Effects of commercially available pulsed electromagnetic field devices on bacterial viability and calcium carbonate precipitation

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by

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Abstract

Biofouling and scaling are two major problems in the operation of reverse osmosis (RO) membranes and other equipment. A variety of control measures are employed in practice, including the use of electromagnetic fields (EMF), which can avoid the use of chemical anti-fouling agents (e.g. halogen-based biocides) that may be toxic to humans or the environment. This is a fairly recent and controversial technology and, from the available documentation and literature, it is clear that the scientific basis for its purported effectiveness is not yet firmly established. In particular, the various conditions under which EMF technologies are likely to be effective for real world applications have not been established. This thesis reviews and collates the relevant literature on the problem of scaling and biofouling in RO membranes and heat exchanger systems (e.g. cooling towers), with a particular focus on the application of *pulsed* EMF technologies, including the broad documentation, relevant scientific studies, proposed mechanisms of action and further research directions. This study confirms that a lot more systematic scientific research is needed in order to validate the application and commercialization of EMF technologies as a pre-treatment method to control fouling and scaling in various applications including RO membrane systems.

Therefore, a number of carefully controlled laboratory experiments have been designed and carried out in order to test the inherent anti-bacterial and anti-scaling claims for two commercially available pulsed electromagnetic field (PEMF) devices, that were demonstrated in this study to operate at ~ 100 kHz but with different waveforms. For example, these commercially available devices are currently being marketed and employed to ostensibly manage biofouling. Since the reliable application and industry acceptance of such technologies requires thorough scientific

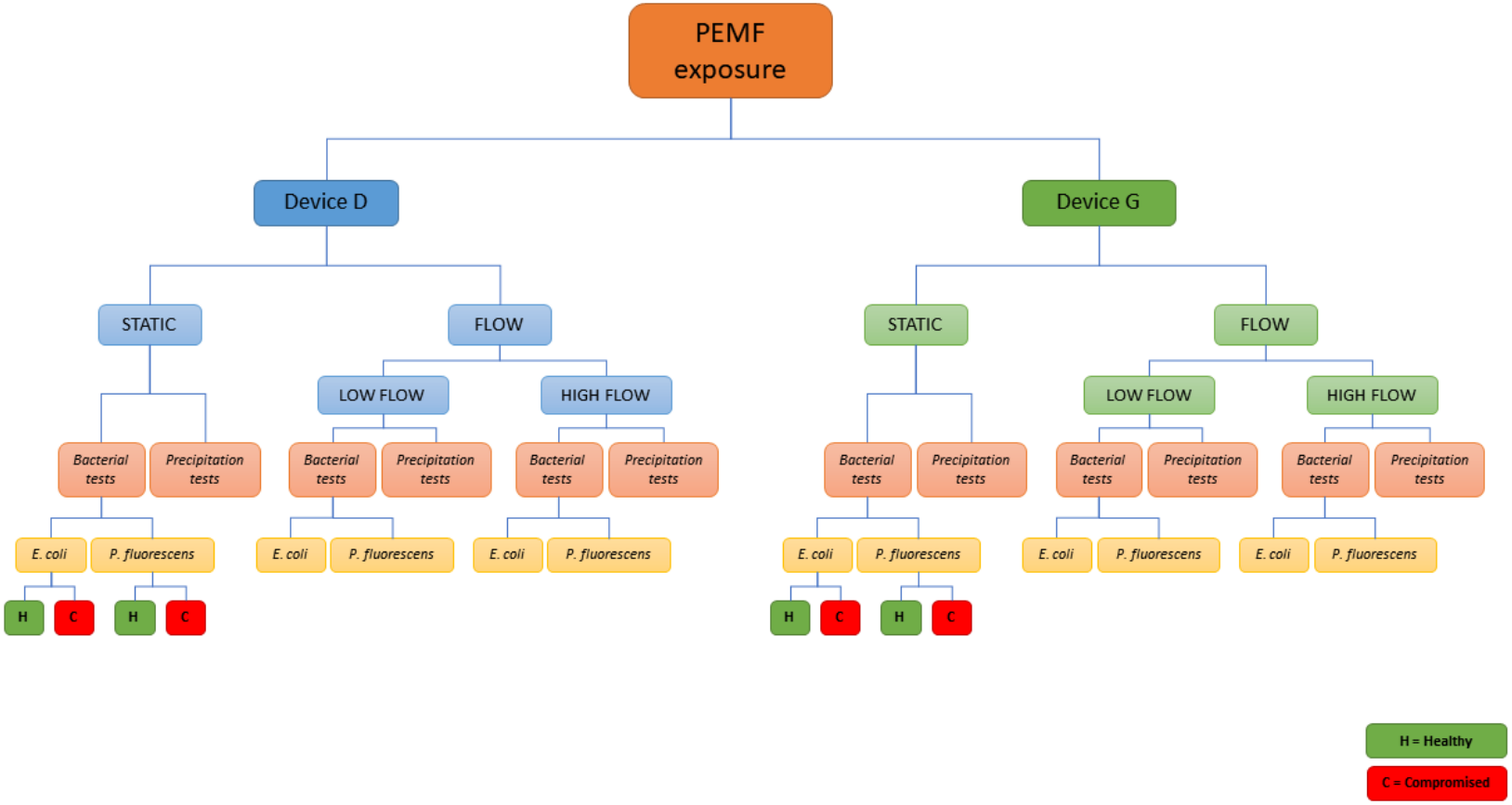
validation – and this is currently lacking, we have initiated proof-of-principle research in an effort to investigate whether such commercially available PEMF devices can influence the viability (culturability) of planktonic bacteria in a pure aqueous environment. Thus, these two devices were first investigated 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. Notably, it has also been observed that under certain conditions bacterial growth is actually stimulated. These studies were then extended to flow conditions and to include another microorganism, *P. fluorescens*. Thus, the effect of the electromagnetic fields generated by the two commercial devices on the bacterial culturability of *E. coli* and *P. fluorescens* under flow conditions has been contrasted with previous static results. Specifically, for *P. fluorescens,* one of the two devices showed no significant inhibitory effect under static conditions but showed significant inhibition under several flow conditions (low and high) and for different exposure times. For the other device, static conditions are actually stimulatory to growth. However, under low flow conditions, the effect is inhibitory and, under high flow conditions, is either inhibitory or stimulatory depending on exposure time.

Also, the marketing and implementation of commercially available pulsed-electromagnetic field (PEMF) devices to, ostensibly, control scaling in processes such as reverse osmosis (RO) and cooling-tower installations, is based on the notion that such devices enhance the coagulation of inorganic particles such as calcium carbonate. In order to provide a scientific basis for these claims,

the precipitation characteristics of calcium carbonate under the influence of the PEMFs from the two devices has also been investigated under controlled conditions. Thus, the rate and profile of calcium carbonate precipitation in the presence and absence of PEMF exposure of parent calcium nitrate and sodium carbonate aqueous solutions were tracked, in parallel, by UV absorption at 350 nm and by turbidity measurements. The morphology of the corresponding crystalline precipitates was also assessed using SEM. From these studies, is apparent that exposure of the parent solutions to the PEMF from one of these devices, but not the other, can influence both the profile of calcium carbonate precipitation and the morphology of the resulting microcrystals, consistent with enhanced particle coagulation.

