Effects of Magnetic Water Treatment on Bacterial Survival in Biofilms

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RESEARCH PROGRESS REPORT
UA-2021-P01
STATUS: Continuing, No Additional Funds Requested
Relationship to Technology Roadmap: Municipal Water Use Practices and Wastewater Reuse

- **Rationale:**
  - Biofilms in cooling towers may harbor pathogenic organisms including *Pseudomonas* and *Legionella*, which may pose a public health risk if not adequately controlled.
  - Biofilms improve the resistance of the population to many chemical disinfectants, reducing the effectiveness of many biofilm control strategies.
  - Application of a magnetic field may reduce the viability of bacteria or the presence of biofilms without the need for additional disinfectants.
- **Objectives:**
  - Assess the effectiveness of Vodaa Technologies magnetic treatment device on *Pseudomonas* and *Legionella* survival/ stress in biofilms.
  - Determine under what conditions, if any, can the effects of the magnetic field be observed.
  - Determine the effects of magnetic treatment on biofilm formation.
  - Narrow down potential mechanisms of action for bacterial removal or biofilm disruption.
- **Approach:**
  - Generate *Pseudomonas* and *Legionella* biofilms on stainless steel coupons and evaluate biofilm associated bacterial survival when exposed to magnetically treated water in a closed loop system.
  - Attempt to generate biofilms in systems containing magnetically treated water to assess effects on biofilm formation.
  - Determine cell viability using dilution and plating, cellular stress responses (LuminUltra ATP and AMP), and microscopy (SEM) to evaluate biofilm structure.
- **Key Findings to Date:**
  - Under certain conditions up to ~3log removal of viable *Pseudomonas* cells from biofilms can be achieved using magnetic treatment of water.
  - Magnetically treated water appears to increase cellular stress though increased AMP:ATP ratios.
- **Project Duration:**
  - January 2020 – March 2022
- **Budget:**
  - Ongoing funding by Vodaa Technologies

Vodaa Technologies

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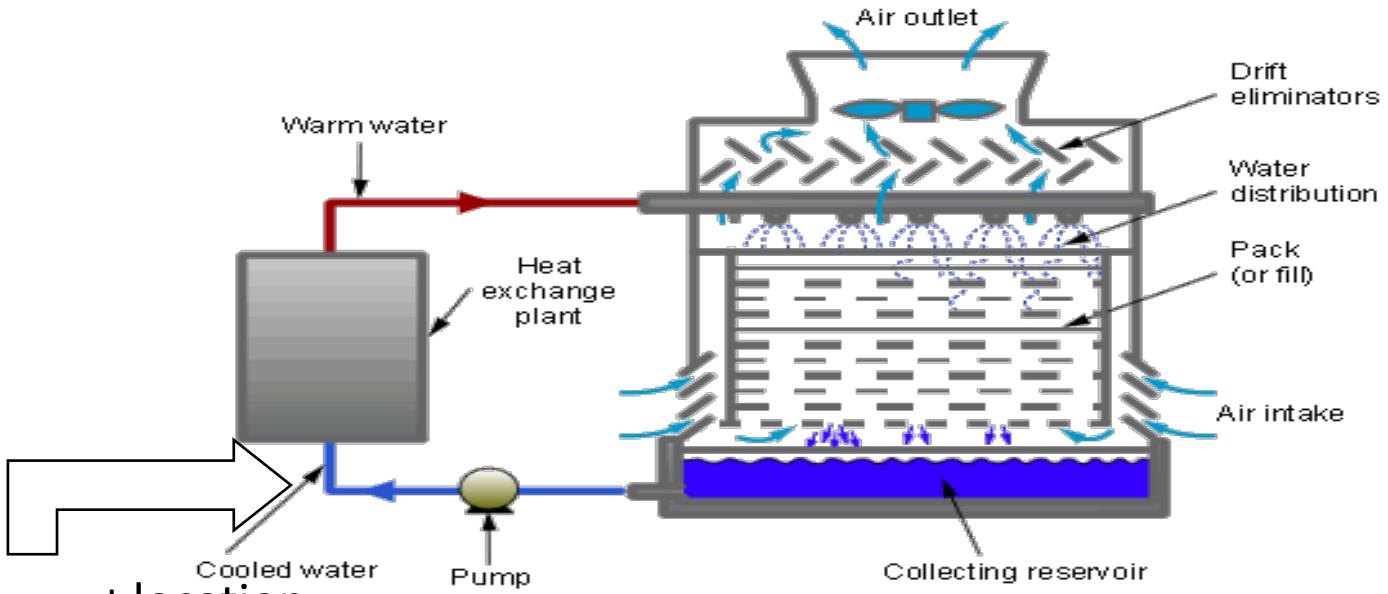
Background – Vodaa Technologies

- Magnetic treatment devices have been utilized in industrial water systems to remove pipe scale.
- When placed on cooling towers Vodaa Technologies noted…
  - Reduced HPC's, *Legionella*, and biofilms in the system without additional biocides



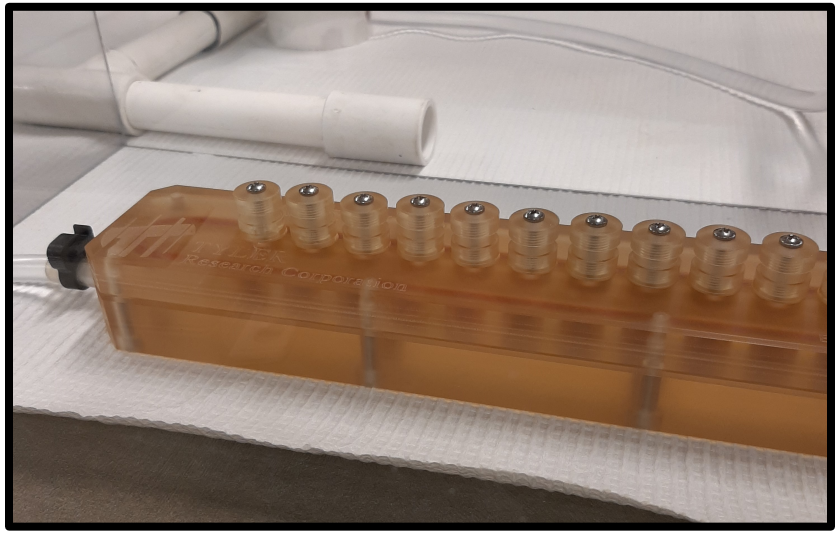
Example magnet location

Vodaa Magnetic Treatment Device
VCU

Background

Biofilms

- Biofilms in water systems may harbor pathogenic bacteria.
  - *ex. Pseudomonas aeruginosa* and *Legionella pneumonia*
- Biofilms increase resistance of bacteria to chemical disinfectants.



