The effect of electromagnetic fields, from two commercially available water treatment devices, on bacterial culturability

Chathuri Piyadasa, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart, Harry F. Ridgway, Con Pelekani and John D. Orbell

ABSTRACT

Commercially available pulsed-electromagnetic field (PEMF) devices are currently being marketed and employed to ostensibly manage biofouling. The reliable application and industry acceptance of such technologies require thorough scientific validation – and this is currently lacking. We have initiated proof-of-principle research in an effort to investigate whether such commercially available PEMF devices can influence the viability (culturability) of planktonic bacteria in an aqueous environment. Thus two different commercial PEMF devices were investigated via a static (i.e. non-flowing) treatment system. ‘Healthy’ *Escherichia coli* cells, as well as cultures that were physiologically compromised by silver nano-particles, were exposed to the PEMFs from both devices under controlled conditions. Although relatively minor, the observed effects were nevertheless statistically significant and consistent with the hypothesis that PEMF exposure under controlled conditions may result in a decrease in cellular viability and culturability. It has also been observed that under certain conditions bacterial growth is actually stimulated.

Key words | biofouling, bacterial viability/culturability, electromagnetic fields, silver nano-particles

INTRODUCTION

Biofouling is a major problem in many water treatment applications, including the operation of reverse osmosis membrane systems for desalination and water reuse (Matin *et al.* 2011), cooling-tower installations and industrial refrigeration plants (Duda *et al.* 2011). Biofouling is primarily due to the accumulation of biofilm on various surfaces. Biofilm is the general term for describing the adhesion and accumulation of bacteria and their associated secretions on a submerged solid surface or at any phase transition interface (Bremere *et al.* 2000; Ambashta & Sillanpää 2010). When a biofilm becomes problematic in the context of one or more operational parameters, such as a loss of flux or solute rejection (Hydronautics 2011), the term ‘biofouling’ is typically used. Primary bacterial attachment progresses to rapid colonization under favorable conditions, such as the presence of trace nutrients – and this later develops into a mature biofilm that is referred to as a ‘structured microbial community’ (Percival *et al.* 1998; Vrijenhoek *et al.* 2001; Hori & Matsumoto 2010). Biofilm communities often contain multiple layers of living, inactive, and dead bacteria along with their associated extracellular polymeric substances (Malaeb & Ayoub 2011). Major groups of bacteria that cause biofouling include *Pseudomonas, Corynebacterium, Bacillus, Arthrobacter, Mycobacterium,* *Acinetobacter, Cytophaga, Flavobacterium, Moraxella, Micrococcus, Serratia, Lactobacillus* (Matin *et al.* 2011) and *Sphingomonas* species (Bereschenko *et al.* 2010).

A variety of biofouling control measures are employed in the water treatment industry, including chemical cleaning, chemical and non-chemical feed-water pretreatment, and optimization of operational parameters (e.g. adjustment of recovery) (Matin *et al.* 2011). The terms ‘non-chemical water treatment systems’ or ‘non-chemical devices’ (NCDs) cover a wide range of physical water treatment technologies, including magnetic, pulsed power, electrostatic, ultrasonic and hydrodynamic cavitation processes (Duda *et al.* 2011). Huchler (2002) has reviewed three specific categories of NCDs: magnetic (permanent/electromagnetic), electrostatic, and alternating current induction.
In so-called ‘electromagnetic field’ (EMF) treatment methods, water is passed through a plastic or stainless-steel conduit which is wrapped by a conductive wire or cable that can be energized. There is no direct connection between the wire coils and the treated solution, and a current that can be varied or pulsed in intensity and frequency is applied within the Hz to MHz frequency range (Huchler 2002). The applied current induces a complex pulsed EMF (PEMF) signal inside the pipe that is claimed by manufacturers to compromise the viability of planktonic and biofilm microorganisms in the water supply (Huchler 2002; J. Dresty, personal communication). Little peer-reviewed research has been published that supports use of commercial PEMF devices for water treatment.

With the limited published literature in this area, the present study faced a number of challenges. However, after an analysis of the available documentation, it was clear that manufacturers tend to make their claims based on uncontrolled laboratory and field conditions. Another problem relating to such devices is that the specifications/manuals omit important technical details such as frequency information and power parameters – and the standard operating procedures are either not available or unclear. Although manufacturer’s websites are often replete with testimonials from laboratory studies and field trials, such reports frequently lack mechanistic (e.g. molecular or biochemical) explanations of how PEMF affects microbial physiology and metabolism (Kitzman et al. 2003; Fitzpatrick 2006) or no information is available on replicability (Alley et al. 2008; Puckorius 2012).

Intellectual property (IP) considerations may also hinder the release of the technical details of PEMF devices, which are often critical for the scientific evaluation of their effectiveness (J. Dresty, personal communication). For example, it is known that there may be two or more coils wrapped around the treatment pipe (Cho et al. 2005). The number and thickness of such coils are often claimed as part of the manufacturer’s IP (J. Dresty, personal communication). Furthermore, if multiple coils are used, they could well be wired in different ways, e.g. either in series or in parallel, which can be unique to a particular device and which might lead to different waveform characteristics and signal strengths (Huchler 2002), and hence different possible outcomes.

