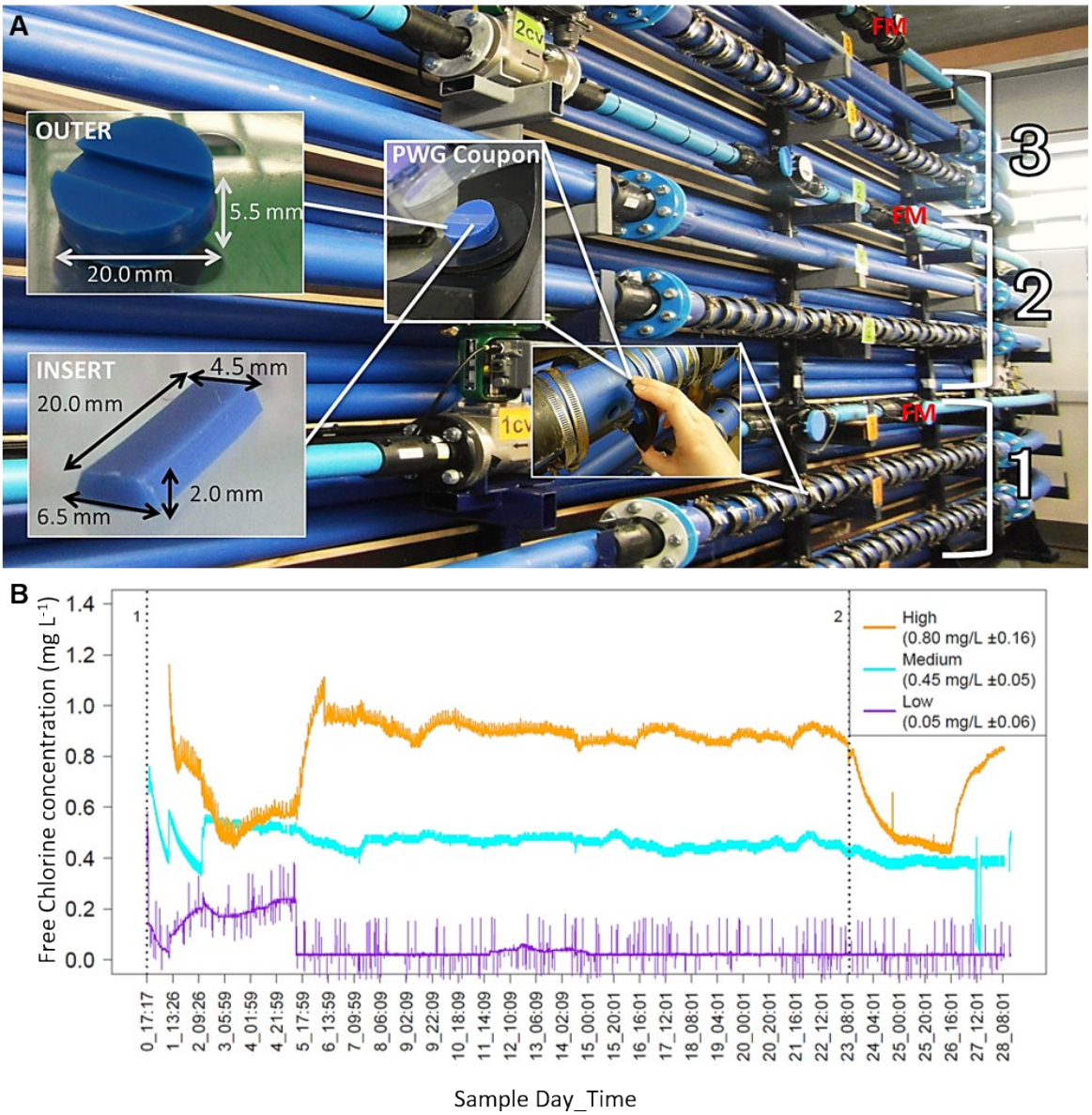

Figure S2 Full-scale pipe loop facility (A) and residual chlorine concentrations (B) during biofilm growth. A. Three independent high-density polyethylene (HDPE) pipes, 203 m long with Pennine Water Group Coupons facilitating biofilm sampling. B: residual free chlorine concentration, raw data is plotted (n≥39986), average ± standard deviation. 1 = dosing started, 2 = dosing interrupted in High-chlorine for ~48 hours. Adapted from Fish et al., 2020.

