

available water treatment devices – controlled studies under static and flow conditions

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Abstract

BACKGROUND: Pulsed-electromagnetic field (PEMF) devices are marketed and utilized, for the non-chemical management of biofouling, with little scientific validation of their effectiveness. We previously initiated proof-of-principle studies, to systematically investigate the effect of two such commercial devices on the culturability of bacteria under controlled static (i.e. non-flowing) conditions and anti-microbial effects were demonstrated under static conditions. However, such effects were small and an expanded investigation, using these devices and including the effect of flow, was deemed necessary.

RESULTS: The effect of the electromagnetic fields generated by the same two commercial devices on the bacterial culturability of *Escherichia coli* and *Pseudomonas fluorescens* under flow conditions has been contrasted with previous static results. It has been found that the effectiveness of PEMF exposure depends on waveform, extent of flow, type of bacteria and PEMF exposure duration.

CONCLUSION: Both stimulatory and inhibitory effects are observed that are uniquely dependent upon device type (i.e. a range of parameters including waveform), species of

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microorganism, presence and degree of flow and PEMF exposure time. For both devices and both microorganisms, stimulatory effects are uniformly observed for one device under static conditions and inhibitory effects are uniformly observed for the other device at low flow and for the former at high flow.

Keywords: biofouling; bacterial viability/culturability; pulsed electromagnetic field; reverse osmosis membrane

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Nomenclature: PEF - Pulsed Electric Field; PEMF - Pulsed Electromagnetic Field; RO - Reverse Osmosis; TSA - Tryptic Soy Agar; TSB - Tryptone Soy Broth; PBS – Phosphate Buffered Saline; NA – Nutrient Agar; CFU – Colony Forming Units.

INTRODUCTION

Water intensive installations such as cooling towers, refrigeration plants and RO membrane systems for desalination and water reuse¹⁻⁴ are susceptible to bio-fouling via a range of environmental microorganisms⁵⁻⁷ including, *Pseudomonas*, *Corynebacterium*, *Bacillus*, *Arthrobacter*, *Mycobacterium*, *Acinetobacter*, *Cytophaga*, *Flavobacterium*, *Moraxella*, *Micrococcus*, *Serratia*, *Lactobacillus*, *Sphingomonas* and *Legionella*. The life cycles of such organisms can lead to the deposition of multiple layers of living, inactive and dead organisms, along with their associated extracellular polymeric substances, so-called biofilms, onto the functional surfaces of such equipment, compromising their performance.⁸ The use and development of measures to combat such biofouling is important and are usually based on chemical methods, that present associated health and environmental risks.^{5,9} To avoid the risks associated with chemical disinfection and cleaning; non-chemical or physical feed-water pretreatment - including magnetic, pulsed power, electrostatic, ultrasonic or hydrodynamic cavitation processes have been investigated. Such methods promise to reduce labour and maintenance costs, improve safety (due to low or no chemical handling) and to reduce toxic breakdown products.¹⁰⁻¹⁴

In particular, water treatment utilizing so-called PEMF has evolved from bacterial decontamination methods using PEFs in relation to the sterilization of food.¹⁵⁻¹⁹ However, PEF and PEMF processes are fundamentally different from each other in that, in the PEF process, the field generating electrodes are in direct contact with the medium.²⁰⁻²³ For the PEMF treatment of water, there is no direct contact with the treated medium and the general method may be defined either as AC induction¹², electromagnetic³ or pulsed-power.²⁴

Thus a typical commercially available PEMF device is composed of two main components; a signal (waveform) generator²⁶ or driver enclosure,²⁷ and a treatment module²⁶ or reaction chamber²⁷ where the water to be treated is passed through a plastic or stainless-steel conduit which is wrapped around by a conductive wire or cable that can be energized²² to generate the encompassing electromagnetic field.³ Notably, manufacturers of such equipment tend to make their claims as to the effectiveness of such products based on uncontrolled laboratory/field conditions and/or unauthenticated testimonials and there is a paucity of controlled scientific research to support such claims or to elucidate potential antimicrobial mechanisms.

We have recently published a paper that thoroughly reviews use of PEMF devices as a pretreatment for scaling and for biofouling control in the water treatment industry²⁷ and we have also initiated a systematic scientific study of the effect of two commercially available PEMF devices on bacterial culturability²⁵. This research demonstrated that for *E. coli* and silver nanoparticle compromised *E. coli*, a small, but statistically significant, inhibition occurred under static (non-flow) conditions for both devices. In addition, under some circumstances, a small but significant *stimulation* of growth was observed. It was clear from the studies that the PEMF was influencing the culturability of this microorganism and that this was sensitive to both waveform and to exposure time. Although the effects observed in this previous work were small, the results represent an important proof of principle and pave the way for the extended studies reported here, where another microorganism, *P. fluorescens*, has been included in the study and flow conditions (low and high) have been introduced into the experiments as additional parameters.

