GRIFFITH UNIVERSITY

FINAL REPORT

# Validation studies and development of the electro-assisted, sound emission technology for improving water treatment – Destruction of *Cryptosporidium* oocysts and prevention of biofilm build up on hardware

## "Development of a new generation sweeping sound frequency (SSF) water treatment system"

By


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# CONFIDENTIAL

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**The Project Team** was lead by Dr Helen Stratton. Dr Steve Okeefe was the team leader for the electromagnetic modelling, Chapter 4. Dr Angela Mordocco lead the biofilm work and assisted in all aspects of the project, including the report writing. The research team also included Lyn Phillips (*Cryptosporidium* assays), and Davide Ostini (modelling).

## EXECUTIVE SUMMARY

The following report outlines Key Findings of the AusIndustry Start project. The effects of the AntiBio system on

- *Cryptosporidium* oocysts in swimming pool water
- biofilm (attachment of cells to surfaces)
- Pilot Swimming Pool studies
- Field – swimming pool at Mt Gravatt Campus, Griffith University
- Modelling of the system.

Each section is summarised with results, conclusions and future work in the following pages.

### *Biofilms*

Three types of biofilm tests were investigated

- o Preliminary biofilm tests in culture tubes
- o Bioreactors, where the cells are recirculated through a device that allows sampling of biofilm on pipes (a modified Robbins device)
- o One in cell culture plates (static experiments)

### Results

- The recirculating biofilm studies have demonstrated reductions and increases in AntiBio treated biofilms.
- Reductions in *Pseudomonas* species and *Zooglea ramigera* cells attaching to surfaces that are treated with the Mark I and Mark II AntiBio System were measured.
- Differences in the amount of cells attached (the biofilm) were noted in raw water, tap water, purified (RO) water and swimming pool water. This suggests that the effects of the ABS on cell attachment were influenced by the physico-chemical properties of the water.
- The ABS effects the attachment of cells to surfaces. Under the correct conditions biofilms can be reduced, depending on the microbial species and other chemicals present in the water.

### *Future work*

- Application of the ABS for biofilm reduction would need to be tested and optimised in each new environment.
- The modelling results suggest an optimal coil configuration for effecting biofilms would be with short coils placed perpendicular to the pipe.

Three different methods used for determining the infectivity and viability of *Cryptosporidium* oocysts in swimming pool water with different concentrations of chlorine.

- Vital staining – indicates oocyst viability or potential to infect
- In vitro excystation – indicates potential to infect
- Cell Culture Real Time PCR – infects a mono-layer of human cells

**Results**

- Different batches of oocysts demonstrated different responses to the ABS. For example, previous studies demonstrated a ~60% decrease in viability of oocysts when treated with Mark II ABS. However subsequent experiments with new batches were not able to replicate this promising result. It is impossible to continually use the same batch of oocysts for a long term study. Age of the culture and batch must be considered in all disinfection studies. Other researchers have experienced variability between batches and different ages of oocysts when testing other disinfection methods/technology.
- Variable results were observed when measuring viability and infectivity of oocysts using the Vital staining and In Vitro excystation methods, with the occasional increase in a log reduction of viability/infectivity.
    - Mark I, with 2 coils attached and 7 mg/L free chlorine resulted in an approximate 5% reduction (<0.1 log) in viability as measured by the vital staining method
    - Mark II, with 1 coil attached and 1 mg/L free chlorine demonstrated an approximate 10% (<0.2 log) reduction in infectivity as measured by in vitro excystation. An approximate 5% reduction in infectivity at 3 mg/L was also recorded during this experiment.
- At this point in time, although we have been encouraged by some of the previous results obtained, the current parameters will need to be optimised in order to obtain more consistent and better results. Therefore, the current frequencies and configuration employed by the ABS can not be recommended as a sole disinfection device for *Cryptosporidium* in swimming pool water at this stage.

***Future work***

As some effects have been measured (using vital staining and excystation methods only), it may be possible to optimise the system and future studies should involve trialing the outcomes of the modelling of the ABS. As suggested by the modelling outcomes coils placed perpendicular to the tubing or pipe wall should be trialed. These coils may take on the form of a disc structure rather than the long coils currently used in the ABS.