18

19

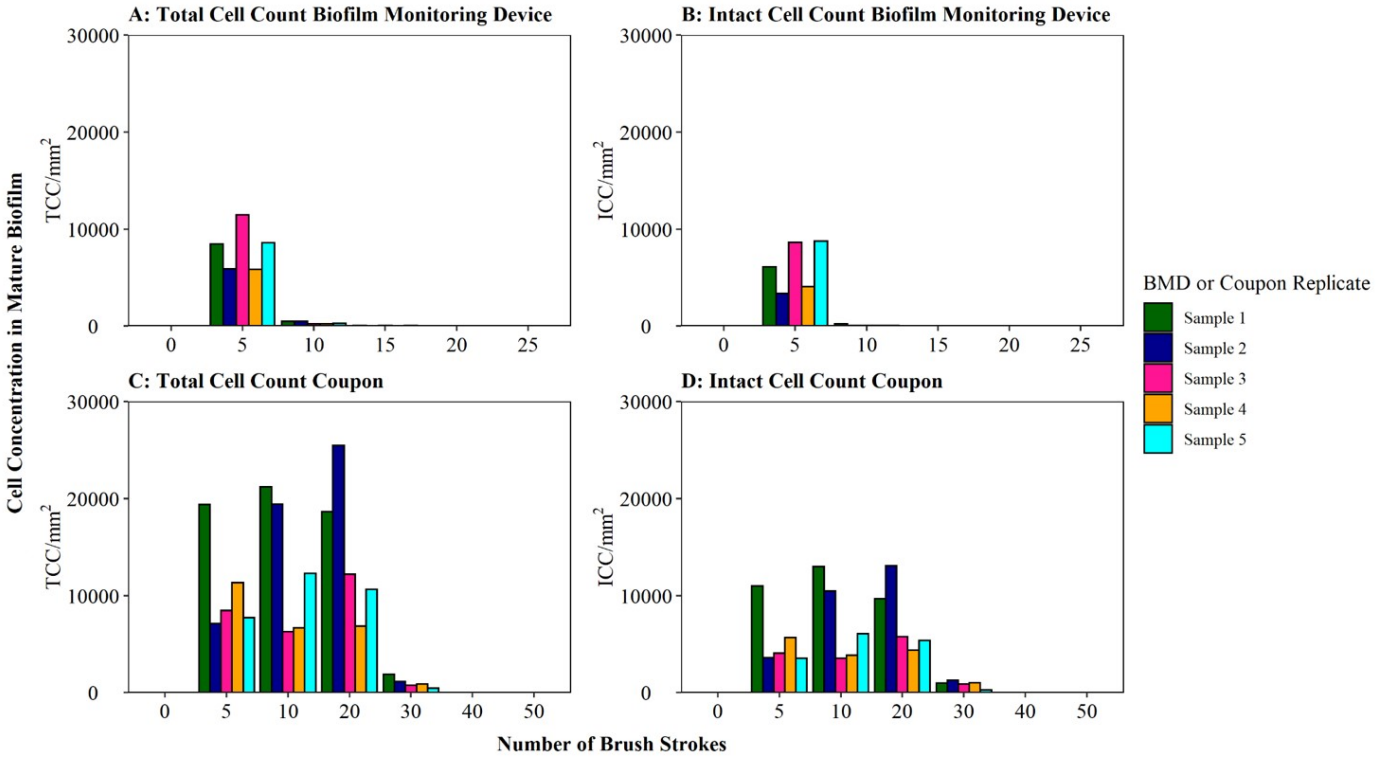

Figure S3 Brushing efficiency to remove biofilm from Biofilm Monitoring Devices (A&B) and High-density polyethylene coupons (C&D). Total cell counts (TCC) and intact cell counts (ICC) obtained from each BMD or coupon (n=5) are presented. Brushes refers to brush strokes. "0" brushes is defined as phosphate buffer solution (PBS) poured over the surface of the BMD or coupon to act as a control.