Modified Robins Device for biofilm formation

Magnetic water treatment

- Magnetic fields may influence charged particles and reactions.
- Utilized in some industrial settings to remove pipe scaling.
- Current knowledge gap on the effects of magnetic water treatment on biofilms.

Stainless steel coupon (surface for biofilm)

Overview and Objectives

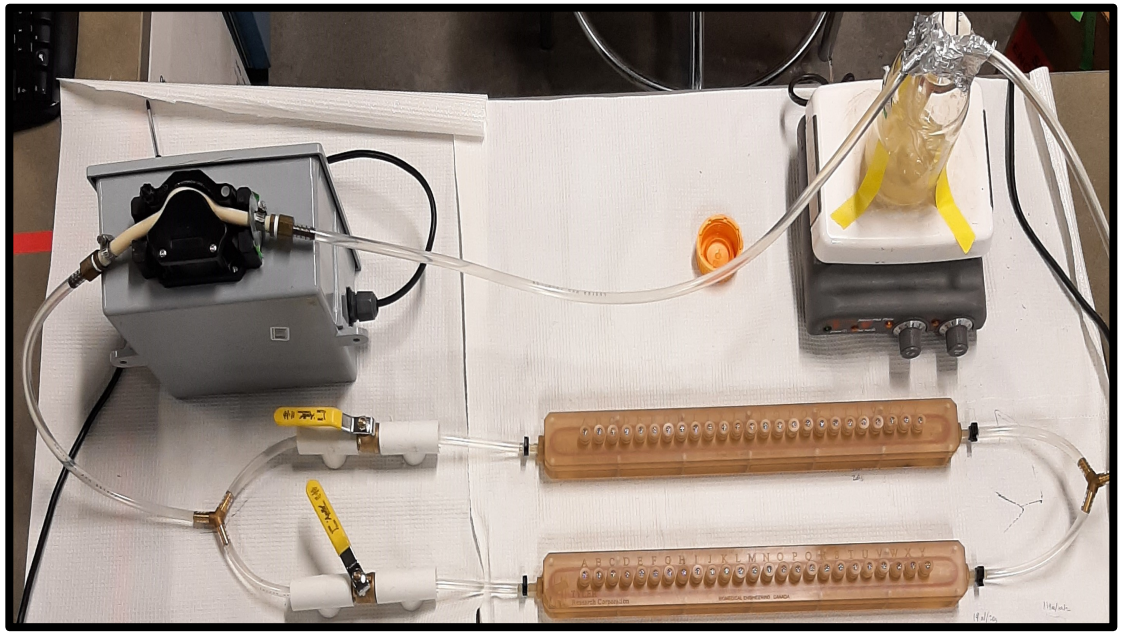
- Evaluate effectiveness of biofilm removal by magnetically treated water.
- Determine the effects of magnetically treated water on viability of common biofilm forming bacteria.
  - *Pseudomonas*
  - *Legionella*
- Assess how this treatment may cause reduced bacterial viability.

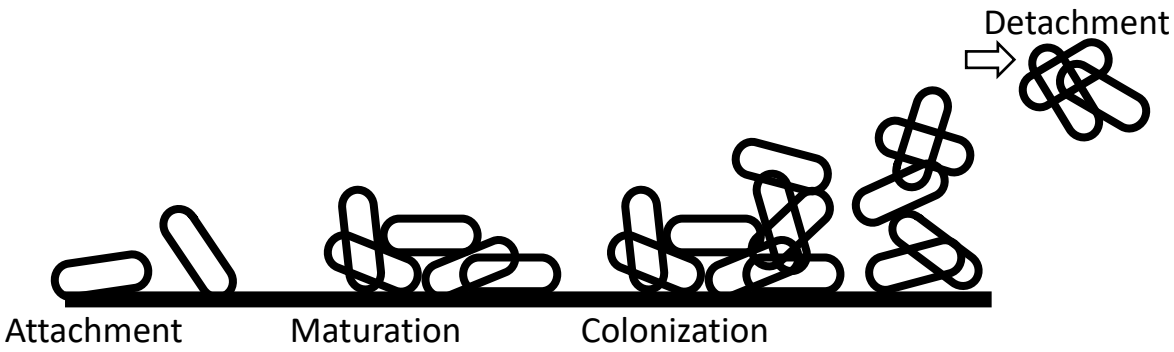
Pseudomonas fluorescence

Biofilm Formation on Modified Robbin's Devices (MRD)

Biofilm formation

- *Pseudomonas fluorescence* cells are grown in nutrient broth, washed, and resuspended in fresh nutrient broth.
- Cells are inoculated into Modified Robbins Device (MRD).
- Biofilm matures for 24 hours under continuous flow (100ml/ min).





