SWEEPING SOUND FREQUENCIES ESTABLISHING AND MAINTAINING WATER QUALITY

HELEN STRATTON[1], and DAVID TELFER[2]
[1]School of Environmental Engineering, Griffith University, Nathan 4111
[2]AntiBio Technologies Ltd, 65 Twelfth Ave, St Lucia 4067

SUMMARY

Maintaining high standards of public health requires the provision of safe water in terms of chemical and biological contamination. Many different methods for improving the hygienic and aesthetic quality of water are already established. These include filtration techniques, biological treatment, chemical such as chlorine and ozone and treatment with UV radiation. Public (and to some extent, professional) confidence in established treatment systems for drinking water and recreational water has recently been compromised. New and improved treatment methods are being sought more often by Water Authorities and usually come at great expense, both in terms of capital and operating costs. A technology that may help solve many problems relating to water treatment is being investigated at Griffith University in relation to *Cryptosporidium* oocysts in water and biofilms that build up on pipes carrying water. This technology uses low frequency sound waves and electromagnetic fields.

Application of this technology in the water industry has been recognised as having significant potential, and an extensive research programme was established this year investigating the use of the technology on swimming pool treatment systems. Preliminary studies have revealed benefits of the system including significantly reduced chemical usage, reduced bacterial counts, reduction in total dissolved solids, and the need for backwashing is minimised. The research programme is investigating the modes of action and efficacy of the system for removal/inactivation of protozoan parasites such as *Cryptosporidium* spp., bacterial pathogens and viruses.

Preliminary investigations into the effect of the sweeping sound system on biofilms forming on pipes have indicated that it may be possible to reduce microbial attachment to pipe walls. More extensive research is required in this area and the work is continuing.

INTRODUCTION

The supply of safe water for drinking, domestic, industrial and recreational purposes is essential for the maintenance of public health. Choices of technology available for disinfection of water in treatment processes are diverse, including chemical disinfection, ozonation, ultraviolet radiation, and pressure driven membrane-based methods such as reverse osmosis. Electro-assisted methods for water treatment are gaining more interest in the water industry but are yet to be widely accepted for various reasons. The principle limitation to acceptance of this technology is the lack of understanding of the mode of action of electro assisted treatment.

Electrochemical, magnetic (Belova, 1972; Demchuck et al., 982) and sonic (Christian, et al., 1998; Ma and Lin, 1998) treatment for removing chemical contaminants from water and preventing scale build up in pipes (Baker and Judd, 1996), has been investigated for a number of years. The effect of electro-magnetic fields on biological cells has also been reasonably

well studied (Goldsworthy, et al., 1999; Mahawaorasilpa, et al., 1996; Schoenbach, et al., 1997). Despite a plethora of both scientific and anecdotal evidence suggesting that electro assisted treatment has merit, the industry and the scientific community remain skeptical of such technology. Probably because the evidence both supports and disputes the beneficial effects of the technology that manufacturers often claim. Despite this skepticism, a thriving industry is growing in the manufacturing and application of electro/magnetic devices. It is of paramount importance that science is involved in the development of these treatment technologies. It is important to ensure that the mode of action on different biological cells and chemical components in water is thoroughly understood.

Applications of electro assisted disinfection are broad ranging from prevention of scale build up in pipes (Baker and Judd 1996) to electrochemical disinfection of marine bacteria using an electro-conductive paint (Okochi, et al., 1995) and many others. It has also been demonstrated that electro assisted chlorination of *Giardia muris* improves the efficacy of chlorination for killing this parasite (Haas and Aturaliye, 1999). Electrical generated fields have also been demonstrated to kill biofouling microalgal cells using a saturated calomel electrode with 1.0 V applied (Okochi, et al., 1999). These are only a few of the examples reported in the literature and the applications extend beyond water treatment to clinical, industrial and waste treatment environments.