It is our contention that a persistent systematic approach to this problem with respect to the variation of parameters such as frequency, waveform, exposure time, temperature, flow rate

and the presence of ionic and other chemical species in solution, will reveal whether a set of optimal conditions can be established that will result in the high level of lethality that is desirable for a practical application of PEMF technology to biofouling - commensurate with current claims made by manufacturers of such equipment.

MATERIALS AND METHODS

Overall experimental design

A schematic showing the overall experimental design is depicted in Fig. 1. For two different commercially available PEMF devices, designated D and G, the culturability (viability) of two microorganisms, namely *E. coli* and *P. fluorescens*, were compared under static, “low” flow (92 mL/min) and “high” flow (460 mL/min) conditions.

>>>>>>> **Insert Figure 1 and Caption here** >>>>>>>

A static mode laboratory system was set up, as described in our previous work,²⁵ that incorporated either of the two commercially available PEMF devices (designated D or G) for exposure experiments on *E. coli* or *P. fluorescens* colonies. The microorganisms were plated onto TSA (Oxoid, Hampshire, England) in triplicate, and incubated at 27 ± 2 °C for 48 hours until the colonies became visible.

Flow mode test apparatus and materials

A schematic of the flow mode apparatus is shown in Fig. 2. This set up was comprised of either of the two commercially available PEMF devices (D or G), PVC arms (tubes), a peristaltic pump (Masterflex, John Morris Scientific, Chatswood, NSW 2067) and a reservoir (a 2 L polypropylene container with screw cap lid, Cospak Pty Ltd, Victoria, AU). The PVC arms

were filled with deionized water along with the treatment chamber of the connected PEMF device and a smaller diameter plastic tube - to carry the microorganism suspension - was passed through the PVC arms and the treatment chamber. As described previously²⁵, the two commercial PEMF devices employed (D and G) shared common features; namely, a signal generator housing the power and control components and a treatment chamber which is connected to the signal generator via an “umbilical” cable. It was noted that the two devices thermally stabilized at different temperatures, namely 40 °C and 27 °C for D and G, respectively, due to their having very different electronics and circuitry, as well as different power specifications and waveforms.²⁵ The temperature of the flow system was taken to be the temperature of the reservoir. Strict temperature control of these experiments is essential and this has been satisfactorily addressed in our experiments, as described herein.

>>>>>>>>> **Insert Figure 2 and Caption here** >>>>>>>>>

Bacterial cultures and materials

The effects of a PEMF on two types of bacteria were investigated. These were non-pathogenic lab strains of *E. coli* (ATCC 25922)²⁵ and *P. fluorescens* (ATCC 17386), that were chosen due to their prevalence in water systems and for their ready availability and ease of culturing²⁶. Fresh colonies from pre-grown plates, obtained from the Victoria University culture collection (Melbourne, AU), were transferred into sterile TSB (Oxoid, Hampshire, England) under aseptic conditions and grown overnight at 35 ± 2 °C for *E. coli* and 27 ± 2 °C for *P. fluorescens* in a shaker/incubator at 120 rpm. The optical density, OD, of an overnight culture was determined at 600 nm using a spectrophotometer (Biochrom, Model Libra S11 Cambridge CB4 0FJ, England) with fresh TSB as the blank. Cultures giving ODs of more than 1 unit at 600 nm were

adjusted to OD 1 with PBS. PBS was prepared by dissolving the PBS tablet in sterile water (Sigma-Aldrich, St Louis MO 63103, USA). The pH of the PBS solution was ~ 7.5.

Exposure of bacteria to PEMF under flow mode

In the flow mode system, the culture was pumped from the reservoir, passed through the device treatment chamber and then was released back into the reservoir. Each PEMF system was stabilized for 4 hours prior to the experiment. Initially, with the field turned off, the pump was started and allowed to run with 990 mL of sterile PBS for five minutes to remove any trapped air. After the five minutes, 10 mL of OD 1 bacterial culture was introduced into the 2 L reservoir and, at time zero ($t=0$, i.e. with no field), samples were taken. The field was then turned back on and the reservoir was left stirring (magnetic stirring) under room temperature conditions. As a result the ‘treated’ liquid flows through a heated chamber, the temperature of the reservoir being slightly elevated ($\sim 27\text{ }^{\circ}\text{C}$) relative to the ambient temperature ($20 - 25\text{ }^{\circ}\text{C}$). This was to minimize cell deposition and to ensure thorough mixing and representative samples.