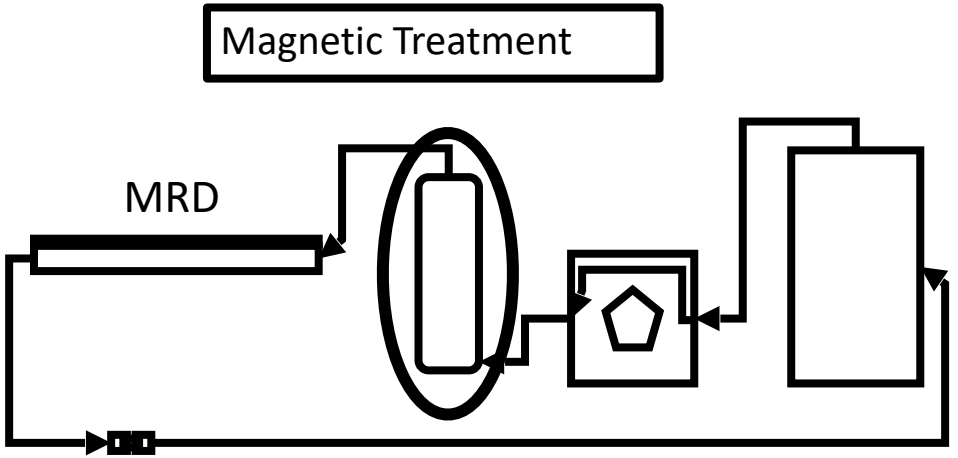
Magnetic Treatment of the System

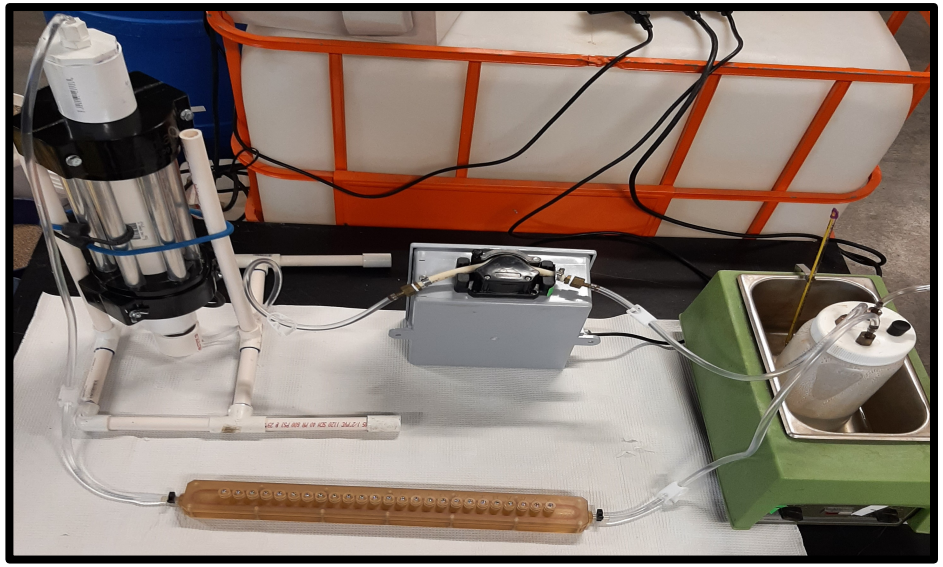
Magnetic water treatment

- Water is magnetically treated and then passed through the MRD.
- Magnetic field does not extend into the MRD (not directly influencing the biofilm).
- Different coupons are assayed every few days.

Evaluation of organisms in the biofilm

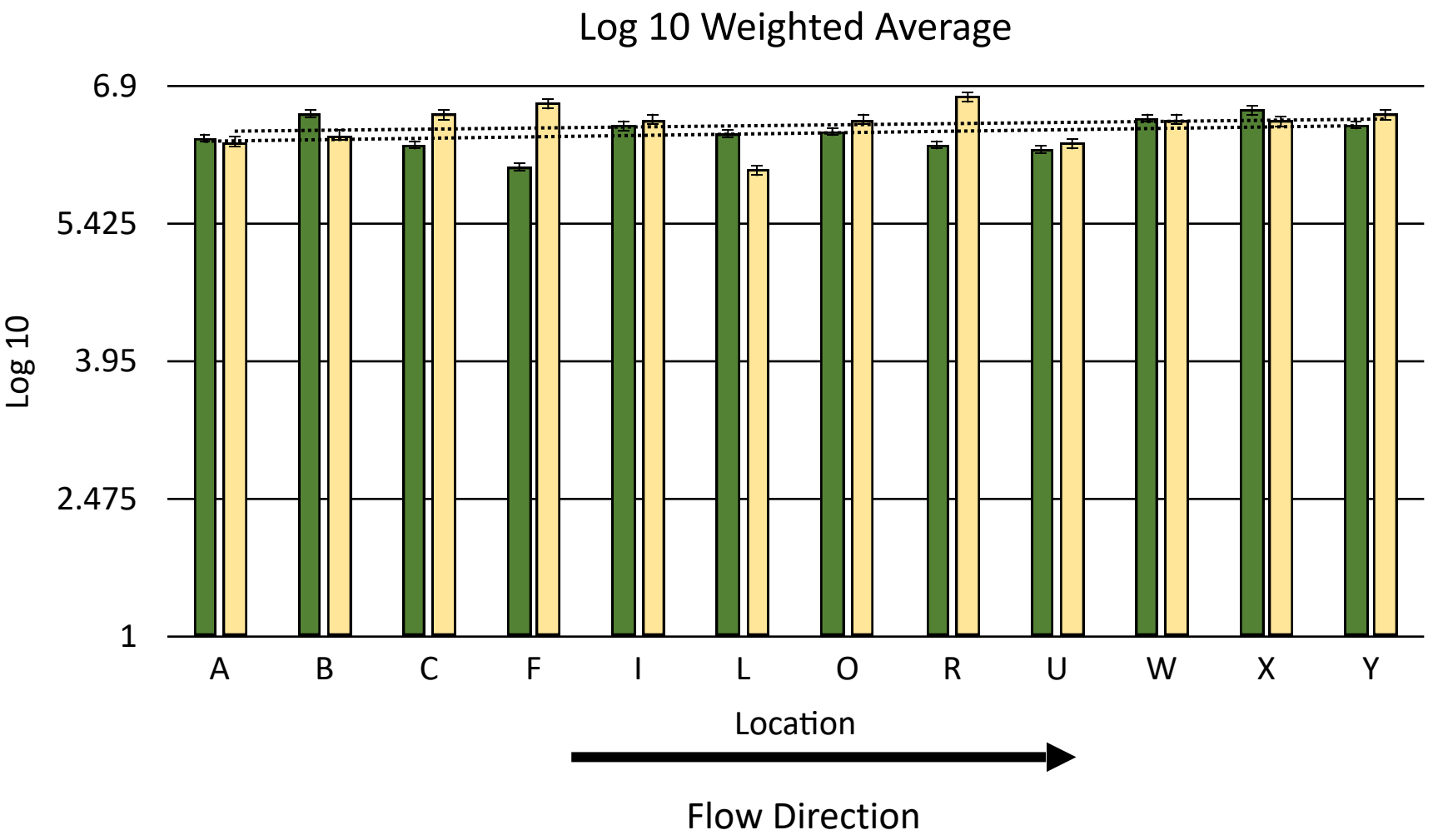
- ATP, AMP, and AMPi (cell stress) are determined through LuminUltra DSA kit
  - $AMPi = AMP/ATP$
- Dilution and plating used to determine culturalable cells.