Anti Bio Technologies Ltd (ABT), an Australian company, have developed a system designed to emit variable sound frequency ranges into surrounding media. The system has previously employed a fixed frequency emittance in the residential swimming pool arena and some commercial pools. At the frequency used, the technology has demonstrated benefits in achieving and maintaining a healthy and safe swimming environment. Preliminary investigations using the Anti Bio System on swimming pools have demonstrated improvements in i) biological water quality, ii) reduced chemical usage, iii) maintenance requirements are minimised.

To achieve the full potential of this technology, further research using a rigorous scientific approach is essential. Anti Bio and Griffith University have developed an extensive research programme involving laboratory and full-scale evaluation and development of the technology. Laboratory scale experiments recently began at Griffith University to determine the efficacy of the system in inactivating the parasitic protozoan *Cryptosporidium* spp in swimming pool water.

Other field studies with other applications using the technology have uncovered the following;

- prevention of biofouling in the brewing industry.
- reduction of bacterial counts in drinking water
- prevention of barnacle growth on boat hulls
- improved water quality in swimming pools

During the field trials carried out in the past, and continuing trials, chemical and biological water quality is always improved, however many questions are yet to be answered. Questions like which organisms and how does the sound frequency affect them; what are the limits of the system? Of most importance to swimming pool operators at present is the efficacy of the system at preventing infection from *Cryptosporidium parvum*.

The water industry is aware that the oocysts of the parasitic protozoan, *Cryptosporidium parvum* are relatively resistant to conventional water treatment (Korich et al., 1990) and this poses a public health risk. Because of this possible risk, there is a sense of urgency to develop a fail-safe water treatment system that ensures that health risks related to parasites in water are reduced or eliminated. Preliminary results indicate that the sweeping sound device has an affect on the viability of *C. parvum* oocysts and are discussed in this paper

METHODOLOGY

Sweeping sound device

For these experiments a device described as sweeping sound technology (AntiBio System - ABS) was employed. The device consists of a control unit and an activator, which are connected by an electrical cable. The activator is placed on the outside of pipe work, in the laboratory the pipe was a silicone tube. The activator does not physically contact the water flowing through the pipe. The control unit is a computer chip that modulates the electromagnetic field and the sonic emission. Two versions of the device were used.

- Mark 1, as referred to in the results, has a set frequency that cannot be altered.
- Mark 2 has an alternating frequency and modulation that can be varied by the user. (NB, the actual frequencies used are commercial in confidence and cannot be discussed in this paper).

Oocysts

Cultures of *Cryptosporidium parvum* oocysts that had passed through mice were purchased from Murdoch University, Western Australia. The culture was stored at 4^{o}C in double distilled water. Viability checks and control experiments were carried out within 24 hours (before/after) of each experiment.

Laboratory set up for *Cryptosporidium* disinfection trials

A purpose built laboratory apparatus for the experiments allowed one variable to be altered at a time. The materials used were inert and sterilisable (Fig. 1). Approximately 300 000 live *Cryptosporidium parvum* oocysts were introduced to 50 L of water (tap or Reverse Osmosis water) in a polypropylene carboy fitted with a triple blade mixer (Fig 1). The oocysts were mixed into the water for 5 min before the AntiBio system was turned on. The contaminated water was removed from the carboy using a peristaltic pump equipped with 12.7 mm ID silicone tubing at a rate of 2 Lmin^{-1}. The coil of the AntiBio system was secured to the outside of the silicone tubing at a point after the pump and before reentering the carboy. The water was removed from the tap at the bottom of the carboy and reentered at the top to assist mixing. After four complete turns of the water (1 h, 40 min), the 50 L of contaminated water was concentrated using the Envirochek (Pall Gelman) system, according to the manufacturer's instructions.

All experiments were carried out at room temperature (21-22^{o}C). The pH of the tap water varied was 7.4-7.6, the pH of the reverse osmosis (RO) water was pH4.6.