Control set up for the flow mode test

In the flow mode system, the culture was pumped from the reservoir, passed through the device treatment chamber and then was released back into the reservoir. As a result the ‘treated’ liquid flows through a heated chamber, the temperature of the reservoir being slightly elevated ($\sim 27\text{ }^{\circ}\text{C}$) relative to the ambient temperature ($20 - 25\text{ }^{\circ}\text{C}$). Recirculation also created some turbulence in addition to the magnetic stirring/mixing. The reservoir was heated to elevate the temperature above room temperature and maintained at $27\text{ }^{\circ}\text{C}$. On separate days, a reservoir of 1L culture was prepared as above. This was re-circulated using the same pump at the same two speeds employed for the PEMF flow mode test. We emphasize that controlling the temperature up to

the required level was considered to be a vital control and was closely monitored, see Tables 1 and 2 below.

Special considerations

The reservoirs were tightly closed and covered with aluminum foil to minimize any effects from the laboratory lights on the bacterial reservoirs, both the treated and the control were covered with aluminum foil. The ambient laboratory temperature was maintained from 20 - 25 °C using an electronic temperature control panel.

Sampling and plating

Samples were directly obtained from the reservoir at the designated sampling times and serially diluted in PBS. *E. coli* were plated in NA in triplicate and incubated at 35 ± 2 °C overnight and *P. fluorescens* were plated in TSA as described earlier. The number of CFUs was used to quantify the results.^{25,34}

RESULTS AND DISCUSSION

Exposure of *E. coli* to PEMF under static, low and high flow conditions

Exposure of *E. coli* to PEMF under static conditions has been reported in our previous study.²⁵ Fig. 3 shows the observed effects of PEMF exposure on *E. coli* culturability, for each PEMF device, under different *flow* conditions. Table 1 shows the monitored temperature variation between the three *E. coli* reservoirs over the duration of the experiment for low flow and high flow conditions for both devices. These slight temperature variations are considered acceptable in relation to the experiments depicted in Fig. 3.

>>>>>>>>> **Insert Figure 3 and Caption here** >>>>>>>>>>>>>>>>>>>>>>>>>

>>>>>>>>>> **Insert Table 1 and Heading here** >>>>>>>>>>>>

Exposure of *P. fluorescens* to PEMF under static, low and high flow conditions

Fig. 4 shows the observed effects of *static* PEMF exposure on culturability of *P. fluorescens* for each device under static conditions for 3 hours and 7 hours. This experiment is analogous to the previously reported experiment conducted for the exposure of *E.coli* to PEMF under static conditions.²⁵

>>>>>>>>>> **Insert Figure 4 and Caption here** >>>>>>>>>>>>

Exposure of *P. fluorescens* to PEMF under low and high flow conditions

Fig. 5 shows the observed effects of PEMF exposure on *P. fluorescens* culturability, for each PEMF device, under *flow* conditions. Table 2 shows the temperature variation between the three *E.coli* reservoirs over the duration of the experiment for low flow and high flow conditions for both devices. These slight temperature variations are considered acceptable in relation to the experiments depicted in Fig. 5.

>>>>>>>>>> **Insert Figure 5 and Caption here** >>>>>>>>>>>>

>>>>>>>>>> **Insert Table 2 and Heading here** >>>>>>>>>>>>

For both devices, both microorganisms, and for the three different conditions of flow, the comparative results across Figs. 3 to 5 are summarized and compared in Table 3 in terms of *to what extent exposure to the PEMF is inhibitory or stimulatory*. This is expressed as the

factors that need to be accounted for in a systematic way, including waveform - an approach strongly suggested by our present research.

Another observation from Table 4 is that, generally, static conditions favor stimulatory effects (S) whereas flow conditions favour inhibitory effects (I). In this regard, it is known that PEF treatment can cause higher inactivation levels in exponentially growing cells than in stationary-phase cells³⁸. In addition, PEF exposure of *E. coli* has been reported to achieve better microbial inactivation with a higher flow rate, attributed to better mixing - allowing uniform treatment. These results are broadly consistent with our observations.

Magnetic fields³⁹, pulsed electric fields⁴⁰ and extremely low-frequency electromagnetic fields⁴¹ have been shown to be effective in controlling *P. fluorescens*, but this species has also shown a positive adaptive response (I to S) to magnetic field treatments.⁴¹ A positive adaptive response has also been observed for *E. coli*³⁸ after exposure to a 50 Hz EMF for 20–120 min. This was manifested as a subsequent increase in cell viability.

