03rd February 2023

John Catton
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Sidings House
Sidings Court
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DN4 5NU

Dear John,

Please find the report with respect to Amecotech 001 – Repeat Testing V1. Please feel free to contact us to discuss these findings further.

Yours sincerely,

Thomas Miles

Head of Technical Operations

Report (Amecotech 001)

1.0 Aim

To screen for the biofilm removal ability of an ultra-fine bubble generating valve.

Study tier: Tier 3b: Customised test method for R&D purposes.

2.0 Materials and Methods

2.1 Test microorganisms

Gram-negative bacteria:
Pseudomonas aeruginosa NCIMB 10434

2.2 Test items

The details of the test items used in this study are outlined in Table 1.

Item name	Item format	LOT number
Control flow system	N/A	N/A
UFB DUAL® Valve	Ultra-Fine Bubble-Generating Valve	WF-20C3WD

Table 1. Test items used throughout the study.

2.3 Equipment and media

Equipment

90 mm Petri dishes – Scientific Laboratory Supplies (SLS), UK
96-well plates – SLS, UK
Autoclave – Priorclave, UK
Masterflex® L/S® Series peristaltic pump – VWR, UK
Masterflex® L/S® Size 36 tubing – VWR, UK
Sonicating water bath – VWR, UK
Sterile universal tubes – SLS, UK
UKAS calibrated multichannel pipettes (P20 and P300) – Gilson®, UK
UKAS calibrated pipettes (0.5 – 1000 µL range) – Proline® Plus, UK
Vortex – Grant-Bio, SLS, UK

Media

Calcium chloride - Merck – SLS, UK
Hard water A (Appendix I)
Hard water B (Appendix I)
Magnesium chloride – Merck, SLS, UK
Phosphate Buffered Saline (PBS) – Acumedia®, SLS, UK
Sodium bicarbonate – Merck, SLS, UK
Test Water (Appendix I)
Tryptone Soya Agar (TSA) – Southern Group Laboratories (SGL), UK
Tryptone Soya Broth (TSB) – Acumedia®, SLS, UK