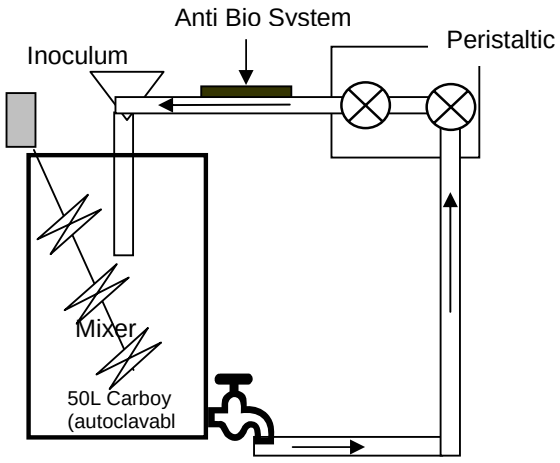

Fig. 1 Schematic diagram of laboratory apparatus used for *Cryptosporidium* dosing trials.

Concentration of sample

Samples were collected from the Envirochek filter following the manufacturer's instructions. After elution and centrifugation, tap water samples required a further purification step using Percoll-Sucrose gradient. The pellet was washed twice and resupsended in PBS pH 7.2, before staining. All centrifugation was carried out at 3000 g for 10-15 min.

Detection and Viability determinations

Detection

The final pellet after concentration was stained with FITC conjugated antibody (Merifluor). Later, samples were stained with an antibody kindly provided by Prof Duncan Veal, Macquarie University, NSW. There was no detectable difference between the two antibodies sources.

Incubation with DAPI and PI

The modified method adopted from that described by (Campbell, Robertson, et al., 1992) using 4',6' - diaminidino-2-phenylindole (DAPI) and Propidium Iodide (PI) for determining viability was used. The Live/Dead kit (Molecular Probes), relies on PI staining, and DAPI staining was used to determine presumptive viability of the oocysts.

Microscopy

Aliquots of 20µl of the stained and fluorescently labeled pellet were viewed under epifluorescence and phase microscopy. The oocysts were recorded as intact or ghosts (empty oocyst), as viewed under x400 phase contrast. The internal structures were viewed under the UV filter block (350nm excitation and 450nm emission) to visualise the sporozoites stained with DAPI. The green filter (500nm excitation and 630nm emission) was used to determine

whether they were PI positive/negative. Intact oocysts that were PI positive were assumed to be non-viable. Oocysts that were empty (ghosts) or ruptured were also considered to be non-viable. At least 100 oocysts were counted in each sample and the results were expressed as a percent of the total count (Figs 2 and 3).

Laboratory set up for biofilm investigations

Pseudomonas aeruginosa was grown in 10mL poly propylene tubes at 35^{o}C. The tubes contained either R2A (Oxoid) broth as the growth media or the cells were inoculated into sterile tap water. The biofilm (attachment of *P. aeruginosa* to the tube wall) was qualitatively examined by removing the liquid culture and staining the tube with crystal violet. Three sets of tubes were examined in triplicate: control tubes were did not have a coil attached; the Mark I device was attached to three tubes and the third set were attached to the Mark II device. All three sets of tubes were grown in separate incubators, in separate rooms where possible.

The tubes were every 3 or 4 days or weekly for attachment of cells to the wall.

RESULTS AND DISCUSSION

Effects of the ABS on *C. parvum* oocysts

When the oocysts were treated with the mark 1 and mark 2 systems their viability was not significantly affected as measured using the fluorogenic dyes. There was a 10-20% increase in the number of empty or ruptured oocysts when the oocysts were treated while in RO water (Fig. 2). The number of oocysts staining with the PI (non-viable) did not significantly increase after treatment with the ABS, Mark 1 or Mark 2.