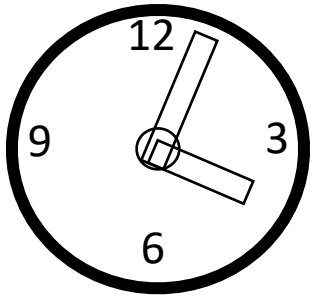
Experimental setup for magnetic treatment

Consistency of Biofilm Results



t-Test: Two-Sample Assuming Equal Variances		
	Weighted Log10 MRD	Weighted Log10 MRD'
Mean	6.444	6.480
Variance	0.039	0.029
P(T<=t) two-tail	0.502	

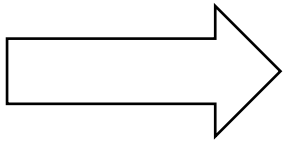
Experimental Variables



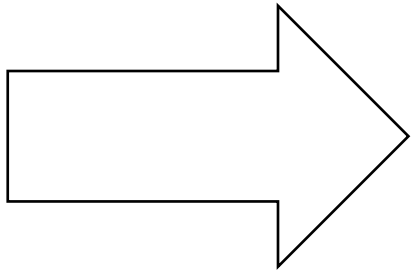
7 days vs 15 days

What is the optimal…

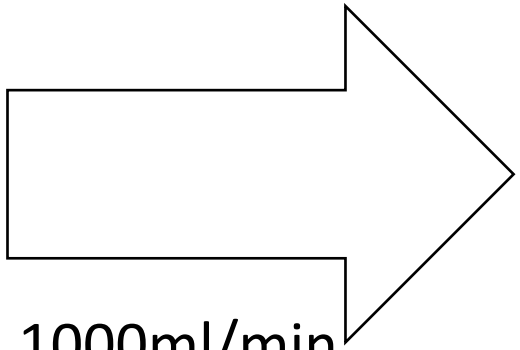
- Time?
- Flowrate?
- Field Strength?