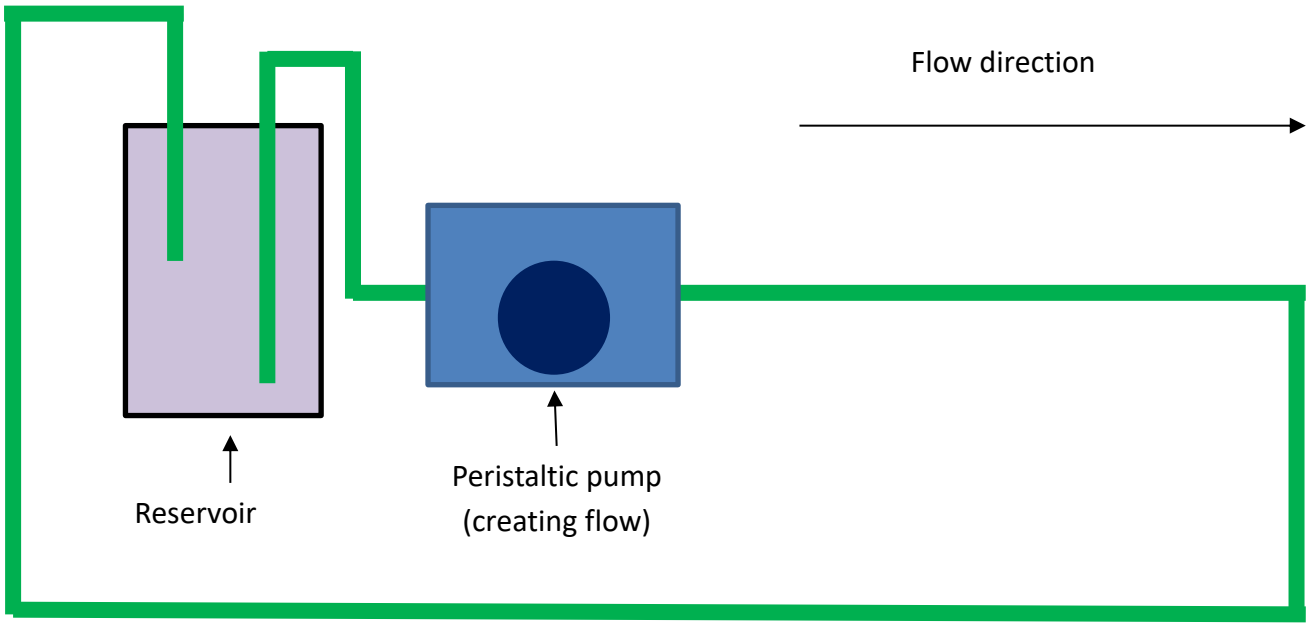
2.4 Method

2.4.1 Determination of the biofilm removal ability of an ultra-fine bubble generating valve (UFB DUAL® Valve) over 14 days

2.4.1.1 Preparation of bacterial inoculum

2.4.1.2 Development of biofilm

A.



B.

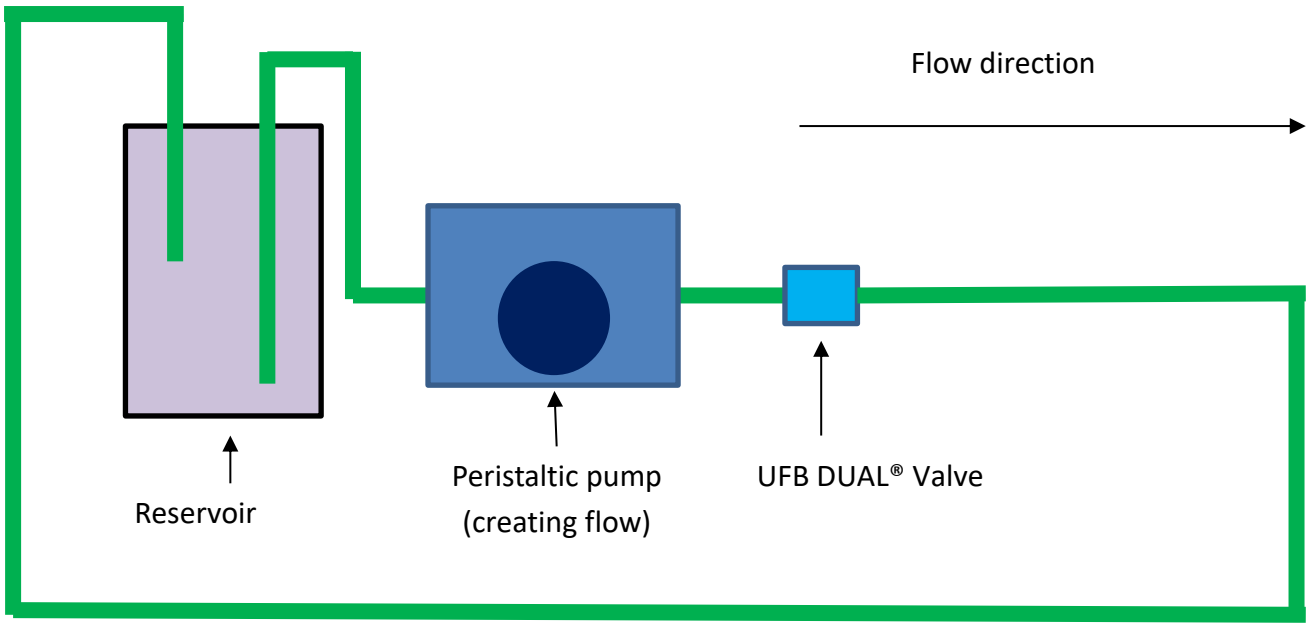


Figure 1. Schematic diagram representing the flow system. A = flow system for growing biofilm and to act as untreated control. B = flow system for UFB DUAL® Valve assessment.