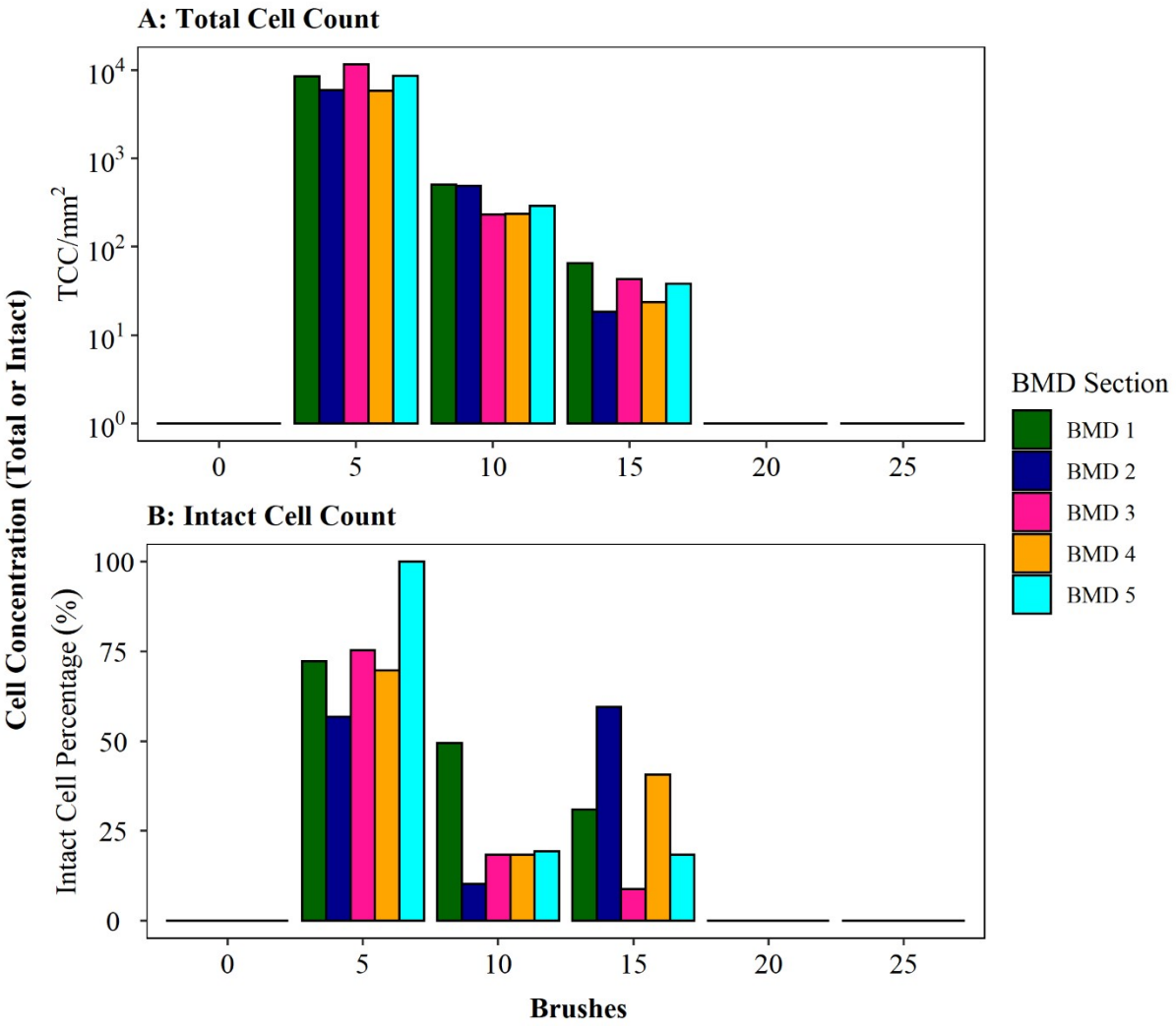

Figure S4. Impact of brushing on cell viability when used to remove biofilm from a Biofilm Monitoring Device (BMD). BMD pipe sections (n=5, see key) were brushed for a series of 0, 5, 10, 15 20 and 25 brushes. Total cell counts (TCC) and percentage of TCC that were intact are presented for each replicate. "0" brushes is defined as phosphate buffer solution (PBS) poured over the surface of the coupon to act as a control.