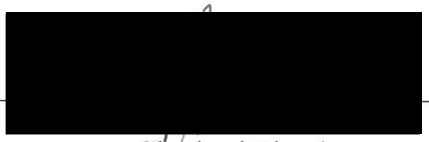
It is evident from these studies that PEMF induced anti-bacterial and anti-scaling effects depend on a wide range of variables such as waveform, extent of flow, type of bacteria and PEMF exposure duration. The effect of other parameters such as frequency, pH, temperature, other dissolved species etc. also need to be considered, but were not addressed in these studies. Our investigations suggest that the uncertainties, if not confusion, in this area are a result of the high level of complexity, due to the aforementioned wide range of possible variables. This can only be addressed by systematically conducting controlled experiments, along the lines of those reported here, in order to properly isolate the effects of all such variables. In particular, it is imperative to define the conditions under which such devices might be commercially viable.

Conceptual framework of the study



Declaration

''I, Chathuri Piyadasa, declare that the PhD thesis by Publication entitled **"Effects of commercially available pulsed electromagnetic field devices on bacterial viability and calcium carbonate precipitation"** is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".



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Date

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‘To my beloved parents, husband, son and to the memory of my late nanny’

Publications

Peer-reviewed journal articles

Chathuri Piyadasa, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart, Harry F. Ridgway, Con Pelekani and John D. Orbell. *The effect of electromagnetic fields, from two commercially available water-treatment devices, on bacterial culturability*. Water Science and Technology, (2016), **73(6)**, 1371-1376.

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Chathuri Piyadasa, Harry F. Ridgway, Thomas R. Yeager, Con Pelekani, Stephen R. Gray and John D. Orbell. *The application of electromagnetic fields to the control of the scaling and biofouling of reverse osmosis membranes - a review*. Desalination, (2017), **418**, 19-34.

Chathuri Piyadasa, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart, Harry F. Ridgway, Con Pelekani, and John D. Orbell. *Antimicrobial effects of pulsed electromagnetic fields from commercially available water treatment devices – controlled studies under static and flow conditions.* Journal of Chemical Technology and Biotechnology, (2017), DOI-10.1002/jctb.5442

Conference presentations

Chathuri Piyadasa, Harry F. Ridgway, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart and John D. Orbell. "*Effects of PEMF technology on bacterial viability & calcium carbonate precipitation"*. 17th IAHR International Conference on Cooling Tower and Heat Exchanger, Gold Coast, Queensland, Australia, 7-11 September 2015 (oral presentation).

Chathuri Piyadasa, Harry F. Ridgway, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart and John D. Orbell. "*Evaluation of pulsed electromagnetic field (PEMF) technology as an anti-biofouling pre-treatment for reverse osmosis membranes"*, 10th International Conference on Membranes and Membrane Processes (ICOM2014), Suzhou, China, 20-25 July, 2014, Paper OR-10-00959 (oral presentation).

Poster presentations

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Chathuri Piyadasa, Harry F. Ridgway, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart and John D. Orbell. "*Pulsed electromagnetic field technology as an anti-scaling pre-treatment for reverse osmosis membrane systems*". 4th Membrane Society Australasia Early Career Symposium, Institute for Frontier Materials, Deakin University, Victoria, (November 2014).

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Chathuri Piyadasa, Harry F. Ridgway, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart and John D. Orbell. "*Evaluation of Non-Chemical Pulsed Electromagnetic technology as an Antibiofouling Pre-Treatment for Reverse Osmosis Desalination Membranes"*. Victoria University Postgraduate Association, Research Poster Session (October 2013).

Note: All conference and poster presentation are provided in Appendices and accompanying CD.

Awards and achievements

Recipient of SECOMB Conference and Travel Fund Award, Victoria University, Melbourne, Australia (August 2015).

Recipient of 3 Year PhD scholarship Award, National Centre of Excellence in Desalination Australia (NCEDA) (2012- 2015).

Winner of best research poster presentation award, 4th Membrane Society Australasia Early Career Symposium, Institute for Frontier Materials, Deakin University, Victoria (November 2014).

Winner of Student Leadership Award, Victoria University, Melbourne, Australia (September 2014).

Finalist of 3 Minute Thesis competition, Victoria University, Melbourne, Australia (September 2013).

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PART A:

DETAILS OF INCLUDED PAPERS: THESIS BY PUBLICATION

Please list details of each Paper included in the thesis submission. Copies of published Papers and submitted and/or final draft Paper manuscripts should also be included in the thesis submission

Item/ Chapter No.	Paper Title	Publication Status (e.g. published, accepted for publication, to be revised and resubmitted, currently under review, unsubmitted but proposed to be submitted)	Publication Title and Details (e.g. date published, impact factor etc.)
Chapter 2	The application of electromagnetic fields to the control of the scaling and biofouling of reverse osmosis membranes - a review	Published	**Desalination, published 13/05/17, IF 5.527**
Chapter 4, part 1	The effect of electromagnetic fields , from two commercially available water-treatment devices, on bacterial culturability	Published	**Water Science and Technology, published 08/12/15, IF 1.197**
Chapter 4, part 2	Antimicrobial effects of pulsed electromagnetic fields from commercially available water treatment devices - controlled studies under static and flow conditions	Published	**Journal of Chemical Technology and Biotechnology. published 22/09/17, IF 3.135**
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Signature: [redacted]

Date: 09/01/18

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List of abbreviations

AC	Alternative Current
AgNP	Silver Nano Particles
ATP	Adenosine triphosphate
$CaCO_3$	Calcium Carbonate
CFU	Colony Forming Units
Device-D	Dolphin PEMF device (D PEMF)
Device-G	WAVE™ PEMF device (G PEMF)
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EMF	Electromagnetic Field
EMS	Electrospray Mass Spectrometry
HCl	Hydrochloric Acid
HVAC	Heating, ventilation and air conditioning
IP	Intellectual Property
LC-MS	Liquid Chromatography-Mass Spectrometry
LED	Light Emitting Diode
MFC	Microscopic flow cells
NCD	Non-Chemical Devices
P. fluorescens	*Pseudomonas fluorescens*
PEF	Pulsed Electric Fields
PEMF	Pulsed Electromagnetic Field
PVC	Polyvinyl Chloride
RO	Reverse Osmosis
RT	Room Temperature
SEM	Scanning Electron Microscopy
SOP	Standard Operating Procedure
UK	United Kingdom
UV	Ultraviolet

Chapter 1 – Introduction

This chapter presents an overview of fouling in RO membranes and cooling tower applications in combination with control measures. The overall aims for the study and the thesis outline are also presented in this chapter.

1.1 Overview

1.1.1 Fouling in reverse osmosis membranes

Membrane technology has played a crucial role in desalination since the mid 1970's (Tsai *et al.* 2011) and it is now widespread (Bellona *et al.* 2004; Melián-Martel *et al.* 2012; Nikkola *et al.* 2013; Vercellino *et al.* 2013) with RO now accounting for more than 60% of the world's desalination capacity (Prihasto *et al.* 2009). Fouling is an issue in the operation of RO systems (Picioreanu *et al.* 2009; Baek *et al.* 2011) and unmanaged fouling can result in dead areas in a membrane element (Dudley 1997). Membrane fouling can involve direct costs such as periodic cleaning, feed water pretreatment and increased energy demand, as well as indirect costs such as product loss due to down time and shortened membrane life (Bereschenko *et al.* 2007; Flemming 2011; Van Geluwe *et al.* 2011; Hashim 2013).

Figure 1.1 summarizes the different types of fouling that may occur on RO membranes. Notably, different kinds of fouling can occur simultaneously (Hydranautics 2011; Valavala *et al.* 2011) and organic fouling and biofouling contribute the most to RO membrane fouling (Armstrong *et al.* 2009b; Armstrong *et al.* 2009a).