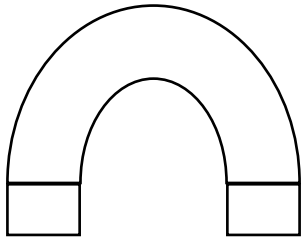
100ml/min



500ml/min



1000ml/min

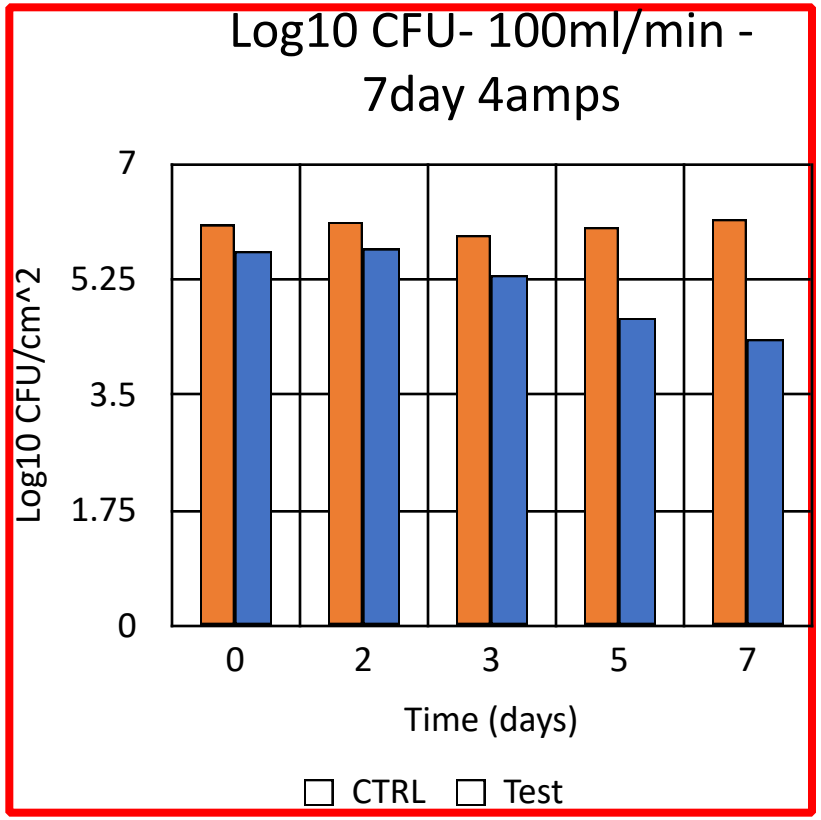


3amp vs 4amp

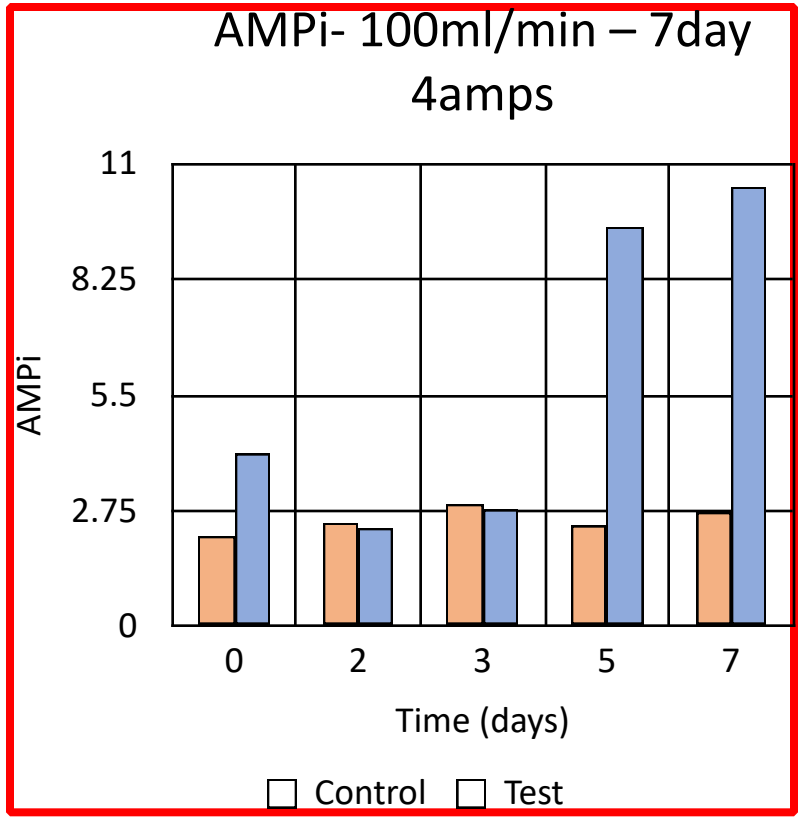
Pseudomonas fluorescence 7-day study

Log removal = $N_{TestFinal}/N_{TestInitial}$

- 100ml/min 4amps → 1.34log removal



High AMPi = Stressed Bacteria



Effects of Flowrate on Magnetic Treatment of Biofilms

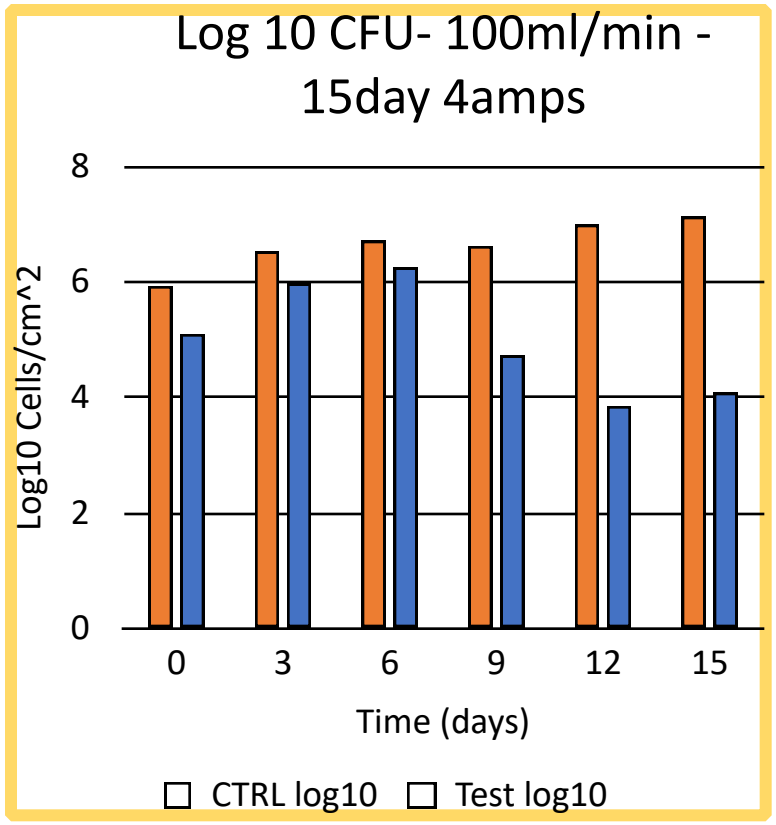
Why evaluate effects of flowrate?

- Increases the rate of interaction between the water and magnetic field
- Increases the turbulence of the water (more interaction across magnetic lines)
- Increased shear forces on the biofilm structures