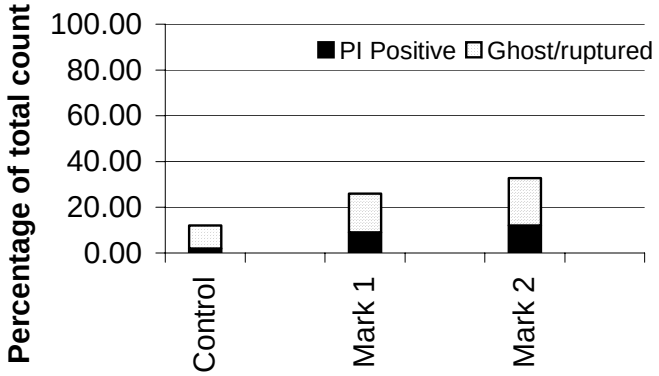

Fig. 2 Percent non-viable *Cryptosporidium parvum* oocysts after treatment with the AntiBio System and using the Molecular Probes Live/Dead kit and phase contrast microscopy. The oocysts were inoculated into RO water.

Repeated trials with the oocysts suspended in RO water did not demonstrate any significant change in the oocysts as viewed under phase microscopy or fluorogenic staining. The next set of experiments was carried out using tap water. Again there were no significant changes in oocysts viability when treated with the fixed frequency Mark 1 system. The oocysts in tap

water and treated with the variable frequency Mark 2 system were affected. Mark 2 treated oocysts in tap water had ruptured under the conditions they were exposed to (Fig 3.). The proportion of PI positive oocysts did not increase while the numbers of empty and ruptured oocysts increased by approximately 60%.

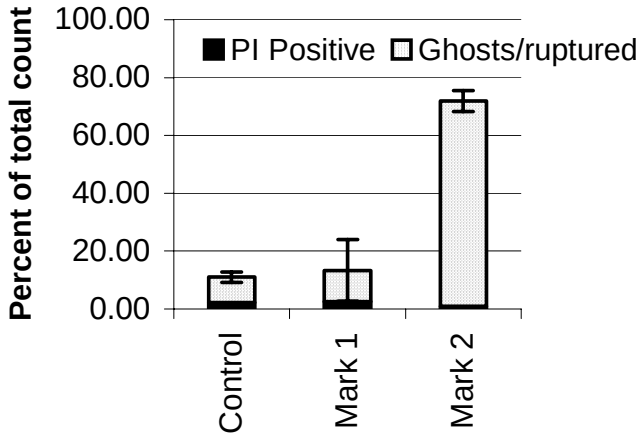

Fig. 3 Percent non-viable *Cryptosporidium parvum* oocysts after treatment with the AntiBio System as measured using Live/Dead kit (Molecular Probes) and phase contrast microscopy. All experiments were carried out in triplicate. The results are expressed as a mean of these 3 experiments, the error bars indicate the standard deviation.

The cyst wall of *C. parvum* oocysts consists of two electron dense layers separated by a thin electron translucent space. It is smooth and averages c.a. 50nm in thickness (Reese, et al., 1982). A faint line along the wall of the oocyst has been identified as a suture that dissolves during excystation (Reese, et al., 1982). The rupturing of the oocysts after treatment with the Mark 2 system appeared to be a result of the opening of this suture. How the suture may have opened when exposed to the frequencies emitted from the ABS remains unknown. Excystation, within a host, requires an acidic (pH 2-4) and anaerobic environment, correct temperature and presence of certain gastric secretions (Reduker and Speer, 1985). It may be that sound waves that the oocysts were exposed to were short enough to vibrate the wall and disrupt the suture, forcing it open. At this stage any suggested mechanism for the mode of action is speculation. Further investigation is required to understand the mode of action of the ABS against oocysts.

The sporozoites (the infective agent) within the oocysts do not survive in the environment, therefore if the oocyst ruptures outside the host; it will no longer be infective. The estimated time of survival of sporozoites in the environment is c.a. seconds (O'Donoghue, personal communication).

Comparison to other studies

While electro- assisted treatment technology has been used to manipulate or control biological cells, a universal theory is yet to be presented. A review is presented by Goldsworthy et al. (1999) on the action of electro-magnetic fields on biological cells, however they only describe the possible impact on membranes and not cyst walls. The mechanism by which magnetic fields effect the colloids in an aqueous environment has also been reviewed (Baker and Judd,

1996). Goldsworthy et al. (1999) suggested that the principles involved in the behavior of colloids in an applied electromagnetic field is similar in a biological membrane, as described below.