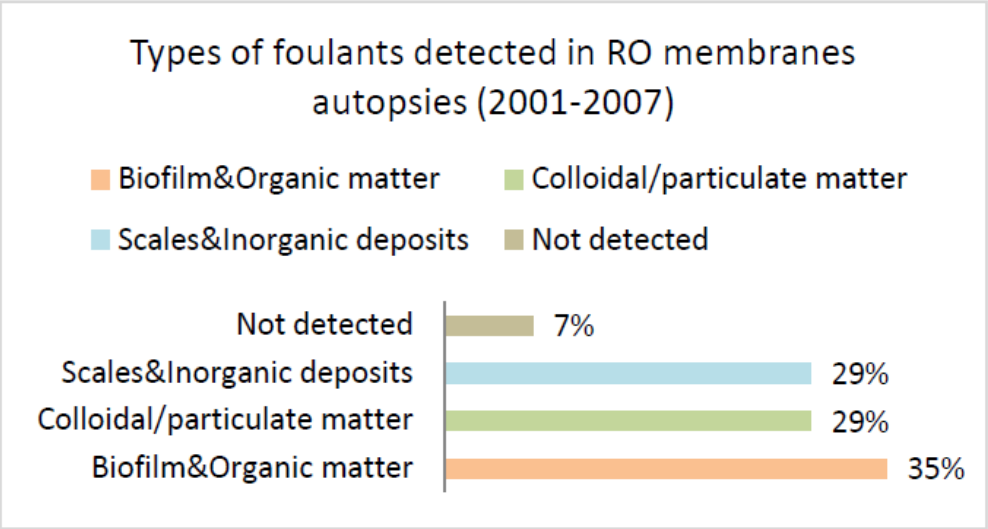

Figure 1.1: Types of foulants in RO membranes

(Armstrong *et al.* 2009b)

1.1.2 Mineral scaling and biofouling management

Scaling (or precipitation) occurs wherever the solubility of any sparingly soluble salt in the feed water is exceeded. The types of scale in RO membranes can be alkaline (e.g. calcium carbonate, $CaCO_3$), non-alkaline (e.g. calcium sulphate) and/or silica based. $CaCO_3$ is the most common scale-forming mineral, which originates in the form of calcium and bicarbonate ions in industrial water, seawater or groundwater sources (Piyadasa *et al.* 2017).

In addition to scaling, biofilm formation is a major concern for RO system performance (Greenlee *et al.* 2009; Nair and Kumar 2013). Biofilm is the general term for describing the adhesion and accumulation of bacteria and their associated exudates on a submerged solid surface or at any phase transition interface (Bates *et al.* 2008; Kim *et al.* 2009). The term 'biofouling' is typically reserved for those situations where a biofilm becomes problematic in

the context of one or more operational parameters, such as loss of flux or solute rejection (Amjad 1996).

Table 1.1 and 1.2 summarize the existing scale and biofouling control methods and their concerns. More details of each method can be found in our recently published review article (Piyadasa *et al.* 2017)

Table 1.1: Summary of existing scale control methods and concerns

Existing scale control methods	Concerns
Low system recovery System running under low recovery	Reduces the likelihood that the solubility of scale-forming salts will exceed the critical value at which precipitation begins - but may not be cost effective
Pretreatment with acid/anti-scalants	The precipitation of sulfates if sulfuric acid is used - otherwise HCl may be used.
Cleaning	The compact design of a spiral wound membrane element makes it very difficult to clean

(Hydranautics 2000; Williams 2003; Bereschenko *et al.* 2007; Hydranautics 2008; Cipollina *et al.* 2009; Valavala *et al.* 2011; Vercellino *et al.* 2013).

Table 1.2: Existing biofilm/biofouling control methods and concerns.

Existing biofilm/biofouling control methods	**Concerns**
Chemical or biological pretreatment of the RO feed water to reduce nutrient loading, inhibit primary bacterial adhesion, inactivate cells through the use of biocides, interfere with quorum signaling compounds that regulate biofilm gene expression.	Bacterial cells might continue to grow better after treatment with biocides showing a positive adaptive response responsive.
Modification of operational/engineering approaches.	Costly.
Membrane module/spacer modifications	Costly.
Physico-chemical cleaning of biofilms using disrupting/denaturing compounds that break down biofilm structure.	Fluid dynamics and compact design of spiral wound membrane elements make cleaning very difficult and it has been found that bacterial cells might in fact grow better after the cleaning process.

From: (Whittaker *et al.* 1984; Al-Juboori and Yusaf 2012).

1.2 PEF/EMF for scaling and biofouling control

PEF technology applied to water treatment has been described as being a pulsed, time-varying, induced electric field generated within a PVC pipe that is incorporated into a recirculating water system (ASHRAE 2013). In this configuration, wires are wrapped around or positioned near an existing PVC pipe through which the treated water flows. There are no electrodes in touch with the treated water and it is considered that, due to the alternating current, an electromagnetic field is induced - hence it is more accurately called PEMF, rather than a PEF method as described for the food systems. Figure 1.2 represents the emergence of PEMF for biofouling control on RO.

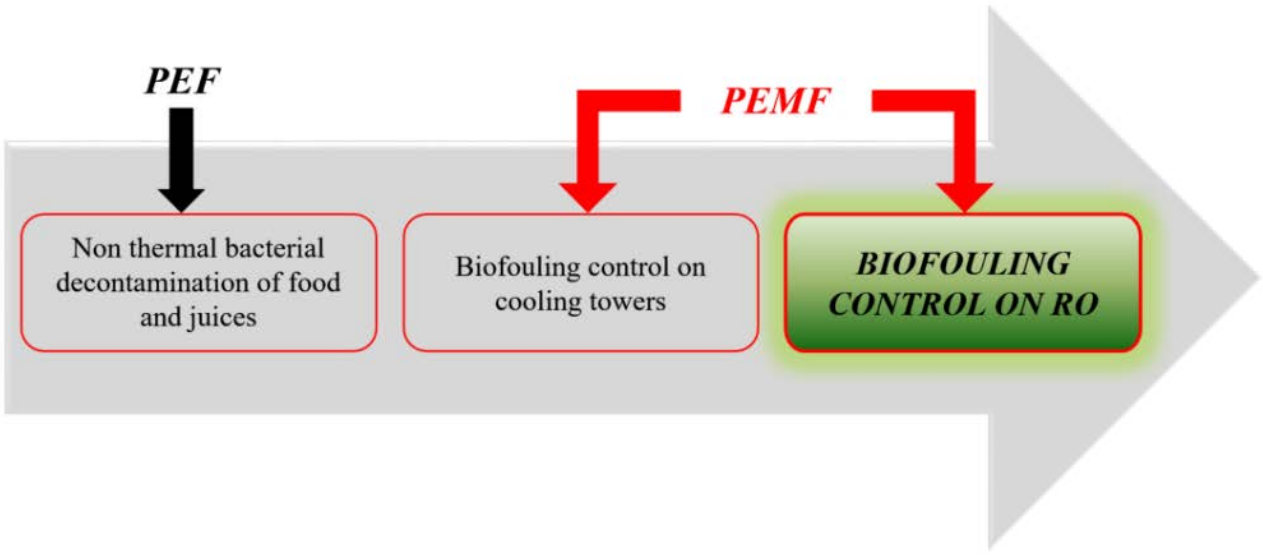

Figure 1.2: Emergence of PEMF use for biofouling control in RO systems.