Such reports are consistent with both the inhibitory and stimulatory effects exhibited here upon exposure to PEMF under different conditions. For example, a positive adaptive response for *P. fluorescens* may be observed in our results when the flow rate is increased from low to high during Device D PEMF exposure for 6-7 h, Table 4. In this case the inhibitory to stimulatory transition is from -38% to +118%, Table 3. A similar positive adaptive response may be observed for *E. coli* when the flow rate is increased from low to high during Device D PEMF exposure, with this effect occurring for both exposure times. In this case the inhibitory to stimulatory transition (I to S) is from -36% to +4% (3-4h) and -42% to +64% (6-7 h), Table 3.

In terms of a negative adaptive response (S to I), Faraj and Muhamad³⁷ have identified a stimulation period followed by a decrease of *E. coli* numbers upon exposure to a high magnetic field. They maintain that the increase in cell numbers might be a result of stimulation in cell division and that the decrease was perhaps due to a change in the permeability of the ionic channels that causes ion imbalance. For *E. coli* PEMF exposure, we observe three examples of such a response (S to I); namely, for Device D upon going from static to low flow over a short exposure time (+9% to -36%) and for Device G upon going from low flow to high flow for both short (+57% to -26%) and long (+55% to -51%) exposure times. Similarly for *P. fluorescens* we also observe three examples of an S to I response; namely, for Device D upon going from static to low flow for both short (+571% to -17%) and long (+169% to -38%) exposure times and for Device G upon going from static to flow conditions for both short (+35% to -34%) and long (+8% to -50%) exposure times.

Table 4 also demonstrates that the effects of Device D are more variable over time than for Device G for both microorganisms. Specifically, for Device G, with increasing exposure time, an inhibitory effect develops with increasing flow and this is more pronounced for *P. fluorescens*. For Device D, the tendency with increasing exposure time is towards stimulatory effects with increasing flow, although this is more pronounced for *E. coli*.

CONCLUSION

The outcomes of these experiments support the findings of other researchers^{40, 41} whereby positive³⁸ or negative^{37, 43} adaptive responses of different microorganisms, upon exposure to magnetic or electromagnetic fields, are observed under various conditions. Via carefully controlled experiments, we have clearly demonstrated that such responses depend, in a sensitive way, on the interplay of numerous factors and parameters such as field generating

device specifications (e.g. waveform, frequency, power etc.), the specific microorganism species, flow rate and exposure time - and possibly other factors. Notably, this complex interdependency of parameters was also apparent in our recent work involving the effect of these same two devices on calcium carbonate precipitation, in relation to the prevention of scaling.⁴² In this latter work, a similarly highly controlled and systematic laboratory study demonstrated that Devices D and G had very different effects on calcium carbonate crystal formation and precipitation. To the best of our knowledge, our work represents the first time that such highly controlled, replicate, experiments have been conducted on commercially available devices.

In order to properly define such effects and to subsequently explore and delineate the mechanisms involved, an ongoing program of highly controlled systematic experiments, such as those conducted here, is required. Given the number of interdependent parameters possible, this will constitute a substantial long-term scientific venture. Indeed, it is suggested that the magnitude and complexity of this task has been a contributing factor to the paucity of scientifically based evidence that is currently available to support or refute the claims of the manufacturers of commercially available magnetic, EMF and PEMF water treatment technologies.

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Table 1: Temperature monitoring of the low flow (92 mL/min) and high flow (460 mL/min) *E. coli* reservoirs for devices D and G. The control tests were performed with no exposure to PEMF but with the same flow rates and heating. The estimated error in temperature measurement is ± 2 °C.

Exposure time (h)	<i>E. coli</i> reservoir temperature (°C) under low flow (92 mL/min)			<i>E. coli</i> reservoir temperature (°C) under high flow (460 mL/min)		
	Control	D PEMF	G PEMF	Control	D PEMF	G PEMF
3-4	30	25	25	30	27	25
6-7	26	26	25	30	27	25

Table 2: Temperature monitoring of the low flow (92 mL/min) and high flow (460 mL/min) *P. fluorescens* reservoirs for devices D and G. The control tests were performed with no exposure to PEMF but with the same flow rates and heating. The estimated error in temperature measurement is ± 2 °C.

Exposure time (h)	<i>P. fluorescens</i> reservoir temperature (°C) under low flow (92 mL/min)			<i>P. fluorescens</i> reservoir temperature (°C) under high flow (460 mL/min)		
	Control	D PEMF	G PEMF	Control	D PEMF	G PEMF
4	28	29	26	27	27	26
6	28	29	27	27	27	26

Table 3: Summary table for exposure of both *E.coli* and *P. fluorescens* to Devices D and G under static, low flow (92 mL/min) and high flow (460 mL/min) conditions (numbers represent % change in growth, i.e. [(value – control)/control] x 100%. Stimulatory (S) is positive; inhibitory (I) is negative.