Despite the scarcity of high-quality scientific support, manufacturers, nevertheless, continue to successfully market PEMF devices to end-users, who often report a reduction in, for example, Legionella counts and/or suppression of biofilm growth in cooling-tower systems and other applications (Patton & Alley 2009). Such ‘successes’ under actual field conditions suggest that further scientific scrutiny of PEMF technologies (that have the advantage of avoiding the use of toxic chemicals) are warranted.

The research described herein represents a ‘proof-of-principle’ study to evaluate the efficacy of two different commercial PEMF devices for their ability to influence the viability (i.e. culturability) of the bacterium Escherichia coli. Both devices were evaluated in terms of their comparative frequencies and waveform characteristics. Two populations of E. coli were treated by the PEMF devices. One (‘healthy’) population was cultivated under standard nutritional conditions prior to PEMF exposure, whereas the other was first pre-treated with a sub-lethal concentration of silver nano-particles (AgNPs) to compromise these cells metabolically. Including the physiologically compromised cells in the experimental design was rationalized by previous findings indicating such cells exhibit enhanced sensitivity to antimicrobial agents. Thus AgNPs, or colloidal silver solutions, are known for their antimicrobial and disinfection properties and have been extensively studied in this regard (Sondi & Salopek-Sondi 2004; Petica et al. 2008; Ruparelia et al. 2008; Gurunathan et al. 2009; Shameli et al. 2012; Mijnendonckx et al. 2013; Morones-Ramirez et al. 2013; Yuan et al. 2013), with a possible mechanism of action being cell membrane damage, referred to as bacterial ‘injury’ (Jung et al. 2008; Köngs et al. 2013). Such ‘injured’ bacteria may remain viable and may still be cultured but are metabolically weakened. Such injured bacteria have been reported to exhibit enhanced sensitivity to antimicrobial agents, such as chlorine (Landeen et al. 1989) and antibiotics (Morones-Ramirez et al. 2013). The inclusion of AgNP-compromised cells in the present experimental design was rationalized on the basis that such cells could be expected to exhibit greater sensitivity to the deleterious effects of EMF compared to non-injured populations.

**MATERIALS AND METHODS**

**Test apparatus**

Two PEMF devices, designated ‘Device-D’ and ‘Device-G’, were purchased from different commercial suppliers. Both units share common features, namely a signal generator housing the power and control components, and a flow-through treatment chamber which is connected to the signal generator via an electrical ‘umbilical’ cable (see Figure 1(a)). However, preliminary testing revealed that
these two devices exhibit different waveform characteristics, Figure 1(b) and 1(c).

**Bacterial cultures**

The effects of PEMF on cell viability were studied by exposing both ‘healthy’ (i.e. cells grown under standard nutrient conditions) and AgNP metabolically compromised bacterial cultures (see below). A non-pathogenic strain of *E. coli* (ATCC 25922) was chosen due to its ready availability, ease of culturing and high degree of biochemical and genomic characterization (Van Houdt & Michiels 2005; Bowman et al. 2012; Aslanimehr et al. 2013). A fresh colony picked from a pre-grown plate, obtained from the Victoria University culture collection (Melbourne, Australia), was transferred into sterile tryptone soy broth (TSB) under aseptic conditions and grown overnight (~18 hours) at 35 ± 2 °C in a shaker/incubator at 120 rpm (‘standard growth conditions’). The optical density of an overnight culture was determined at 600 nm (OD_{600nm}) using a spectrophotometer (Biochrom, Model Libra S11, Cambridge, UK) with fresh TSB as the blank. Cultures giving an OD_{600nm} of >1 unit were adjusted to ~1 (OD1) with phosphate buffered saline (PBS) with a pH of approximately 7.5. PBS was prepared by dissolving PBS tablets in sterile water (Sigma-Aldrich, St Louis, MO, USA).

**Preparation of ‘healthy’ and metabolically compromised *E. coli***

The effect of AgNPs on *E. coli* viability was determined by the method of Jung et al. (2008). Briefly, 1.0 mL of an overnight TSB culture (adjusted to OD_{600nm} ~ 1.0 with fresh PBS) was added to 99 mL of sterile PBS with and without addition of 0.2 ppm AgNPs (Jung et al. 2008). For the purposes of this investigation, ‘healthy’ cells were defined as those that were not exposed to AgNPs, whereas ‘compromised’ cells were exposed to AgNPs. Both flasks were incubated at 37 °C with shaking at 120 rpm. At t = 0, 1, 2, 3, and 4 hours, 1.0 mL aliquots were removed from each culture, serially diluted in PBS, plated on nutrient agar (Oxoid, Hampshire, UK), in triplicate, and incubated at 37 °C overnight. Following incubation, colony forming units (CFUs) were manually enumerated with a laboratory colony counter (GallenKamp, UK). The number of CFUs was compared for the ‘healthy’ and AgNP-treated (‘compromised’) cultures. Results from this comparison indicated substantial (~99%) but not complete cellular inactivation within 1 hour by the AgNPs compared to the untreated control (data not presented). In contrast, untreated control cells underwent normal cell division and a marked population increase, presumably at the expense of endogenous nutrients. Based on this analysis, AgNP-compromised cultures were routinely prepared by exposing cells for 1 hour to 0.2 ppm AgNPs at 37 °C in PBS (see Figure 2).