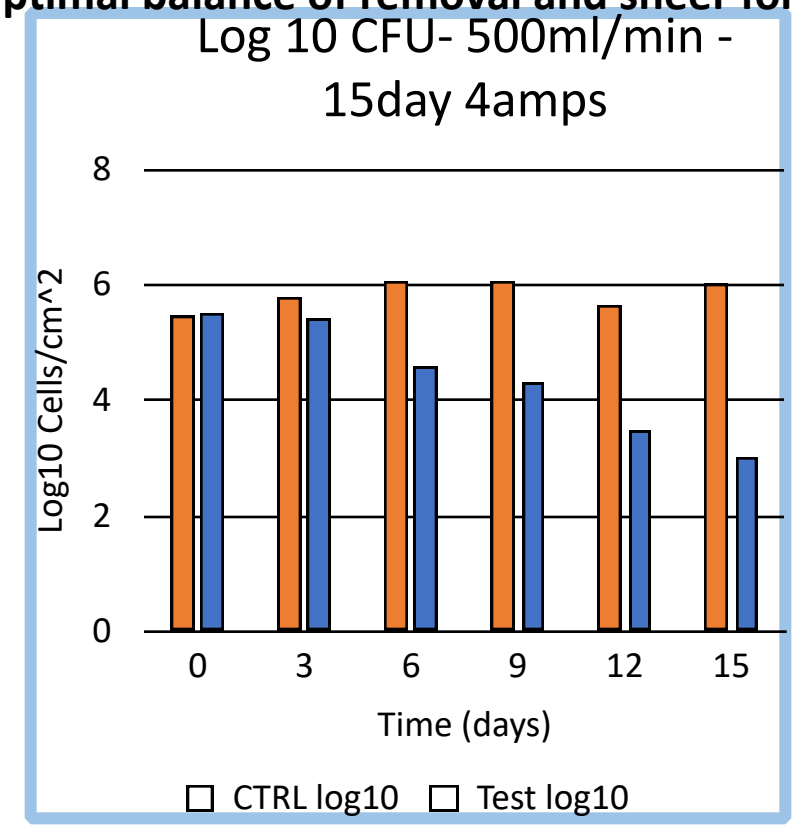
Effects of Flowrate on Culturable Cells

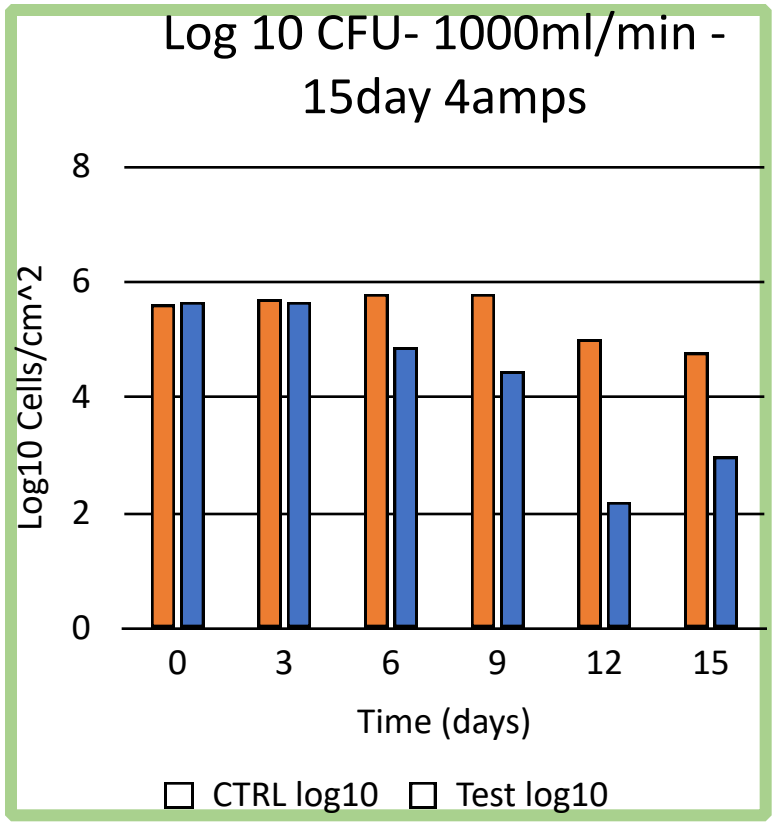
Log removal = $N_{TestFinal}/N_{TestInitial}$

- 100ml/min 4amps → 1.06log removal
- 500ml/min 4amps → 2.46log removal
- 1000ml/min 4amps → 2.71log removal