1.3 Significance of this study

Based on available literature there is a great deal of interest in the use of PEMF as a potential pre-treatment strategy for water treatment. In this regard, the use of commercial PEMF devices appear to be more common for scaling control in the cooling tower industry than for fouling control in RO membrane systems. However, efficacy is still a controversial question in the water treatment industry and manufacturers continue to market PEMF units despite the lack of peer-reviewed laboratory data, proof-of-principle studies or documented field studies in order to demonstrate that the electromagnetic fields generated by such units are actually effective in producing significant antimicrobial and/or anti-scaling effects. In addition, standardized operating procedures, such as the operating time needed for a particular scale reduction and/or reduced bacterial counts are often not reported. There is a lack of baseline data from controlled scientific experiments and it is not clear what the effects might be on the water chemistry itself or what conditions are required for optimal performance.

Generally, manufacturers make claims based on uncontrolled laboratory and field conditions. In most of the available documents the experimental designs are scientifically questionable with minimal emphasis, if any, on reproducibility (Opheim 2000).

Manufacturers of AC induction/EMF devices do not disclose all of the relevant technical information on such devices/systems due to intellectual property issues and this hinders a full characterization of the devices and their operation. For example, the number of coils and arrays around the pipe may vary and be design specific. There may be two or more coils wrapped around the pipe, wired either in series or in parallel and, when two or more coils are used, they may be wired or wound such that the fields generated at one coil are in opposition

with one or more of the remaining coils. Also, each coil may be divided into two or more parts and the parts of two or more coils may be arranged in various alternating arrangements (EVAPCO 2005) which can be unique to a particular device - that cannot be observed from outside due to thick and compact housing and/or sealing that would require an autopsy to be performed of the device. Electromagnetic field characteristics vary significantly between the commercial PEMF devices (Huchler 2002).

These and other considerations prompted us to make a thorough investigation on two commercially available PEMF devices. This study was designed to be proof-of-principle research ultimately aimed at understanding the underlying mechanisms involved in biofouling and scaling control by commercially available PEMF units, when such effects can be demonstrated and validated. Thus, our approach here is to initiate systematic laboratory scientific investigations, in replicate and with the highest levels of control.

1.4 General aims

1. To investigate whether PEMF exposure changes the culturability of bacteria and if so, in what ways?
2. To investigate whether PEMF exposure alters calcium carbonate precipitation behaviour *at all* under controlled conditions and if so how?

1.5 Thesis outline

This thesis contains six chapters as described below. For those chapters published in peer-reviewed journals, declarations of co-authorship and co-contribution for these papers are included at the start of the respective chapters.

Chapter 1: Introduction

This Chapter presents an introduction to the thesis, use of PEMF for controlling bacterial growth and precipitation as well as outlines the research questions and the thesis structure.

Chapter 2: Literature review

This Chapter presents an extensive literature review with detailed insight into mineral scaling and biofouling and their control strategies and the emergence of PEMF devices for scaling and biofouling control. Chapter 2 has been published in the journal *Desalination* as a review paper.

Chapter 3: PEMF exposure – system design

The System Design (experimental set-up) Chapter sets the stage for the two data collection Chapters 4 and 5 on the bacteria and precipitation studies, respectively. This Chapter details the experimental set-ups used to quantify the effects of the PEMFs produced by two commercial devices; namely the Dolphin (Device D) and the WAVE™ (Device G). Descriptions of the specific experiments and variations made to the basic set-up are included in the subsequent Chapters 4 and 5. Each of Chapters 4 and 5 is self-contained as published papers. As the System Design chapter is relevant to both Chapters 4 and 5 there is necessarily some overlap. However, different aspects were explored and different research questions were addressed in each of these Chapters.

Chapter 4: Effects of PEMF on bacterial culturability

This Chapter, that discusses the bacterial studies, has been published in the Journal of Water Science and submitted for publication to the Journal of Chemical Technology and Biotechnology. The former publication represents an investigation of 'healthy' and 'compromised' bacteria exposed to the two PEMF devices under static conditions where the

culturability of bacteria were assessed in replicate following standard protocols. In the latter paper, the experiments were extended to flow exposure conditions and an addition microorganism species.

Chapter 5: Effect of PEMF on $CaCO_3$ precipitation

This Chapter has been published in Environmental Science: Water Research and Technology. Here, calcium carbonate precipitation is shown to be significantly affected if the parent solutions are pre-exposed to PEMF from one device but not the other. The precipitation profiles and the morphology of the formed crystals are shown to be influenced by a certain kind of PEMF exposure.

Chapter 6: Conclusions and recommendations

This project serves to establish a forward path for research in this area. The findings from point to the possibility of using of PEMF for biofouling and scaling prevention in RO systems, based upon further systematic scientific studies. The design and construction of customized laboratory-based PEMF devices for future research is suggested.

Appendices contain the supplementary materials for each of the data chapters.

The writing and referencing style of the literature review and data chapters has followed the requirements of the journals to which they were submitted to and/or published in, however, the overall format, section headings, numbering and referencing have been amended to be consistent across the thesis. A combined reference list is presented at the end of the thesis.

Chapter 2 – Literature review

2.1 Overview

Chapter 2 presents a literature review in relation to the use of PEMF to control scaling and biofouling of RO membranes. The existing control and management strategies of scaling and biofouling and the emergence of PEMF methods as a non-chemical control method is discussed in detail.

The review article (Paper 1) entitled “The application of electromagnetic fields to the control of the scaling and biofouling of reverse osmosis membranes - a review, by Chathuri Piyadasa, Harry F. Ridgway, Thomas R. Yeager, Matthew B. Stewart, Con Pelekani, Stephen R. Gray and John D. Orbell has been published in the journal *Desalination*, (2017) **418**, 19-34. The declaration of co-authorship for this paper is as follows which is then followed by the paper itself.

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Engineering advance

The application of electromagnetic fields to the control of the scaling and biofouling of reverse osmosis membranes - A review

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ABSTRACT

Scaling and biofouling are two major problems in the operation of reverse osmosis (RO) membranes. A variety of control measures are employed in practice, including the use of pulsed electromagnetic fields (EMF), which can avoid the use of chemical anti-fouling agents (e.g. halogen-based biocides) that may be toxic to humans or the environment. This is a fairly recent and controversial technology and, from the available documentation and literature, it is clear that the scientific basis for its purported effectiveness is not yet firmly established, although some studies suggest that beneficial effects could be possible. In particular, the various conditions under which EMF technologies are likely to be effective for real world applications have not been scientifically established. This review collates the relevant literature on the problem of scaling and biofouling in RO membranes and heat exchangersystems (e.g. cooling towers), with a particular focus on the application of pulsed EMF technologies, including the broad documentation, relevant scientific studies, proposed mechanisms of action and further research directions. This review demonstrates that a lot more systematic scientific research is needed in order to validate the application and commercialization of EMF technologies as a pretreatment to control fouling in RO membrane systems.

1. Introduction

Desalination is a general term that refers to the removal of salts from saline or brackish water to produce fresh water [1–4]. Desalination may be achieved via thermal processes based on distillation [5,6], ion exchange methods [2] and membrane-based processes [7,8]. Membrane technology has played a crucial role since the mid 1970s [9] and it is now widespread [10–13], with RO now accounting for more than 60% of the world's desalination capacity [3]. Macedonio, et al.[14] and Amjad [15] summarize and compare thermal and membrane-based desalination technologies and state that RO tends to be favored over distillation - due to better system performance, user friendliness and economic feasibility [16,17].