2.4.2 Statistical Analysis

3.0 Results

3.1 Assessment of the biofilm removal ability of an ultra-fine bubble generating valve (UFB DUAL® Valve) over 14 days

3.1.1 Control flow system

Following 24 hours biofilm development (0 hours), an average viable *Pseudomonas aeruginosa* recovery of 6.97 ± 0.08 $Log_{10}CFUmL^{-1}$ was observed for the Control flow system. Following a further 24 hours, 7 days and 14 days, average viable *P. aeruginosa* recoveries of 6.70 ± 0.15, 6.62 ± 0.17 and 6.84 ± 0.08 $Log_{10}CFUmL^{-1}$ respectively, were observed for the Control flow system. These were average Log reductions in viable *P. aeruginosa* of 0.28 ± 0.15 ($p < 0.05$), 0.36 ± 0.17 ($p < 0.05$) and 0.13 ± 0.08 $Log_{10}CFUmL^{-1}$ respectively, compared to the Control flow system at 0 hours (Table 2, Figure 2).

3.1.2 Test flow system

Test condition	Time point	Average recovery ± SD ($Log_{10}CFUmL^{-1}$)	Average reduction ± SD ($Log_{10}CFUmL^{-1}$)	Percentage reduction ($CFUmL^{-1}$)
Control flow system	0 hours	6.97 ± 0.08	N/A	N/A
	24 hours	6.70 ± 0.15	0.28 ± 0.15*	44.54%
	7 days	6.62 ± 0.17	0.36 ± 0.17*	54.75%
	14 days	6.84 ± 0.08	0.13 ± 0.08	26.52%
Test flow system	0 hours	6.83 ± 0.10	N/A	N/A
	24 hours			
	7 days			
	14 days			

Table 2. Average Log recovery, average Log reductions and percentage reductions in the quantity of viable *Pseudomonas aeruginosa* from the Control and Test flow systems following 24 hours biofilm development (0 hours), and 24 hours, 7 days, and 14 day treatment, when compared to the Control and Test flow system at 0 hours, respectively. SD = standard deviation, CFU = colony forming units, N/A = not applicable. *= $p < 0.05$, *** = $p < 0.001$.