Treatment duration	No flow (static treatment)		Low flow (92 mL/min)		High flow (460 mL/min)	
	Device D	Device G	Device D	Device G	Device D	Device G
3-4 hours <i>E. coli</i>	<i>Stimulatory</i> 9% (Ref. 25)	<i>Stimulatory</i> 68% (Ref. 25)	<i>Inhibitory</i> -36% Fig 3 (a)	<i>Stimulatory</i> 57% Fig 3 (a)	<i>Stimulatory</i> 4% Fig 3 (b)	<i>Inhibitory</i> -26% Fig 3 (b)
6-7 hours <i>E. coli</i>	<i>Inhibitory</i> -55% (Ref. 25)	<i>Stimulatory</i> 5% (Ref. 25)	<i>Inhibitory</i> -42% Fig 3 (a)	<i>Stimulatory</i> 55% Fig 3 (a)	<i>Stimulatory</i> 64% Fig 3 (b)	<i>Inhibitory</i> -51% Fig 3 (b)
3-4 hours <i>P. fluorescens</i>	<i>Highly Stimulatory</i> 571% Fig 4 (a)	<i>Stimulatory</i> 35% Fig 4 (a)	<i>Inhibitory</i> -17% Fig 5 (a)	<i>Inhibitory</i> -34% Fig 5 (a)	<i>Inhibitory</i> -31% Fig 5 (b)	<i>Inhibitory</i> -40% Fig 5 (b)
6-7 hours <i>P. fluorescens</i>	<i>Highly Stimulatory</i> 169% Fig 4 (b)	<i>Stimulatory</i> 8% Fig 5 (b)	<i>Inhibitory</i> -38% Fig 5 (a)	<i>Inhibitory</i> -50% Fig 5 (a)	<i>Stimulatory</i> 118% Fig 5 (b)	<i>Inhibitory</i> -17% Fig 5 (b)

Table 4: An overview of the stimulatory (S) or inhibitory (I) effects as a result of exposure to the PEMFs from two different commercial devices (D & G) as a function of the device itself, the flow conditions (static, low (92 mL/min) or high (460 mL/min)), the microorganism species and the exposure time.

Device	Flow			Organism	Exposure (h)
	Static	Low flow	High flow		
D	S	I	S	<i>E.coli</i>	3-4
	I	I	S		6-7
	S	I	I	<i>P. fluorescens</i>	3-4
	S	I	S		6-7
G	S	S	I	<i>E.coli</i>	3-4
	S	S	I		6-7
	S	I	I	<i>P. fluorescens</i>	3-4
	S	I	I		6-7

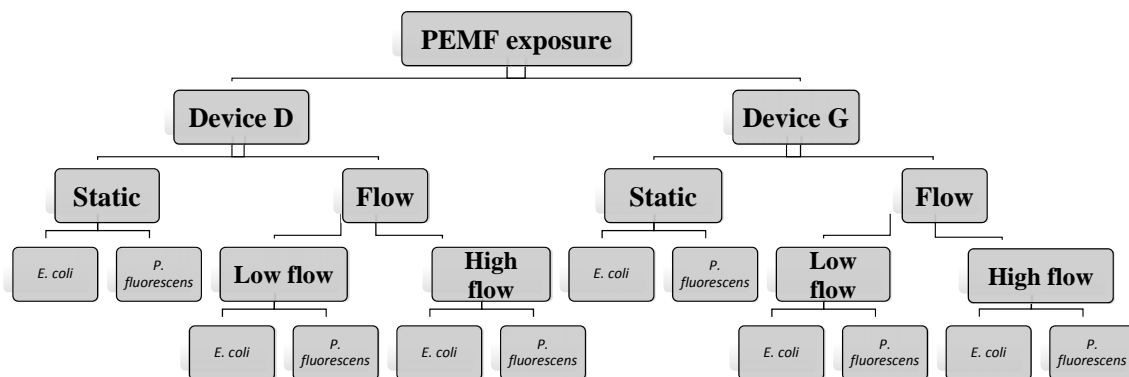


Figure 1. Schematic showing the overall experimental design.