Optimal balance of removal and sheer forces

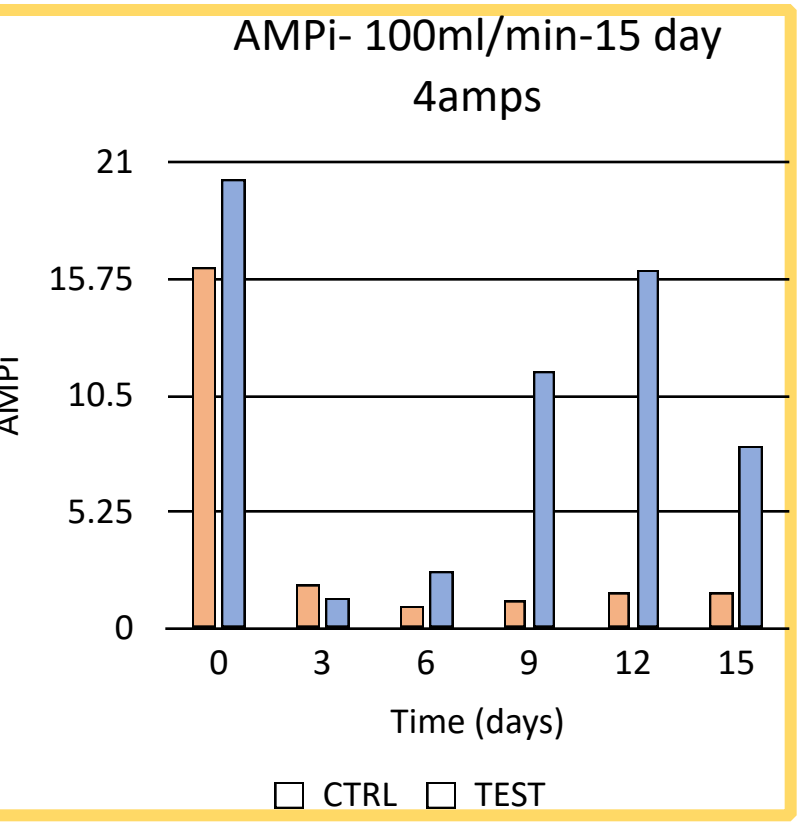


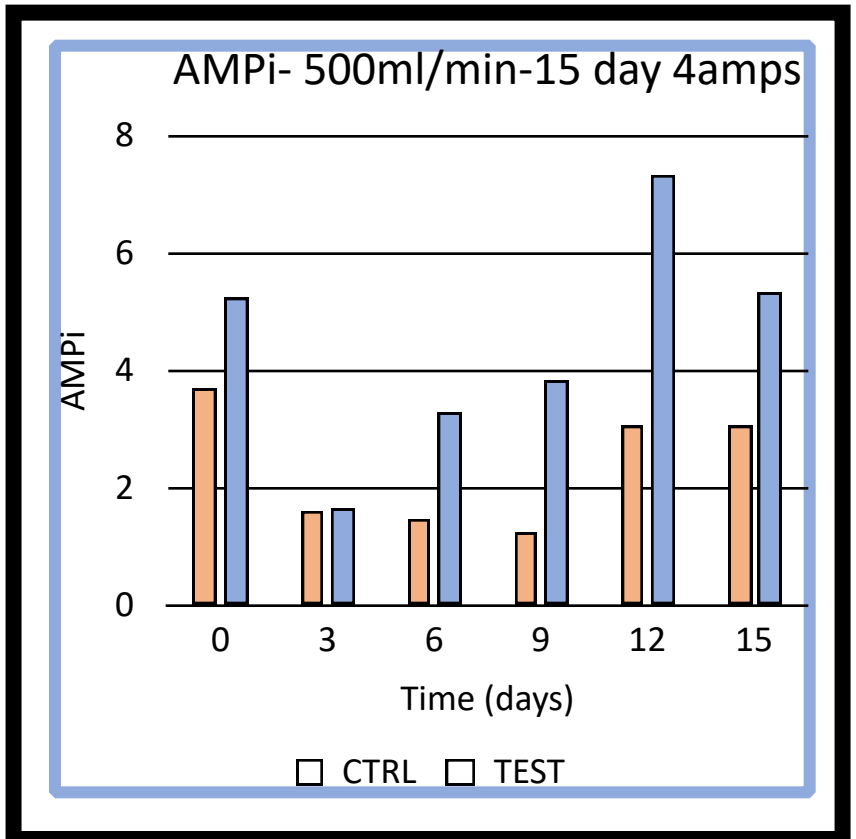


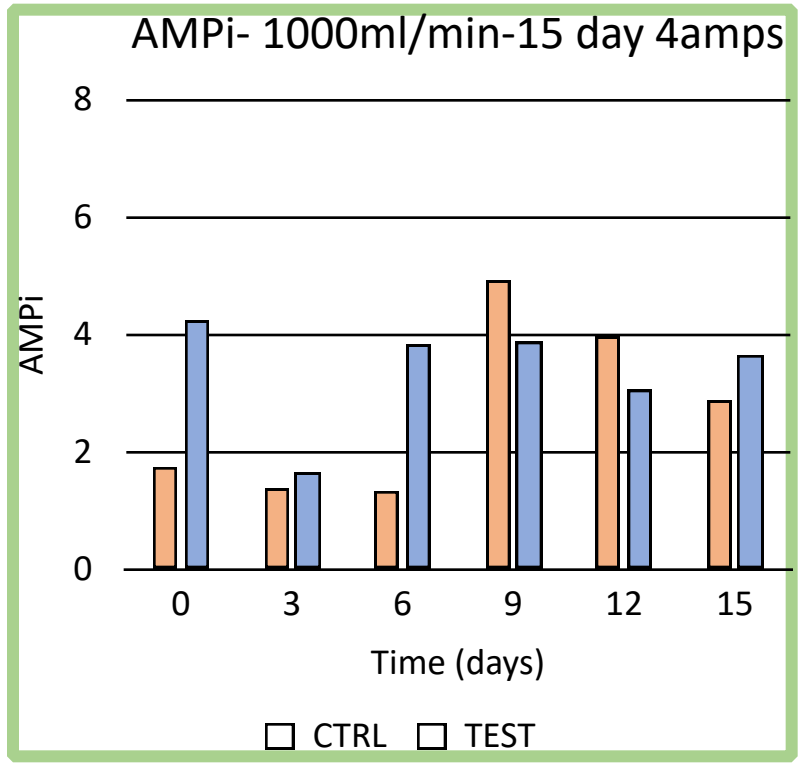
Effects of Flowrate on AMPi

- AMPi increases in test system ~day 6
- Trend of increased stress occurs in test system at low flow rates
- Increasing flowrates likely remove unhealthy cells from the biofilm
  - selects for healthier population