The explanation of Goldsworthy et al. (1999) does not necessarily apply to an oocyst wall as the structure and components of an oocyst wall are quite different to a semi-permeable membrane. They suggest that the membrane permeability of biological cells increase within an electro-magnetic field. Biological membranes are stabilised by a monolayer of calcium ions, which cross-link the phosphate moieties of the phospholipid bilayer. If the exposure to an electro-magnetic field results in the removal of some of the calcium ions, or other colloids attach to them, the membrane structure is disrupted and becomes increasingly permeable. Increased permeability of walls or membranes presents an advantage in the presence of toxins or biocides used as disinfectants. The observation that less chlorine was required to inactivate *Giardia muris* cysts after exposure to an electric field (Haas and Aturaliye, 1999) may be the result of a more permeable cyst wall. However, no explanation was given as to why the wall may have become more permeable.

Effect of the ABS on Biofilms

Pseudomonas aeruginosa was grown in poly propylene tubes for up to 2 weeks. The results from examining the biofilm were inconsistent an inconclusive. However, under certain conditions it does appear that the sweeping sound device may have reduce biofilm formation during these experiments (Fig 3 and 4).

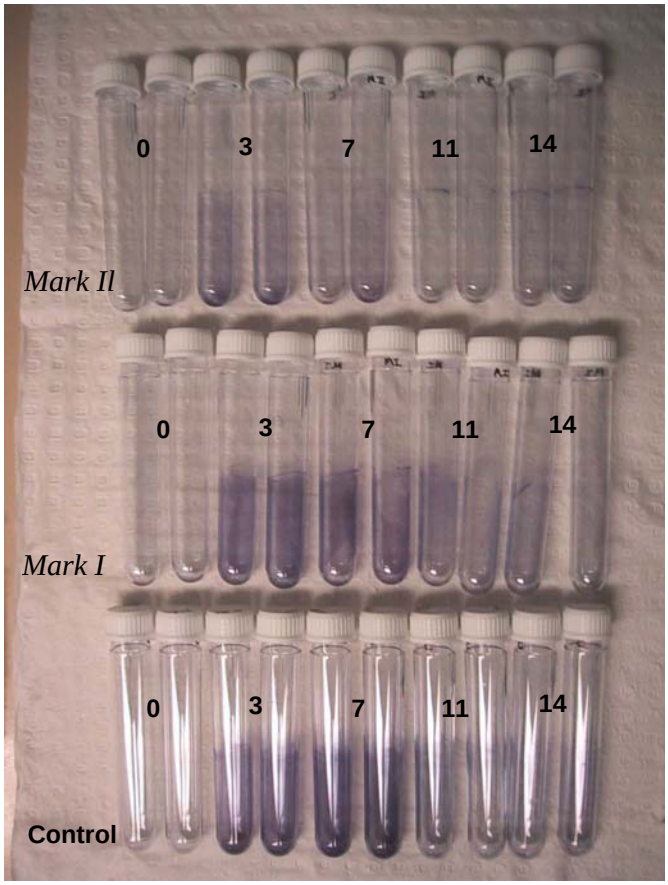

Fig 3. Established biofilms of *Pseudomonas aeruginosa* in tap water. Biofilm growth was checked at Day 0, 3, 7, 11 and 14. Duplicate tubes were stained biofilm formation using crystal violet.

The system was not able to completely inhibit the attachment of cells when they were in tap water, however the biofilm was reduced when the tubes were attached to a Mark II coil (Fig 3). The biofilm formed when the cells were grown in nutrient broth was significantly reduced when treated with the Mark I system (Fig 4). During these experiments, the Mark II system was no different to the control.

Fig 4 Biofilm formation of P. aeruginosa growing nutrient broth in tubes treated with the Mark 1 device (left) and the control tubes (right).