**Exposure of healthy and compromised *E. coli* cultures to PEMF**

The basic experimental protocol is outlined in Figure 2. During the 1 hour pre-incubation, the healthy and AgNP-compromised cells both grew to approximately 4.0–6.0 × 10⁵ CFU/mL (data not shown). After the 1 hour pre-incubation period, 5 mL of each cell suspension was introduced to two 10 mL sterile screw-cap graduated tubes...
(Techno Plas, St Mary’s, Australia) which were then placed inside the treatment chamber of each temperature-stabilized PEMF device. The cultures were exposed to PEMF under non-flowing (static) conditions for either 3 or 7 hours (conducted on different days) (Figure 2). As a ‘non-PEMF’ control, a portion of each cell suspension was also incubated in a separate temperature-controlled water bath equilibrated at the same temperature as the PEMF device sample chamber.

**RESULTS AND DISCUSSION**

Test apparatus and characterization

Since the commercial PEMF devices have preset currents and frequencies, exposure duration was the only parameter manipulated. The waveform characteristics, as determined by an oscilloscope, were found to be very different for each device, Figure 1(b) and 1(c), and it remains unclear how differing waveforms per se affect cell viability. This will be the subject of future enquiry. The waveform obtained for the Device-D PEMF unit was consistent with manufacturer’s stated specifications although we were not able to obtain such information from the manufacturer of Device-G. Both devices were found to have frequencies that were determined to be in the order of ~100 kHz. It was also noted that the two devices thermally stabilized at different temperatures, namely at 40 °C and 27 °C for D and G, respectively. This is due to their having very different electronics and circuitry as well as different power specifications.

**Culturability of healthy and compromised *E. coli* when exposed to PEMF treatment**

Under the experimental conditions described above in the ‘Materials and methods’ section, in the absence of AgNPs, *E. coli* grew as expected, demonstrating exponential growth after ~4 hours. Under the same growth conditions, it was established that a 0.2 ppm concentration of AgNPs was sufficient to inhibit this growth and debilitate (i.e. injure) the micro-organisms within about 1 hour – but at the same time leaving them sufficiently viable for further study.

Figures 3 and 4 summarize the observed effects of PEMF exposure for 3 or 7 hours, respectively, on the bacterial culturability of the healthy and AgNP-compromised organisms for each PEMF device.

The data presented in Figure 3 indicate PEMF exposure in both devices for 3 hours resulted in a statistically significant growth enhancement of the healthy cell populations compared to the non-PEMF water-bath controls. In contrast, for both PEMF devices, the AgNP-compromised populations underwent a substantial (approximately 50%)
electric fields may become less pronounced (Mazurek et al. 2004). Thus, longer-term exposure to PEMFs may partially ameliorate the inhibitory effects, suggesting that organisms might develop an adaptive response (Gaafar et al. 2006), possibly linked to the expression of heat-shock proteins (Inhan-Garip et al. 2011). This is also consistent with the results obtained in this study, whereby after a prolonged period of exposure of 7 hours, the stimulatory effect appeared to have relaxed.

CONCLUSIONS

Although relatively minor, the observed growth-inhibitory effects for 3 hour PEMF-exposed, AgNP-treated cell populations, compared to their non-PEMF controls, were statistically significant for both devices – and this is consistent with the hypothesis that PEMF exposure under controlled conditions may result in a decrease in cellular viability and culturability, when the organisms have been otherwise compromised. The observed growth responses of healthy *E. coli* cells exposed to PEMF energy for a 7 hour period also indicated a statistically significant inhibition of growth (compared to the non-PEMF control). The results show a stronger growth-inhibitory effect for Device-D relative to Device-G, which could be attributed to the effect of different PEMF waveforms and applied energies between the two devices. This is an obvious direction for further enquiry. However, it is also apparent that under certain conditions bacterial growth is actually stimulated by the PEMF.

The observed growth-inhibitory effects, albeit small, are consistent with the application of such devices for the control of microbial growth in various industrial settings. Nevertheless, based on these current proof-of-principle investigations and outcomes, we do not deem it appropriate at this stage to make recommendations to manufacturers or buyers of such equipment, since such effects could be enhanced, or otherwise, under actual operating conditions such as under flow conditions. However, this research does encourage more scientific investigation into NCDs in general and emphasizes the importance of carefully controlled laboratory-based enquiry into this area of research.

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