It is not possible to dose full scale or pilot plants with viable *Cryptosporidium parvum* oocysts. To determine the incidence of Cryptosporidiosis in swimming pools using the ABS, a thorough epidemiological study could be undertaken. As Cryptosporidiosis is a reportable disease in Queensland and has been since 1998, information for the long term incidence of this disease can be compared to all pools in Queensland with and without the ABS installed and operating.

Monitoring of the Griffith University Mt Gravatt swimming pool was carried out over the summer of 2001/2002. The AntiBio Device Mk II was installed 6 February 2002.

**Results**

- There was a significant difference (improvement) in the overall water chemistry between the control (before ABS) and treated (with ABS)
- Free chlorine levels were higher during the ABS treatment period – it was impossible to calculate liquid chlorine dosing rates due to a mechanical problem in the dosing equipment
- The pH of the water remain at the desired level 7.6 during the treatment period – the amount of $CO_2$ used after installation of the ABS was reduced by 30% compared to the control period.

A pilot plant swimming pool operating in the AntiBio workshop, Ashmore has been tested for the number and viability of bacteria in the water. The pilot pool was designed as a scaled down 25m pool. It was operated without filtration but with swimming pool chemicals and nutrients (to simulate a bather load) added. Different water types have been tested for two weeks without ABS operating and then two weeks with ABS operating.

**Results**

- Marked decreases in bacterial numbers in the “mock pool water” were recorded when the water was being treated with the ABS, as compared to the controls without ABS treatment.
- The ABS controlled an overgrowth and attachment of cells to the walls of the pool and equipment
- High numbers of bacteria were counted in the control and treated pool waters, however the numbers were significantly lower in the tap water and pool water that were treated with the ABS.

***Conclusions/Future Work***

The ABS does not kill microorganisms in swimming pool water, however reduced numbers of bacteria were counted in tap and pool water samples that had been with nutrients added and treated with the ABS. The ABS assists in reducing the number of microorganisms growing in water. This depends on the chemical composition of the water.

An additional task for the AusIndustry project was included in the study. The modelling task was implemented to assist in understanding the ABS and optimise its effectiveness in water treatment. The model was developed to investigate the magnetic fields emitted from the coils and transported through pipes.

The results of this study have shown a number of insights into the deployment of magnetic solenoids for the purpose of creating magnetic fields for the treatment of liquids flowing in a pipe. The issue of where the fields should be concentrated in the pipe is still to be clarified. The options are to maximise the fields across the entire pipe cross-section, or to maximise the fields around the bio-film region on the inside of the pipe.

- The single parallel coil method of excitation is the most ineffectual. It should only be considered for pipes of under 10 mm in diameter.
- The shorter coil gives the higher density at coil ends.
- If a high flux density is required over a long length of the pipe then the parallel coil structure is of advantage

**Future Work**

- If the magnitude of the field is of importance and influences the biological effects of the ABS, then the perpendicular arrangement of the coils to the pipe should be chosen. This arrangement will give very high flux densities in the bio-film
- two or more coils provides the best results.
- The number of coils that are deployed on a pipe is very important. From the results obtained, for the 8 mm ferrite coils used, a rule of thumb has been determined. *To keep the minimum biofilm region flux density to approximately 50% of the maximum then the circumference of the pipe (mm) should be divided by 20 (mm). This will give the approximate number of coils required when used in the parallel configuration. The perpendicular arrangement requires similar spacing although due to the peaky nature of the flux density at the pole end the spacing may need to be closed up slightly if the very high flux densities produced are required around the whole circumference.*
- For very large pipes, >100mm, The perpendicular arrangement will continue to

provide high flux densities in the bio-film region. Obtaining a high flux density in the centre of these pipes will be difficult and can only be obtained with the existing structures by using very high currents.

- Future studies into the biological effects of the ABS should attempt to optimise all three components of the system (SONIC, MAGNETIC and ELECTRIC fields). These optimisation modelling studies should be performed before any further biological effects are investigated.