RO technology, which produces water essentially free of pathogens and pollutants [18–23] is often categorized into brackish water reverse osmosis (BWRO) and sea water reverse osmosis (SWRO) [24–26]. BWRO membranes generally have higher product water flux with lower salt rejection, whereas SWRO membranes have higher salt rejection but need to be operated at higher pressures [1]. A typical membrane desalination process is composed of intake, pre-treatment, RO and post-treatment [26]. Spiral-wound RO modules are more commonly employed [27] than plate/frame and hollow-fiber modules due to the balance between ease of operation and better fouling control - and the spiral wound module has been standardized amongst many membrane companies ensuring competitive pricing [28,29]. In the spiral-wound module, the semipermeable membranes are separated by feed spacers and permeate spacers and are wound around a central porous tube in a spiral fashion. Due to the separation by the feed spacers, turbulence in the tangential cross-flow is enhanced and the product water which permeates through the membrane is collected into a central permeate tube [30]. In addition to desalination, RO membranes are also used in wastewater treatment applications [31–35].

Fouling is an issue in the operation of RO systems [18,36]. Expenditure associated with membrane fouling can include direct costs such as periodic cleaning, feed water pretreatment, and increased energy demand, as well as indirect costs such as product loss due to

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down time and shortened membrane life [37–40]. Berenchko and co-workers [39] have stated that the fluid dynamics of spiral wound membrane elements combined with their large exposed surface areas render them especially prone to microbial attachment followed by biofilm formation.

When product water continuously passes through the membrane, rejected dissolved solutes and suspended solids accumulate in a viscous sub-layer (i.e. boundary layer) near the membrane surface, a process referred to as concentration polarization (CP) [41]. Within this boundary layer, salts may exceed their solubility limits and precipitate from solution resulting in mineral scaling on the membrane surface. Suspended solids can undergo adsorption to the membrane surface [42], forming a gel-like fouling layer that can act as a secondary membrane [18,43–46]. The resulting fouling layer can limit membrane performance [47–50] by reducing permeate flux and increasing solute passage (via CP) into the permeate [43,51–53]. Goosen [54] includes scale formation, cake formation and biofilm formation as being external fouling or surface fouling because the various substances comprising the fouling layer do not penetrate into the membrane substructure. Internal fouling or fouling within the membrane material can result in a change in membrane structure due to physical compaction or chemical (solute) interactions, altering solute and solvent transport. RO membranes are considered to be non-porous since they appear as homogeneous polyamide (PA) networks even under high-resolution transmission electron microscopy. Thus, fouling is considered to be localized mainly at the membrane surface [1]. Many authors have discussed the fouling of RO membranes, including a critical review by Goosen et al. [54]. Table 1 summarizes the different types of fouling that may occur on RO membranes. Notably, different kinds of fouling can occur simultaneously [55–57].

Metal oxide and colloidal fouling tend to occur in lead elements, whereas mineral and polymerized silica scaling tend to be more common in the last stage [39]. Biological fouling can occur at any stage [57] and on all surfaces [44] in an RO facility [20]. Armstrong et al.[58] state that organic fouling and biofouling contribute the most to RO membrane fouling and further details of RO fouling can be found in Malaeb and Ayoub [59].

1.1. Scaling

Scaling or precipitation occurs wherever the solubility of any sparingly soluble salt in the feed water is exceeded [60,61]. Antony et al.[61] have published an excellent review on scale formation and its control in high pressure membrane water treatment systems. This review includes discussion on scale forming mechanisms, factors affecting scale formation and types of scale. These authors categorize the types of scale in RO membranes as being alkaline (e.g. calcium carbonate), non-alkaline (e.g. calcium sulfate) and/or silica based. Calcium carbonate, $CaCO_3$, is the most common scale-forming mineral, which originates in the form of calcium and bicarbonate ions in industrial water, seawater or groundwater sources. When the water temperature increases, the solubility of calcium carbonate decreases which results in precipitation onto heated surfaces [62]. However, heated surfaces are not required for calcium salts to form scale [63], but scale can occur whenever the solubility concentration is exceeded [64]. It should be noted that barium and strontium salts often co-precipitate with calcium carbonate [1]. Some workers [65–67] have reported that trace amounts of Zn can significantly inhibit the nucleation rate of $CaCO_3$ and promote the formation of aragonite.

When dissolved or suspended minerals precipitate they are attracted to the membrane surface due to their natural charges [68,69] and crystalize [70]. Once a nascent scaling layer develops, it can exacerbate CP near the membrane surface by reducing fluid convective forces proximal to the membrane surface [42]. Conway [71] and Antony et al. [61] have constructed flow diagrams that attempt to explain such scale formation. Mineral scaling results in permeate flux decline and crystals can damage the active membrane layer [57]. In addition, harsh chemical cleaning cycles can also damage the membrane and shorten its lifetime [71]. Fig. 1 summarizes the key steps in scale formation.

Calcium carbonates, iron and silica can be present naturally in water in dissolved form [62,72]. Calcium carbonate is usually the main precipitate in seawater RO [1,71] and crystallizes in three different crystal forms: calcite, aragonite and vaterite. Calcite usually gives rise to hard scale whereas aragonite and vaterite give rise to softer types of scale that are more easily removed [70]. Calcium sulfate scale is much harder than calcium carbonate, and calcium phosphate scale is common when treating wastewaters. Metal oxides and hydroxides can occur due to oxidation of soluble metal ions or aluminum-based coagulants [57]. Silica is a general term which refers to crystalline, amorphous, hydrated or hydroxylated forms of silica [72–74]. The highest silica levels are typically found in ground waters [74]. Super-saturation and polymerization of soluble silica can form a silica gel coating which is very difficult to remove. This is different from 'silica-based colloidal foulants', which may be associated with either metal hydroxides or organic matter [57]. Ca^{2+} and Mg^{2+} ions have a strong influence on the formation of filterable silicate and on the kinetics of formation of silicate species [73].

Table 1
A summary of the different types of foulants in RO membranes.

Fouling category	Symptoms	Representative references
Mineral scaling (Inorganic fouling or scaling)	Flux decline Damage to membrane Loss of solute rejection	[3,33]
	Increase of salt passage into the permeate	[42,58]
Particulate fouling or colloidal fouling	Flux reduction	[3,33]
Organic fouling	Increase or decrease of salt passage	[42]
Biological fouling or biofouling	Increases the resistance to water permeation through the membrane	[3]
	Increased pressure differentials Flux reduction	[2]
	Damage to the membrane	[33]

1.2. Biofilms and biofouling

In addition to scaling, biofilm formation is a major concern for RO system performance [75–77]. Biofilm is the general term for accumulation of bacteria on a surface [4,44,72], while 'biofouling' is when a biofilm becomes problematic in the context of an operational definition [56]. A biofilm is a structured community [47,77,78] containing multiple layers of living, inactive, and dead bacteria along with their associated extra-cellular polymeric substances (EPS). EPS is important for the development and maintenance of biofilm structure [79] and accounts for roughly 50–90% of the total organic carbon of biofilms [79]. It is composed primarily of polysaccharides and proteins and is often accompanied by nucleic acids, lipids or humic substances [80]. The quantity and composition of a biofilm may change according to the environment [81]. It tends to be a slimy material [82] that may or may not uniformly cover the membrane surface [83]. A biofilm stability study by Mayer et al.[79] showed that electrostatic forces, hydrogen bonds and interactions such as van der Waals forces were possible molecular interactions responsible for the gel structure in a biofilm. Biofilm can also trap other deposits [20,84] resulting in a diffusion-transport barrier that limits the penetration of antimicrobial agents into the deeper layers [83]. This makes the biofilm essentially irreversible under a variety of environmental conditions [81] such as low flow [47]. Biofilm population and dynamics can also be affected by permeate flux