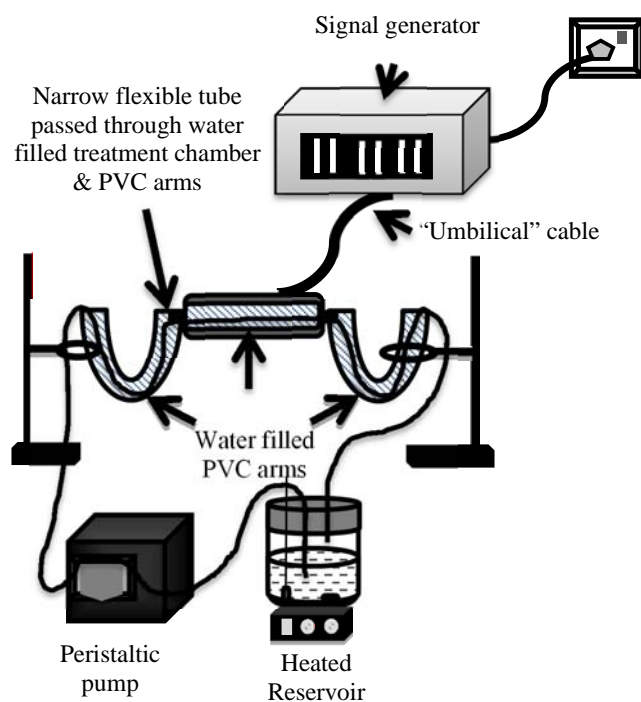


Figure 2. The "flow mode" apparatus incorporating the PEMF device(s).