The results indicate that the Mark 1 Anti-Bio-System also could not completely prevent the formation of a biofilm but a reduced growth was demonstrated in two out of three tubes. The frequency used with the Mark 1 device seems to be more effective to treat bacterial biofilm formation than the alternating frequencies used with the Mark 2 device. However, more data is necessary to obtain statistically reliable results and further research is necessary to identify the underlying mechanisms, which inhibit the bacteria from forming a biofilm.

Mode of action of electromagnetic water conditioners

Magnetic conditioners have been used to some degree of success for improving water quality (Baker and Judd, 1996), however they rely on the flow of water through the magnetic field. Electric based water conditioners apply an alternating or pulsed electromagnetic field via and antenna or coil attached to the outside of the pipe (Goldsworthy, et al., 1999), similar to the system trialed here. A moving electromagnetic field moves through the water and does not depend on flow rate (Goldsworthy, et al., 1999). However, it appears from other studies that the level of conditioning is increased at low flow rates which would give a higher contact time for the particulates and colloids within the water.

Waveforms generated with different frequencies and energy levels vary, which may explain why some so-called water conditioners are more successful than others. Electrical generated waves are usually sharp pulses or audiofrequency square waves, that are rich in harmonics, often extending into the radio-frequency (RF) spectrum (Goldsworthy, et al., 1999). The ABS employs frequencies that do extend into the radio frequency region. It was proposed that the RF component an emitted signal may be important, since the rapidly moving RF field should

compensate for the much lower field strengths employed by electromagnetic water conditioners (2 υT). RF signals have been demonstrated to affect the ζ- potentials of colloidal calcium (Chibowski, et al., 1994), which led Goldsworthy et al. (1999) to suggest it is the calcium in the cell membrane that affects the permeability.

Which component of the ABS responsible for the rupturing of the oocyst wall is unknown. As both an electromagnetic field would be generated and sound waves, it could be a combination of both. It was interesting to observe a marked affect when the treatment was carried out in tap water, as compared to the minimal affect when the oocysts were in RO water. The presence of colloids in tap water that would be expected to be absent in the RO water may have influenced the results. Further work is required to understand how the device works.

Several studies have shown that particular frequencies encourage and other discourage microbial growth. Promotion of growth of bacteria that cause black spot on leaves of fruit trees was achieved by exposure to very high frequency electromagnetic field (Alexander, 1996). Yeast cultures were inhibited by strongly conditioned water and growth was encouraged by weakly conditioned water (Goldsworthy, et al., 1999), where increasing the contact time increased the strength of the conditioning. Similar observations have been made with the ABS in the field where yeast cells were encouraged at a particular applied frequency and inhibited at another (unpublished). The Mark 1 and Mark 2 devices are operated a different frequencies and as demonstrated here, the Mark 2 was the only one that affected the oocyst wall by rupturing it at the suture.

Potential in the water industry

The potential application of the ABS technology discussed here in the water industry is extensive. Based on anecdotal evidence to date and other reports (Baker and Judd, 1996; Goldsworthy et al. 1999), the applications could range from: prevention of biofilm build-up on pipes and other hardware; to improved filtration; to a disinfection aid. Some of the applications where the sweeping sound technology may be applied include:

- Improved performance for UV disinfection by preventing scale and organics building up on the bulbs and walls
- Improved filtration as shown by the field trials in swimming pools.
- A disinfection aid to be used with chemical disinfection methods
- A method to combat parasitic protozoans in water

CONCLUSIONS

- The sweeping sound technology trialed affects the viability of *C. parvum* oocysts. The Mark 2 device set at a particular set of frequencies resulted in the oocyst wall rupturing under the conditions used.
- The work presented here is considered to be preliminary. Further testing is required to confirm the results obtained to date and to decipher the mode of action of the ABS system on biological cells and cysts.