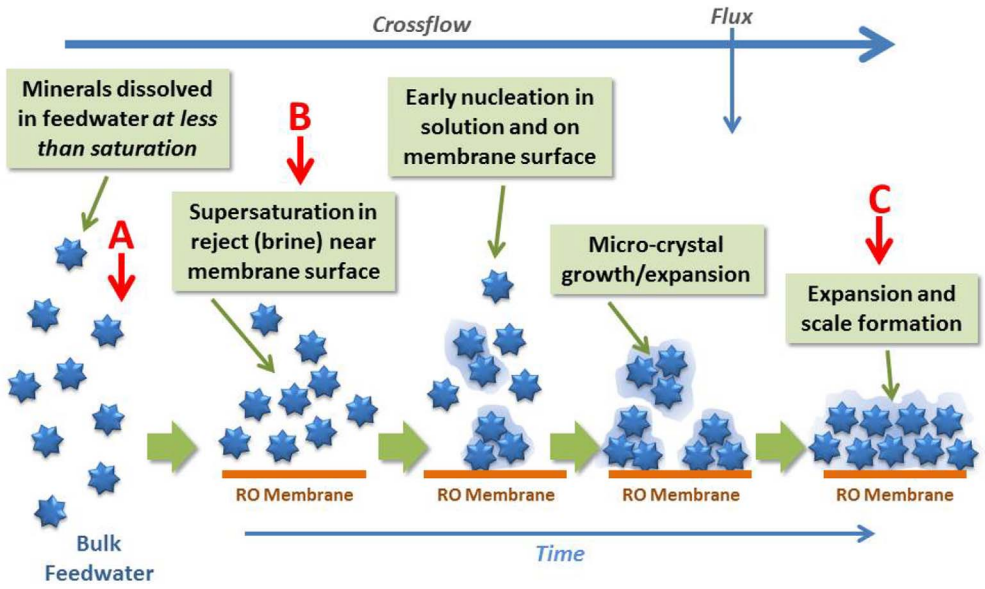

Fig. 1. Schematic illustration of the principle stages of mineral scale formation in RO membrane systems. Blue stars represent sparingly soluble salts or ions. Salts generally remain in a dissolved form in the bulk feed water (far left), but as solutes are rejected by the membrane, they accumulate in the viscous sub layer near the membrane surface where they may exceed their solubility limits (left to right). Nucleation and micro-crystal formation takes place both in solution (primarily in the viscous sub layer) as well as on the membrane surface. Micro-crystals can grow and expand over time; eventually resulting in confluent mineral scaling that can impede water transport and damage the active semipermeable layer. Red letters and arrows indicate potential intervention points where scaling can be retarded or reversed by various methods including: (A) Introduction of anti-scalants and chelating agents to maintain solubility; (B) use of chemical dispersants that obstruct micro-crystal and/or floc aggregation in suspension, or that interfere with association of micro-flocs with the membrane surface, and (C) cleaning (i.e. reversal) of established mineral scale by treatment with acids, surfactants, and/or proprietary commercial products. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[85]. Biofouling is more serious in warm climates as this is more favorable for microorganism growth [86] and the membrane surface changes rapidly once the biofilm layer starts to develop [86].

Various types of microorganisms may be involved in development of membrane biofilm, including bacteria, fungi, protozoa and micro-algae [59]. However, there is no typical biofouling organism [56] since they are opportunistic [20] and tend to adhere to membrane surfaces [86] depending on a variety of environmental factors [75] such as feed water composition, membrane chemistry and microbial type. Herzberg and Elimelech [75], Nguyen et al.[87] and Redondo [88], summarize the various factors involved in biofilm formation and the main steps associated in this process are given in Fig. 2. Matin et al. [46] identifies some of the major groups of bacteria that cause biofouling as *Pseudomonas*, *Corynebacterium*, *Bacillus*, *Arthrobacter*, *Mycobacterium*, *Acinetobacter*, *Cytophaga*, *Flavobacterium*, *Moraxella*, *Micrococcus*, *Serratia*, and *Lactobacillus*. More recently Berenchko [19] reported that *Sphingomonas* species are often observed in wastewater and sea water RO membrane biofouling. A report on scaling, fouling and corrosion parameters [89] provides a well summarized table on the microorganisms present in process waters. The broad steps in biofilm formation are depicted in Fig. 2.

The first step in biofilm formation involves pre-conditioning of the membrane surface by the adsorption of organic macromolecules. A more complex multistep process of biofilm development has been described by many authors [19,44,82,90–97]. Some describe a pre-initial state involving the adsorption of macromolecules such as proteins, polysaccharides and nucleic acids, and smaller molecules such as humic acids, fatty acids and lipids – as well as pollutants such as polyaromatic hydrocarbons and polychlorinated biphenyls, resulting in a 'conditioned', nutrient-enriched surface that may promote primary bacterial adhesion [82]. Generally, points of low water velocity are targeted [82] by the biofilm forming bacteria for their initial attachment, and film formation begins mainly in places behind the spacer filament crossings as they provide a shielded environment [97]. This has been demonstrated by the simulation study by Radu [52]. For interaction between the cell and the target surface, and for cell adhesion to occur, bacteria sometimes produce proteinaceous cell appendages such as pilli and nanofibers [98].

2. Current approaches to the control of scaling and biofouling

2.1. Scale control

Existing scale control methods can be broadly categorized under, (i) low system recovery, (ii) pretreatment with acid/antiscalants, and (iii) cleaning. Operation at low system recovery enables the solubility of scale forming solutes to not exceed the critical value at which precipitation begins [16,99]. However, running at a low recovery rate decreases the efficiency of the system, can waste water resources and may not be cost effective. If the system recovery is required to be higher, as is often the case, then scaling may be controlled by injecting acid, e.g. sulfuric acid [12], into the feed, or by adding antiscalants/dispersants to the feed water [57,100,101]. Alternatively, hydrochloric acid (HCl) may be used if sulfuric acid leads to precipitation of sulfates [57,101]. Acid solutions can also be used to re-solubilize residual accumulations of calcium carbonate scale [101]. Greenlee [1] states that anti-scalants prevent precipitation by disrupting one or more stages of the crystallization process mentioned previously. MacAdam [102] provides a table which summarizes chemical and non-chemical treatment options available for scale control.

Anti-scalants are chemicals such as organophosphonate, polyphosphate- or acidic polymers such as polyacrylic acid [1,99]. However, the use of anti-scalants is limited to low concentrations (< 10 mg/L) since some can serve as a source of carbon and trace elements, such as inorganic phosphate, that can promote biofilm growth [72]. Also, anti-scalants do not completely control scaling if the ion concentrations are high and, with increasing salt concentrations, precipitation will eventually occur [1]. Lin et al. [103] provide a well summarized and thorough paper on various aspects of membrane cleaning.