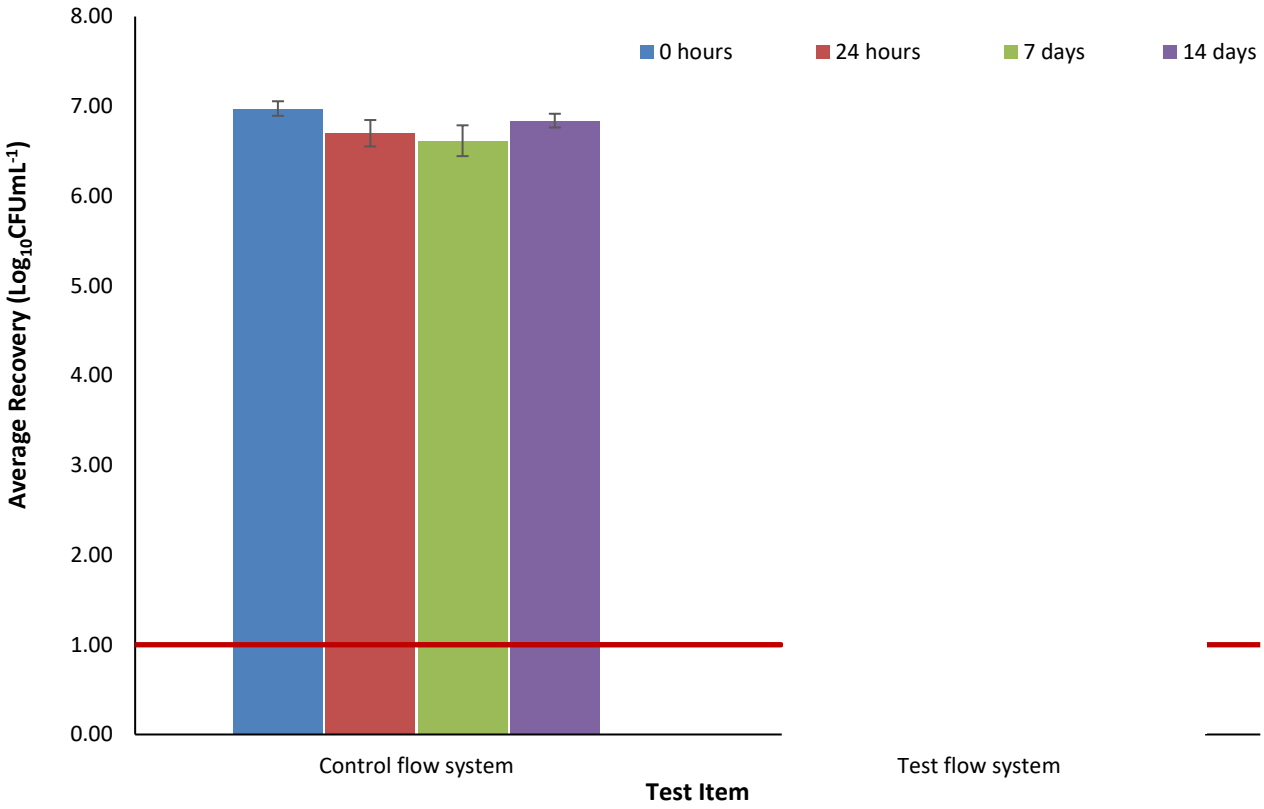


Figure 2. Average recovery of viable *Pseudomonas aeruginosa* for Control and Test flow systems following 24 hours biofilm development (0 hours) and 24 hours, 7 days, and 14 days treatment. CFU = colony forming units. Error bars represent the standard deviation. Red line represents the limit of detection (1.0 Log).

4.0 Discussion

Biofouling is referred to as the unwanted deposition and growth of biofilms. This phenomenon can occur in an extremely wide range of situations, from the colonisation of medical devices to the production of ultra-pure, drinking and process water and the fouling of ship hulls, pipelines, and reservoirs. Biofilms are the causes of chronic infection and contamination of water sources and are often able to tolerate treatment with antibiotics and biocides. This study aimed to investigate the potential of a new technology that employs water containing ultra-fine bubbles to reduce the biofouling associated with industrial and domestic water systems.

5.0 Future Testing

- Assessment of the efficacy of UFB DUAL® Valve on a wider range of organisms associated with biofouling of industrial and domestic water systems.
- Assessment of synergistic effects when combining the UFB DUAL® Valve with chemical disinfectants or biocides.
- Assessment of the stability of a batch of the solution produced by the UFB DUAL® Valve.
- Assessment of the efficacy of the solution produced by the UFB DUAL® Valve using a range of standardised methods.
- Use of molecular techniques to assess the change in expression of key biofilm genes following treatment with the UFB DUAL® Valve.

Project Start Date: 10th January 2023

Project Completion Date: 26th January 2023

Appendix I

Production of Test Water (TW)

Hard Water A recipe

Reagent	Amount
Calcium chloride	
Magnesium chloride	
Deionised water	

Table A. Ingredients required to make 500 mL Hard Water A.

Hard Water B recipe

Reagent	Amount
Sodium bicarbonate	
Deionised water	

Table B. Ingredients required to make 500 mL Hard Water B.

Test Water recipe

Reagent	Amount
1/3 strength TSB	
Hard water A	
Hard water B	
Deionised water	

Table C. Ingredients required to make 1000 mL Test Water.

Appendix II

Raw data

Time Point	Test condition	Average recovery ($Log_{10}CFUmL^{-1}$) N=1	N=2	N=3	Average	SD
0 hours	Control Flow System	6.94	6.92	7.07	6.97	0.08
	Test Flow System					
24 hours	Control Flow System	6.82	6.74	6.54	6.70	0.15
	Test Flow System					
7 days	Control Flow System	6.43	6.65	6.77	6.62	0.17
	Test Flow System					
14 days	Control Flow System	6.88	6.75	6.89	6.84	0.08
	Test Flow System					

Table D. Log recoveries of total viable *Pseudomonas aeruginosa* from individual replicates of Control and Test flow systems following 24 hours biofilm development (0 hours) and 24 hours, 7 days and 14 days. CFU = colony forming units. SD = Standard Deviation.

End of report.