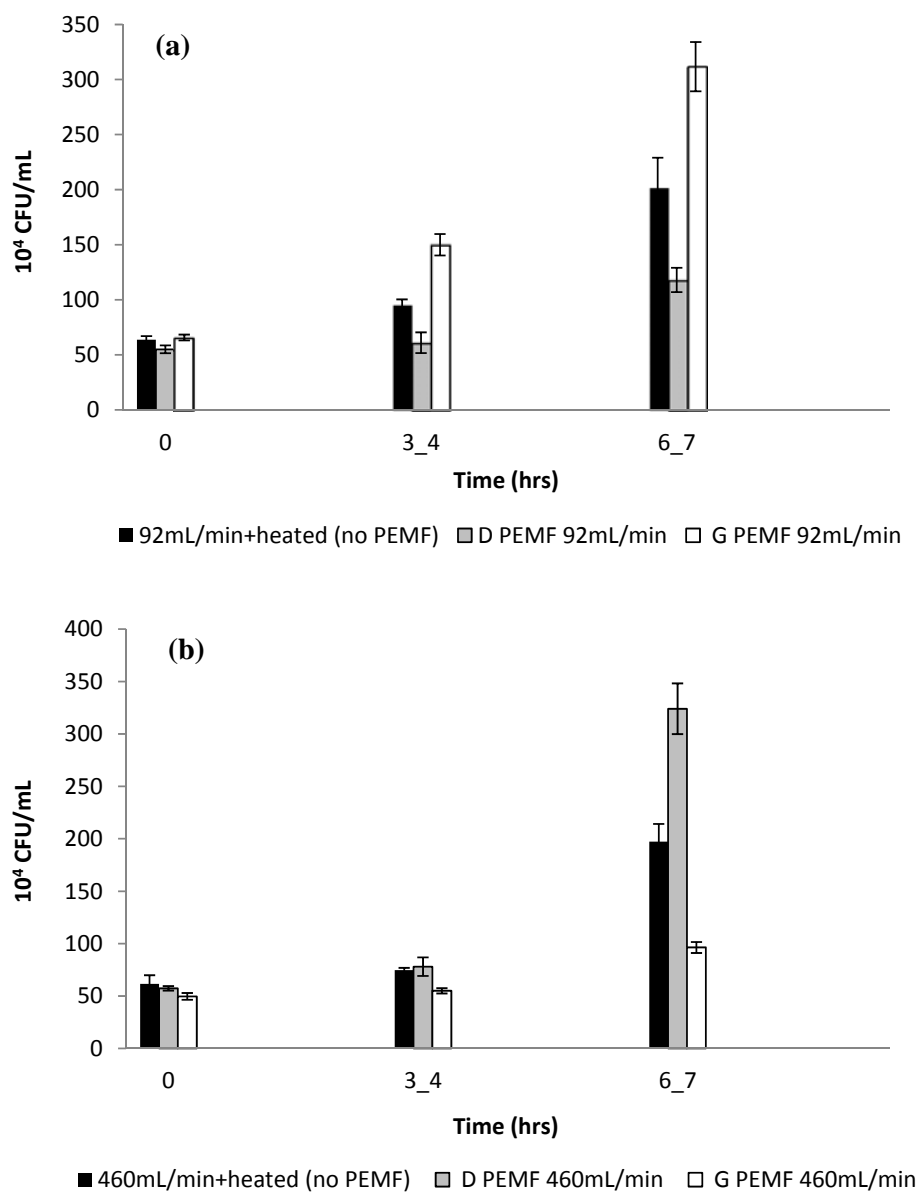


Figure 3. Enumeration of *E. coli* populations (expressed as CFUs/mL) for the control and for exposure to PEMF by devices D and G under (a) low flow rate (92 mL/min) (b) high flow rate (460 mL/min). Experiments for device D, G, and control (for all static, low flow and high flow) were freshly started and performed separately on different dates. The error bars are standard errors for three replicate platings. Bars at $t=0$ represent the ‘establishment stage’ after about 1h of growth where the bacteria are introduced into the experiments – note that for these three bars there is no PEMF applied.

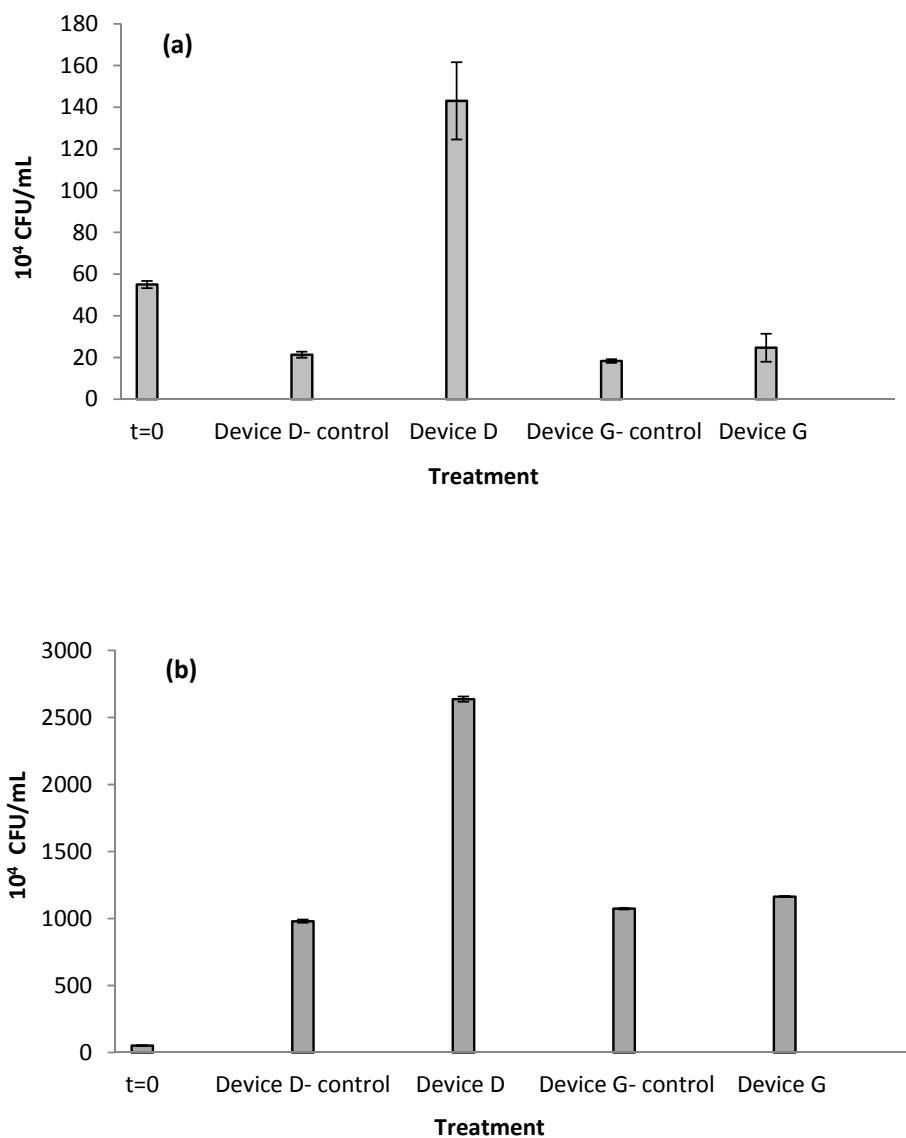


Figure 4: Enumeration of *P. fluorescens* populations (expressed as CFUs/mL) following exposure for (a) 3 hours (b) 7 hours to PEMF for Device-D or Device-G and their respective non-PEMF temperature pre-equilibrated water-bath controls. Error bars are standard errors for three replicates. The 3 hour and 7 hour exposure experiments were conducted on 2 separate days due to the difficulties of sampling, hence these are presented in two graphs. *Notes:* (i) bars at t=0 represent the 'establishment stage' after about 1 hr of growth where the bacteria are introduced into the experiments.