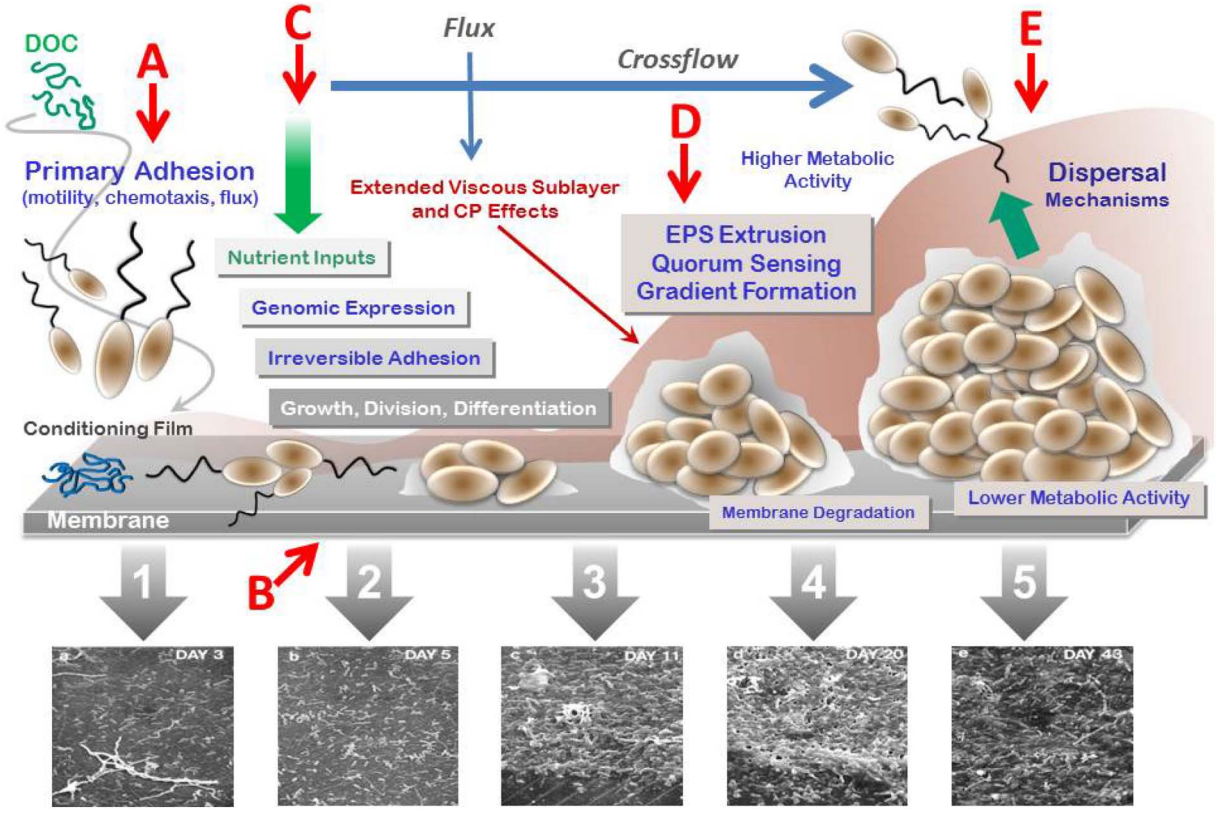

Fig. 2. Schematic illustration of the main stages of biofilm formation and biofouling in RO membrane systems. Primary bacterial attachment is preceded by rapid adsorption of NOM and other biogenic compounds on the membrane surface, a process referred to as 'conditioning film' formation. The conditioning film may or may not facilitate primary bacterial adhesion. Primary adhesion from the planktonic phase triggers the expression of many genes that mediate biosynthesis of EPS (and sometimes pili and other extracellular appendages) that mediate and strengthen adhesion to the membrane surface, which results in 'irreversible adhesion'. Biofilm genome expression is regulated in part through quorum signaling mechanisms that are stimulated by enhanced cell density at the membrane surface. Over time, the adherent bacteria grow into micro-colonies and a confluent biofilm using dissolved feed-water nutrients that become concentrated in the polarization layer. Growth of the biofilm, which possesses hydrogel-like properties, retards convective mixing near the membrane surface and effectively extends the viscous sub layer, which in turn further amplifies CP effects causing declines in water flux and solute rejection. The red arrows and letters indicate potential points where biofouling can be controlled by various means, including: (A) Introduction of biocides or other chemical agents that inactivate cells or that interfere with primary cell adhesion; (B) chemical modification of the membrane surface to discourage primary cellular adhesion or to metabolically inactivate bacteria as they approach the membrane; (C) removal of nutrient sources from the feed water resulting in starvation and cell death; (D) chemical and/or hydrodynamic cleaning of the biofouled membrane surface; and (E) interference with or destruction of quorum signaling compounds (e.g. acylated homoserine lactones) involved in biofilm growth and maintenance. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. Biofilm control

Existing biofilm control measures include: (i) physical cleaning of biofilms, (ii) pretreatment of feed water, e.g. nutrient reduction, bacterial adhesion control methods, use of biofilm gel disrupting/denaturing compounds and addition of compounds that eradicate/control living planktonic bacteria, (iii) modification of operational/engineering approaches and (iv) membrane module/spacer modifications [104,105].

Membrane cleaning disrupts and removes the biofilm layer [59]. Although membrane cleaning helps to restore permeate flux and decrease salt passage, the design configuration of spiral-wound membranes makes physical methods of cleaning of fouling layers either ineffectual or impossible [106]. The efficacy of chemical cleaning depends on several factors, such as membrane fouling type, the choice of cleaning agents, the duration of cleaning and procedural cleaning conditions (e.g. temperature, pH, duration, mechanical shear) [107]. Unit shut down and replacement is usually necessary which is both labor intensive and costly [108]. A typical chemical cleaning process involves a low pH cleaning (to remove foulants such as mineral scale), followed by a high pH cleaning (to remove organic material) [102]. Chelating acids and other sequestering agents create a low pH shock and react with the inorganics of the biofilm. Caustic chemicals enhance the solubility of bio-organic molecules [109]. Sometimes this procedure can be carried out with or without detergents or chelating agents (e.g. EDTA) added to aid remove biological material [102]. EDTA complexes cations such as Ca^{2+} and Mg^{2+} within biofilms [110]. Loosened films are then sloughed off by the bulk water. Following cleaning, biofouling layers may actually grow faster due to some interesting factors explained in Bereschenko [19]. Bereschenko [19] further states that the regrown biofilms could be more complex in terms of structure and composition compared to the communities forms on the fresh RO membrane surfaces. Physical cleaning techniques as described in Nguyen et al.[88] include hydraulic cleaning, pneumatic cleaning, and the use of ultrasound, electrical fields and self-collapsing air bubbles. It has been shown that cleaning at an early stage of biofouling could be more efficient in removing biomass than cleaning performed at a later stage [111].

Yang et al.[84] suggest that scaling is always affected by biofouling as microbial secretions might enhance inorganic ion retention on the membrane [112], resulting in enhanced concentration polarization [54]. Similarly, Yongping and Qiang [113] reported that precipitated salts can contribute more than microorganisms to initial biofilm buildup. Thompson et al. [69] indicated that mineral salt crystallization was induced in the presence of a pre-existing biofilm. These authors also noted that surface scale coverage and crystal density increased, and the rate of individual mineral crystal growth can be significantly higher in regions with higher biofilm density. These arguments are in agreement with Herzberg and Elimelech [112] who identified that the biofouling layer hinders back diffusion of accumulated solutes into

the bulk flow, leading to elevated osmotic pressure near the membrane surface - a phenomenon referred to as biofilm enhanced osmotic pressure [108].