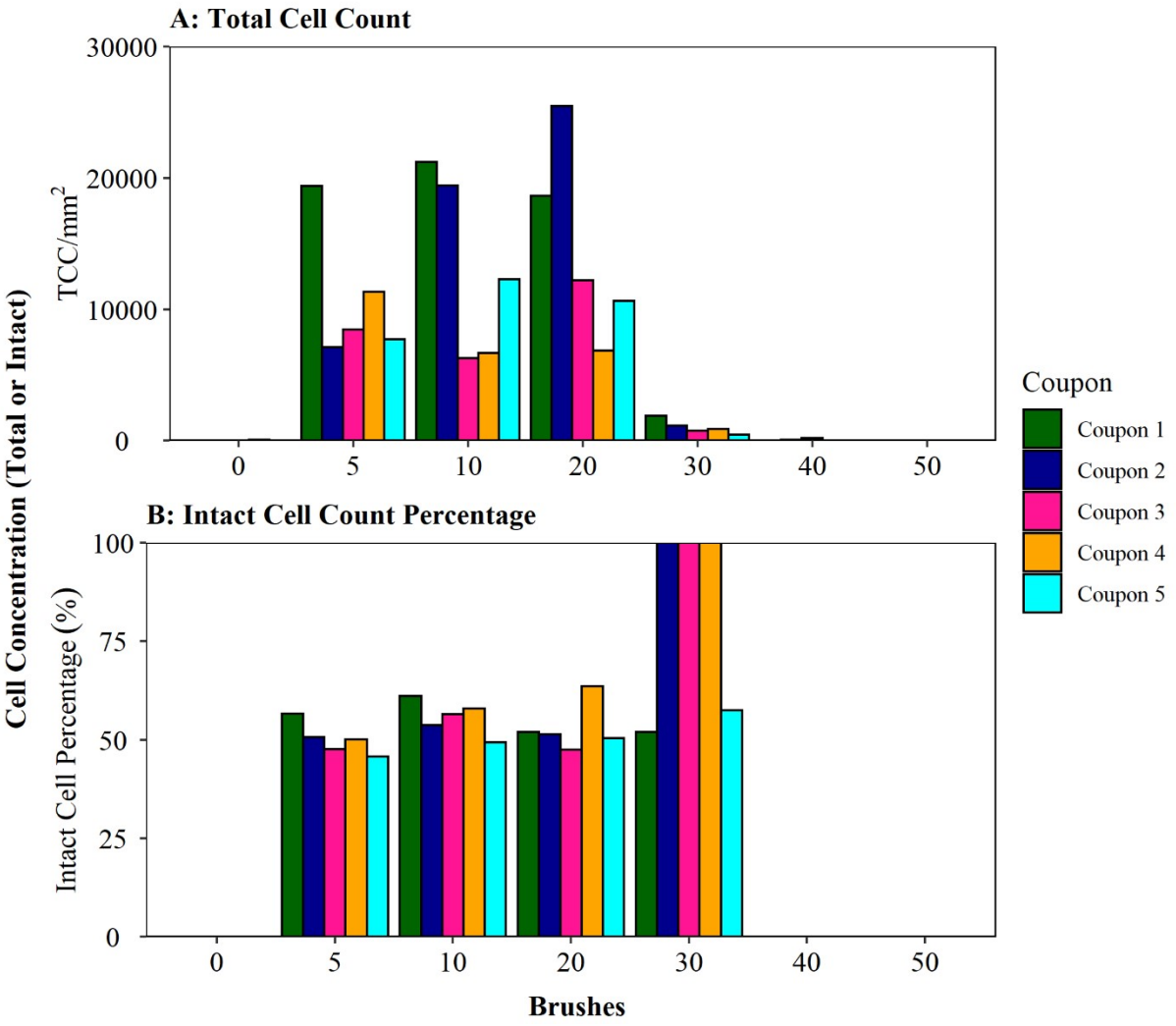

Figure S5. Impact of brushing on cell viability when used to remove biofilm from coupons. Coupons (n=5, see key) were brushed for a series of 0, 5, 10, 20, 30, 40 and 50 brushes. Total cell counts (TCC) and percentage of TCC that were intact are presented. 0 brushes is defined as phosphate buffer solution (PBS) poured over the surface of the coupon to act as a control.

Table S2: Singlet-doublet percentages of 3-month old drinking water biofilm samples homogenised via vortexing for different longevities (triplicate results are listed in the table). Biofilms were previously removed from the Biofilm Monitoring Device pipe sections using the optimised brushing protocol or sonication via a water bath with glass beads**.** Control samples were not vortexed. Corresponding total (TCC) and intact cell count (ICC) results are plotted in Figure 5. *Excluded from statistical analysis as an outlier value.

