

## **Summary - Major findings of the Research Project**

### **Application Of The Anti Bio System (Abs) On Biofilms (Fouling) In Pipes Carrying Drinking Water.**

#### **ARC SPIRT**

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#### **1. Field test studies on the effect of the AntiBio system (ABS) on biofilm growth and development.**

- effects of the ABS extended to biofilms 20 m sampled 20 m down the piping from the magnetic field emitting device. This implies that a residual effect of magnetic field exposure occurred.
- Examination by scanning electron microscopy and transmission electron microscopy showed no morphological variation between ABS treated biofilms compared to control biofilms.
- Examination by confocal scanning laser microscopy showed that ABS treated biofilms were around half the thickness of untreated control biofilms.

#### **2. Effect of Magnetic fields on enzymatic Activity and ion transport on *Pseudomonas aeruginosa*.**

- Cytochrome c oxidase activity in planktonic cultures of *P. aeruginosa* exposed to the ABS was on average 75.46% that of unexposed control cultures of *P. aeruginosa*.
- NADH activity in planktonic cultured of *P. aeruginosa* exposed to the ABS was on average 110.6% that of unexposed control cultures of *P. aeruginosa*.
- No change could be determined in the ion transport of H<sup>+</sup>, K<sup>+</sup> or Ca<sup>2+</sup> between planktonic and biofilm cultures of *P. aeruginosa* exposed to the ABS compared to unexposed controls.
- The ion traffic of Ca<sup>2+</sup> and H<sup>+</sup> was indicative of an antiporter system that has not previously identified in *P. aeruginosa*.

#### **3. Surface analysis of Biofilms and conditioning films.**

- Analysis by X-ray Photoelectron Spectroscopy (XPS) found that there was an increase of oxygen on present on surfaces treated with the ABS. This oxygen was predominantly in the form of C-O.
- XPS analysis also showed there was more Chloride detected on ABS treated surfaces. As the substrate used to analyse biofilm and conditioning film accumulation was polyvinylchloride (PVC), which is high in detectable chloride, an increase of chloride detected indicates a decrease in film deposition on the surface.
- The electrostatic interactions of silica oxide surfaces (negative charge) and poly-L-lysine coated surfaces (positive charge) and silica oxide bead in the presence and absence of an electromagnetic field was analysed by atomic force microscopy. It was found that the presence

of an electromagnetic field the Debye length ( $K^{-1}$ ) was reduced to nearly half on all charged surfaces tested in 1 mM NaCl solutions. This would have a large effect on the physicochemical adhesion forces as it reduces the electrostatic repulsive force for a required for a particle to attach to a surface.

- Surface adhesion forces of *P. aeruginosa* cells in the presence and absence of an electromagnetic field were assessed using the MATH (microbial adhesion to hydrocarbons) assay. This assay showed that the ABS treated cells adhered better to hexadecane and while the untreated cells had stronger adherence to toluene and xylene. Again, this implies that electromagnetically treating *P. aeruginosa* cells changes the surface physicochemistry of the cell.

#### **4. Transcriptional analysis of planktonic and biofilms of *Pseudomonas aeruginosa* exposed to electromagnetic fields by real-time RT-PCR.**

- Exposure to the ABS caused increases in mRNA transcription of *dnaK*, a gene encoding a protein part of the stress response system, in planktonic cells but not biofilms.
- Exposure to the ABS field caused a slight reduction in the transcription of *rpoH*, the gene encoding the alternative sigma factor that regulates the heat-shock response.
- Exposure to the ABS caused variable results in the expression of *algU*, the gene encoding the alternative sigma which regulates the production of exopolysaccharides. In two of the three replicates the experiment transcription of *algU* was underwent a slight downregulation in response to electromagnetic exposure while in the third the expression was upregulated. The expression of *algU* is negatively regulated by the transcription factor MucA. A well documented point mutation in the *mucA* gene which results in the upregulation in the *algU* could be responsible for this disparity.
- The expression of *rpoS*, the gene encoding the stationary phase sigma factor, was downregulated upon exposure to electromagnetic fields. The regulon of  $\sigma^S$ , the gene encoded by *rpoS*, includes many genes essential for biofilm formation and maturation.