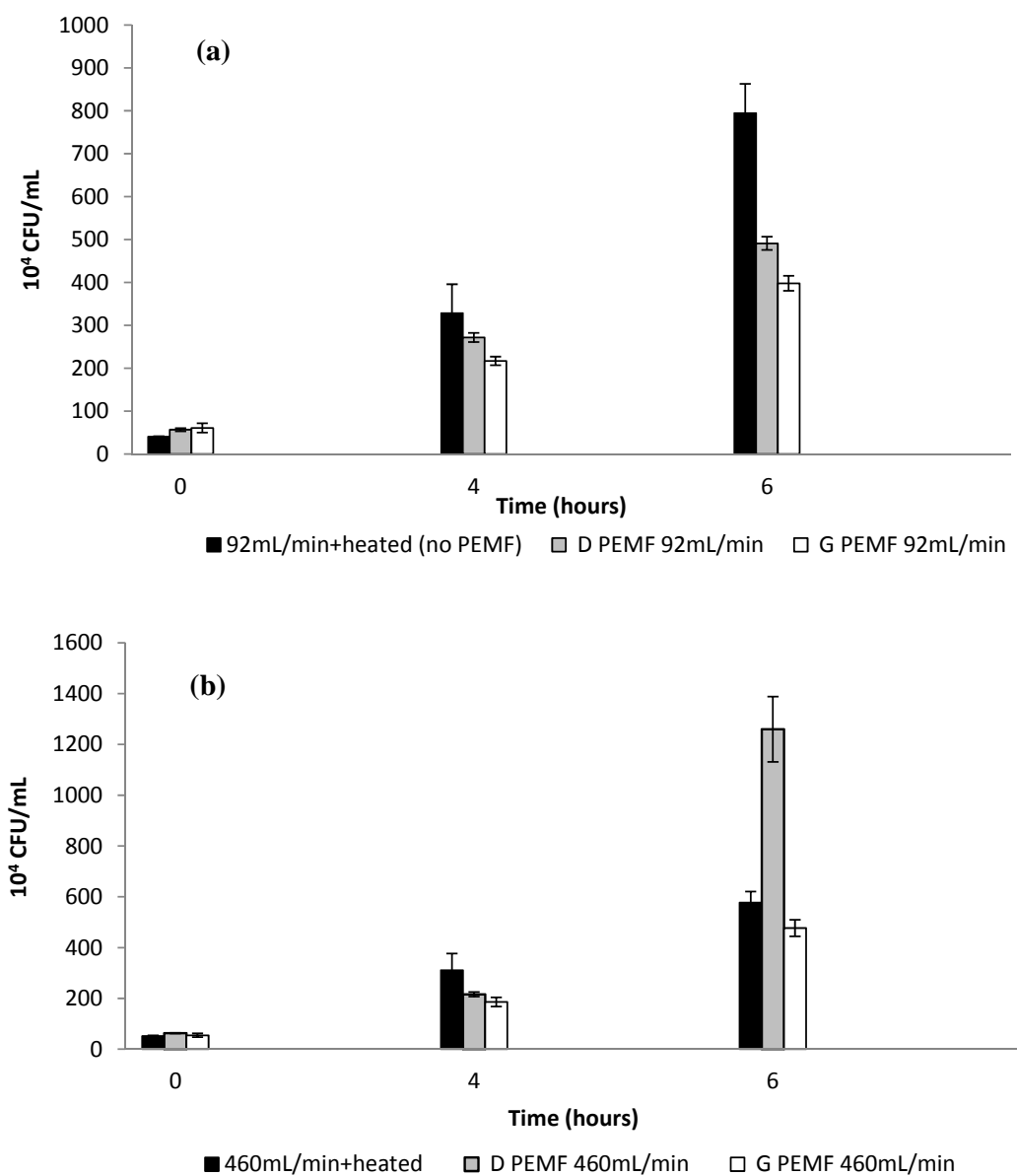


Figure 5: Enumeration of *P. fluorescens* populations (expressed as CFUs/mL) for the controls and for exposure to PEMF by devices D & G under (a) low flow rate (92 mL/min) (b) high flow rate (460 mL/min). Experiments were performed separately on different dates and the error bars are standard errors for three replicate plating. Bars at t=0 represent the ‘establishment stage’ after about 1 hr of growth where the bacteria are introduced into the experiments, note that for these bars there is no PEMF.