Biofilm Removal Method	Biofilm Homogenisation Method	Time (minutes)	Singlet-doublet % TCC	Singlet-doublet % ICC
Brushing	Control (no vortexing)	0.5	99, 100, 99	99, 100,100
		1	98, 99, 99	99, 100, 100
		2	99, 100, 100	100, 100, 100
		4	99, 100, 100	99, 100, 100
		8	97, 99, 99	99, 99, 88*
	Vortex	0.5	99, 100, 100	99, 100, 100
		1	99, 100, 100	99, 100, 100
		2	99, 100, 100	99, 100, 100
		4	98, 99, 99	98, 100, 99
		8	69*, 99, 99	99, 100, 100
Glass beads & sonicating water bath	Control (no vortexing)	0.5	100, 100, 100	100, 100, 89
		1	100, 99, 99	87, 100, 92
		2	99, 100, 100	96, 100, 100
		4	99, 100, 100	100, 100, 100
		8	97, 99, 100	100, 100, 100
	Vortex	0.5	100, 100, 100	99, 100, 100
		1	100, 100, 99	99, 100, 99
		2	99, 100, 100	99, 99, 100
		4	99, 100, 100	98, 99, 100
		8	100, 100, 99	99, 100, 99

Table S3: Singlet-doublet percentages of 3-month old drinking water biofilm samples homogenised via sonication (needle) for different longevities (triplicate results are listed in the table). Biofilms were previously removed from the Biofilm Monitoring Device pipe sections using the optimised brushing protocol or sonication via a water bath with glass beads**.** Control samples were not vortexed. Corresponding total (TCC) and intact cell count (ICC) results are plotted in Figure 6.

Biofilm Removal Method	Biofilm Homogenisation Method	Time (minutes)	Singlet-doublet % TCC	Singlet-doublet % ICC
Brushing	Control (no sonicating needle)	0.5	98, 99, 99	98, 100, 100
		1	98, 100, 99	99, 100, 100
		2	99, 99, 99	99, 100, 100
		4	97, 99, 99	99, 100, 100
		8	98, 99, 99	99, 100, 100
	Sonicating Needle	0.5	98, 99, 99	99, 100, 100
		1	99, 99, 99	99, 100, 99
		2	98, 99, 99	99, 100, 99
		4	95, 99, 98	98, 100, 99
		8	90, 98, 99	95, 99, 99
Glass beads & sonicating water bath	Control (no sonicating needle)	0.5	100, 100, 100	90, 100, 99
		1	96, 100, 100	100, 100, 99
		2	100, 100, 99	100, 100, 100
		4	100, 100, 100	100, 100, 96
		8	100, 100, 97	100, 100 91
	Sonicating Needle	0.5	100, 100, 100	98, 99, 100
		1	100, 100, 100	98, 99, 100
		2	100, 100, 100	98, 99, 100
		4	100, 100, 100	97, 100, 100
		8	99, 100, 100	97, 98, 98

Table S4: Singlet-doublet percentages of mature drinking water biofilms homogenised via vortexing or sonicating (needle) (triplicate results are listed in the table). Biofilms were removed from sample surfaces by brushing. Control samples were not homogenised via vortexing or sonicated with a sonicating needle but were quantified at the same timepoints as samples which did have homogenisation methods applied. Corresponding total and intact cell count results are plotted in Figure 7.

Biofilm Removal Method	Biofilm Homogenisation Method	Time (minutes)	Singlet-doublet % TCC	Singlet-doublet % ICC
Brushing	Control (no vortexing)	0.5	93, 99, 99	95, 99, 100
		1	95, 99, 99	98, 100, 100
		2	97, 99, 99	95, 100, 100
		4	97, 99, 99	95, 99, 100
		8	97,100, 99	96, 100, 100
	Vortex	0.5	96, 99, 99	97, 100, 100
		1	96, 100, 100	98, 100, 100
		2	97, 100, 100	97, 99,100
		4	99, 100, 100	98, 100, 100
		8	99, 100, 99	97, 99, 100
	Control (no sonicating needle)	0.5	98, 100, 100	96, 99, 100
		1	99, 100, 100	97, 100, 100
		2	99, 100, 100	97, 99, 100
		4	99, 100, 100	97, 99, 100
		8	99, 99, 100	97, 99, 100
	Sonicating Needle	0.5	100, 100, 100	99, 100, 100
		1	100, 100, 100	99, 100, 100
		2	100, 99, 100	99, 100,100
		4	100, 100, 100	99, 100, 100
		8	100, 100, 99	98, 100, 99