Flowrate enables stress levels to be observed





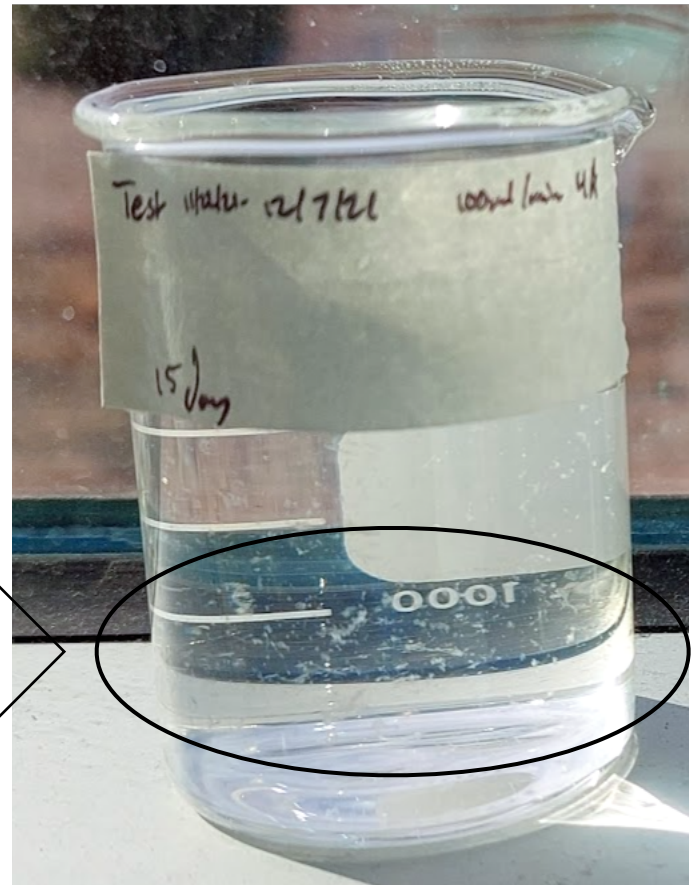


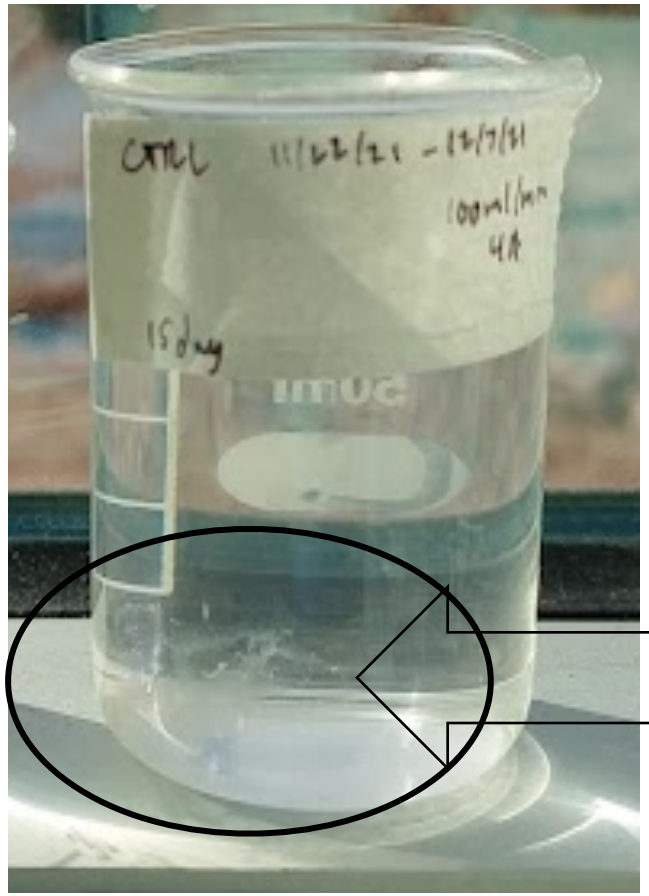
Test system basin

- 100ml/min
- 15days
- 4amps

Control system basin

- 100ml/min
- 15days
- 4amps



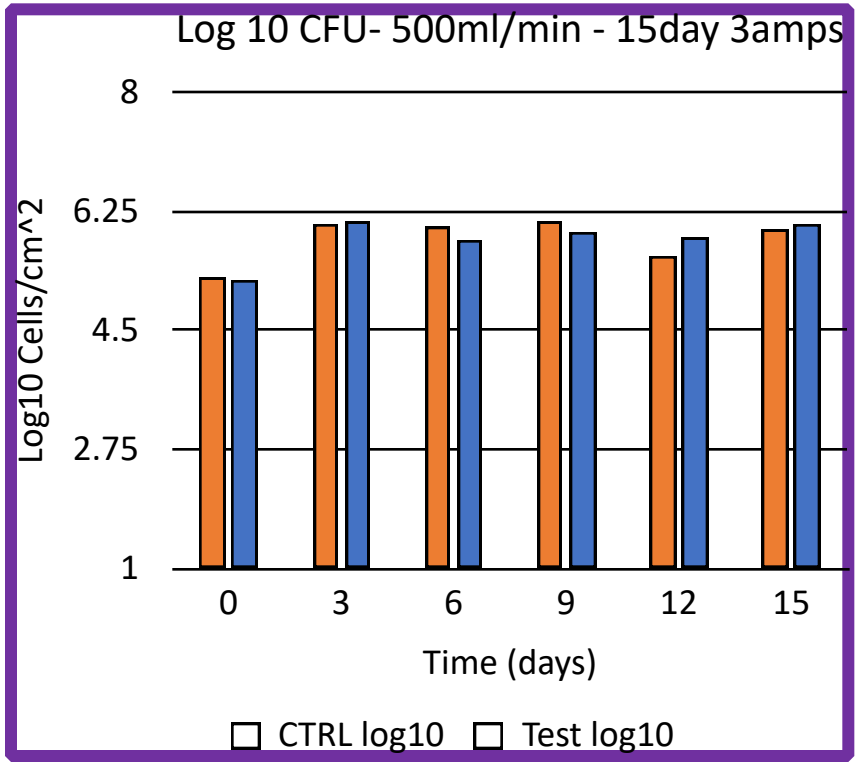


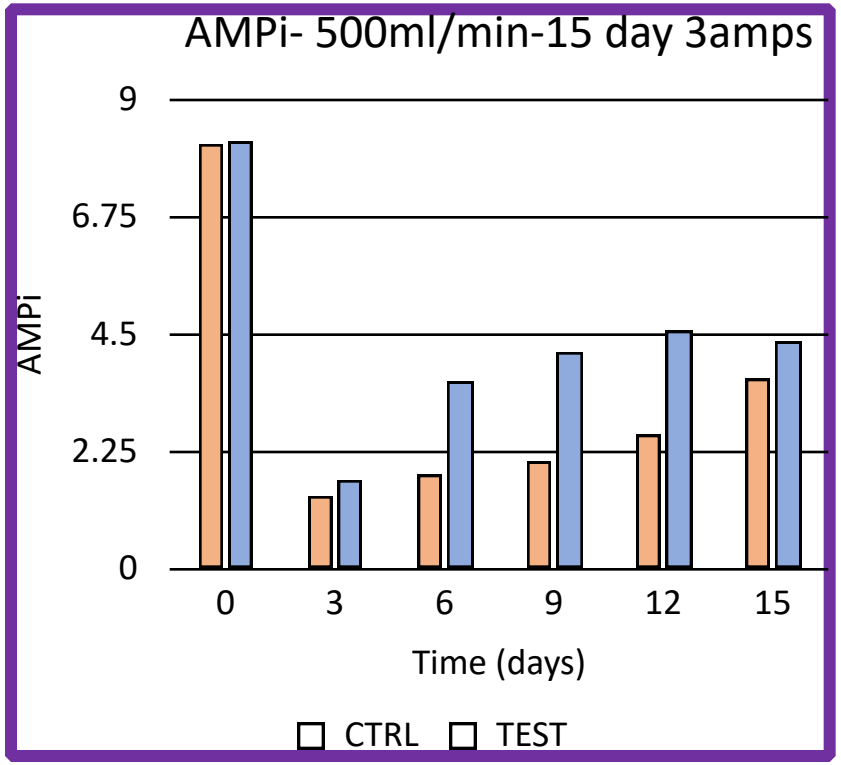
Effects of Field Strength on Biofilm Removal

Reducing amperage from 4amps to 3amps

- No obvious effect on culturable cells
- Increased stress levels in the treated system

Field strength appears to determine the effectiveness of the treatment





Future Work

- Effect of magnetic treatment on biofilm formation
  - Do biofilms form under magnetic treatment?
  - Are biofilms formed under magnetic treatment less robust?
- Effects of magnetic treatment on biofilm structure
  - Scanning electron microscopy (SEM)
- Effect of magnetic treatment on *Legionella* and *Pseudomonas* mixed biofilms

Conclusions

- Increasing treatment time from 7 days to 15 days shows clearer trends of removal
- Higher flowrate increases the effect of the magnetic treatment
  - 100ml/min = 1.1log removal
  - 500ml/min = 2.5log removal
  - 1000ml/min = 2.7log removal
- The strength of the magnetic field impacts effect of the treatment
- The AMPi gives an accurate value of stress for what is sampled
  - But as flowrate increases unhealthy cells are likely removed from the population

Acknowledgements

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