Future work

Clearly, a lot more work needs to be carried out to understand the mechanisms by which the sweeping sound technology achieves the observed benefits. At present, we are concentrating on understanding the mode of action for the effects the device imposes on *Cryptosporidium* oocysts. A combination of methods including *in vitro* excystation and fluorogenic staining and electron microscopy will assist in determining how the sound waves/electromagnetic fields are rupturing the oocyst wall. Therefore the research programme will:

- Determine infectivity using in vitro excystation
- Determine whether remaining 30 % of oocysts are still viable using excystation and possibly molecular techniques
- Attempt to understand the mechanism by which the sweeping sound waves result in a ruptured oocyst wall. Electron microscopy may be of assistance here.

The study is to be extended to investigate the effect of the ABS on bacterial cells and virus particles in water.

REFERENCES

BAKER, J. S. and JUDD, S. J. (1996). Magnetic amelioration of scale formation. Water Research 30 (2), 247-260.

CAMPBELL, A. T., ROBERTSON, L. J. AND SMITH, H. V. (1992). Viability of *Cryptosporidium parvum* oocysts: Correlation of *in vitro* excystation with inclusion of fluorogenic dyes. Applied and Environmental Microbiology 58 (11), 3488-3492.

CHIBOWSKI, E., HOLYSZ, L. AND WOJCIK, W. (1994). Changes in - potential and surface free energy of calcium carbonate due to radio frequency electric field. Colloids Surf. A: Physicochemical Engineering Aspects 92 79-85.

CHRISTIAN, P., YI, J. AND LAMY, M.-F. (1998). Ultrasound and environment: Sonochemical destruction of chloroaromatic derivatives. Environmental Science and Technology 32 (9), 1316-1318.

GOLDSWORTHY, A., WHITNEY, H. AND MORRIS, E. (1999). Biological effects of physically conditioned water. Water Research 33 (7), 1618-1626.

HAAS, C. N. AND ATURALIYE, D. N. (1999). Kinetics of electroporation-assisted chlorination of *Giardia muris*. Water Research 33 (8), 1761-1766.

KORICH, D. G., MEAD, J. R., MADORE, M. S., SINCLAIR, N. A. AND STERLING, C. R. (1990). Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. Appl Environ Microbiol 56 (5), 1423-1428.

MA, Y.-S. AND LIN, J.-G. (1998). Effect of pre-sonication on removal of organic matter resulting from chlorinated humic acids. Water Science and Technology 38 (6), 253-260.

MAHAWAORASILPA, T. L., COSTER, H. G. L. AND GEORGE, E. P. (1996). Forces on biological cells due to applied alternating (AC) electric fields. II. Electro-rotation. Biochimica et Biophysica Acta (BBA) - Biomembranes 1281 (1), 5-14.

OKOCHI, M., NAKAMURA, N. AND MATSUNAGA, T. (1995). Electrochemical disinfection of marine bacteria, using an electro-conductive paint and its application to prevent biofouling. Denki-Kagaku 63 (12), 1200-1204.

OKOCHI, M., TAGUCHI, T., TSUBOI, M., NAKAMURA, N. AND MATSUNAGA, T. (1999). Fluorometric observation of viable and dead adhering diatoms using TO-PRO-1 iodide and its application to the estimation of electrochemical treatment. Applied Microbiology And Biotechnology 51 (3), 364-369.

REDUKER, D. W. AND SPEER, C. A. (1985). Factors influencing Excystation of *Cryptosporidium* oocysts from cattle. The Journal of Parasitology 71 (1), 112-116.

REESE, N. C., CURRENT, J. V., ERNST, J. V. AND BAILEY, W. S. (1982). Cryptosporidiosis of man and calf: A case report and results of experimental infection in mice and rats. American Journal of Tropical Medicine and Hygiene 31 226-229.

SCHOENBACH, K. H., PETERKIN, F. E., ALDEN, R. W. I. AND BEEBE, S. J. (1997). Effect of pulsed electric fields on biological cells: Experiments and applications. IEEE Transactions on Plasma Science 25 (2), 284-292.