Effective feed water pretreatment is often considered as the most important factor for successful long-term operation of an RO membrane [114]. Pretreatment for RO biofouling includes ultrafiltration (UF) [114–118] to remove colloidal material and particles which can form a filter cake on the membrane surface and lead to cake enhanced osmotic pressure [56], and amendment of the feed-water chemistry with coagulants, dispersants and anti-scalants, for the removal of organic compounds to reduce subsequent organic fouling. Coagulation and activated carbon are considered conventional pretreatment, whereas microfiltration (MF), UF and NF are considered advanced pre-treatments for RO [119]. Acid addition, disinfection, media filtration and cartridge filtration are also considered as conventional pretreatments [1]. Electrokinetic methods [88] are fouling control techniques that use an electric field [120]. Al-Juboori et al. [121] have tested the potency of thermo-sonication (using a commercial ultrasonic horn device at a 60 kHz frequency) as a pre-treatment technique to deactivate microorganisms to reducing biofouling in batch RO. The efficiency of this technique was influenced by the type of the medium in which the microorganisms were suspended. When distilled water was used as the medium, the concentration of the remaining bacteria was significantly lower than when a broth medium was used under same treatment conditions.

Perhaps the earliest and still widely employed pretreatment strategy to eradicate and/or control planktonic bacteria in the feed water was dosing continuously with low concentrations of biocides/bactericides [122], or periodic high-dose applications to avoid microbial adaptation and resistance [89]. Chloramine and chlorine, with its removal via sodium meta bi-sulfite, before the RO membrane treatment, are the most widely used disinfectants. Non-oxidizing biocides (e.g. DBNPA) are a preferred option as they are considered safe for the polyamide membranes and very effective at low concentrations against aerobic bacteria, anaerobic bacteria, fungi and algae [123].

Bio-dispersants are non-biocidal surface-active agents, which are slug-dosed at high concentration, or continuously or periodically applied at lower dosages to break up the fouling layer rather than to kill microorganisms [99]. Synthetic water-soluble polymers which act as surfactants are preferred over natural dispersants such as lignins and tannins as the latter might act as food for microorganisms [124]. Anionic surfactants, such as alkyl aryl sulfonates are the most widely used surfactants, due to their excellent detergent capacity, their availability and low-cost, and their formulation potential. Examples of non-ionic surfactants are *n*-octyl glucoside, Triton-X100 and related polyethylene oxides [124]. Quaternary ammonium compounds (QAC) represent the cationic surfactant group [124], but these should be used with extreme caution since they can bind irreversibly to RO membranes causing permanent loss of flux.

In spite of the widespread use of chemical pretreatments, their effectiveness against sessile and planktonic cells is questionable [79], as resistant strains can develop [125] and eventually microbial growth occurs [126] that can alter membrane chemistry [127]. In addition, appropriate design criteria are needed in order to minimize unwanted effects such as residual chlorine [128] and improper selection of a cleaning chemical, or an improper sequence of chemical introduction that can make the fouling problem worse [102]. Therefore, for disinfection of feed water, some authors recommend processes such as fluorescent light photocatalytic disinfection over UV and chlorine [129]. With respect to UV, the radiation has to penetrate through water and will be less effective if the suspended solids content is high [130]. Broekman et al. [131] have shown that a combination of shear, microbubbles, and high frequency/low power ultrasound could control bacteria and algae in industrial water systems. Kim [26] provides a comparison chart for physical disinfectants (UV, membrane, sand filtration) used for bio-fouling control of SWRO membranes. Yu and co-workers [132] have suggested the use of dichloroiso-cyanurate (DCC) as a potential disinfectant.

Cell-to-cell signaling between biofilm microorganisms by quorum-sensing (QS) compounds has been shown to be related to biofilm development, structure, and detachment [133–135]. The importance of pretreatment with respect to biofouling control has been highlighted above [18,19,51,105,136,137] and one possible strategy that has been investigated is to disrupt biofilm QS processes as a means to discourage primary bacterial adhesion and subsequent biofilm growth. For example, Dobretsov et al.[138] have demonstrated that it is possible to control bacterial density and community structure of biofilms using QS blockers. Kalia [139] explains the mechanisms.

Redesigning the RO membrane and/or module has also been proposed as a technique to reduce biofouling effects [20]. Rougher membrane surfaces result in higher rates of microbial adhesion [105]. Membrane/spacer modifications include a change of chemistry and coatings - and have been tested and used for biofouling management [14,44,56,140–144]. Flemming [37] has tabulated possible approaches to minimize primary biofilm formation by surface modifications. Belfer et al.[86] state that the possibility of membrane modification by sequential grafting of two oppositely charged monomers in nanofiltration membranes may be of some advantage in reducing biofouling [87]. FilmTec has designed biofouling resistant elements for potable and non-potable water productions [89]. A study by Sagle [143] focuses on the use of polyethylene glycol (PEG) based surface-coating materials for RO membranes to reduce membrane fouling. They also summarize previous attempts of other scientists to modify membranes (e.g. smooth coating of a hydrophilic, neutrally charged material, use of polyether–polyamide commercial block copolymers to coat UF and RO membranes). Gorey et al.[145] have developed microbial 'sensing membranes' where fouling-resistance is achieved by attaching a stimuli-responsive polymer film onto the surface. A decrease in temperature causes the film to expand into a hydrophilic state and vice versa.

From the above discussion, it is clear that membrane biofouling management is mainly achieved by balancing pre-treatment and membrane cleaning practices [107] and a given method/s should be selected according to the stage of biofilm development [146]. Ridgway [146] has shown schematically the control/management methods associated with each stage of biofilm development [147]. Cleaning remains the most widely used control method, even though chemical treatments can change water composition and produce toxic by-products [51,148] - and adjusting the characteristics of the RO membrane does not always control microbial adhesion, due to the complexity of the microbial surface structure and the microorganisms' adaptability for different environmental conditions [105]. Therefore, non-chemical methods and devices may offer advantages for the prevention of scaling and biofouling, with prevention being promoted over cleaning [148]. This review will focus on the application to water treatment of non-chemical Pulsed Electric Field (PEF) technology and Electromagnetic fields (EMF).

3. Pulsed electric field (PEF) and magnetic/electromagnetic field (EMF) technologies for water treatment

The terms "non-chemical water treatment systems" or Non-Chemical Devices (NCDs) cover a wide range of technologies. Duda [149] states that physical water treatment systems include magnetic, pulsed power, electrostatic, ultrasonic, and hydrodynamic cavitation processes. Huchler [150] focuses on three specific categories of devices: magnetic (permanent/electro-magnetic), electrostatic and alternating current (AC) induction. Pandey et al. [32] provide an excellent review that describes the fouling of RO membranes and evaluates pretreatment options available for each type of fouling. However, there is no mention of non-chemical treatment methods available for feed water treatment.

Anti-scale magnetic treatment of hard water has been employed for more than half a century [151] or over 100 years according to some

authors [152,153]. Magnetic water treatment consists of passing water through a magnetic field (MF) of certain characteristics [154]. Many studies have been performed on various aspects of magnetic treatment for scaling control, including those of Tai et al.[155,272] who also summarized successful and unsuccessful studies of anti-scaling effects of magnetic fields. A number of studies suggest that, when magnetically treating industrial hard water, colloidal silica present in the water can be activated leading to adsorption of calcium, magnesium or other metal ions that then precipitate from the solution as a coagulated agglomerate that interferes with the crystallization of calcite [67,144–147]. A study by Coey and Cass [156] concluded that magnetic treatment of $CaCO_3$ produces aragonite rather than calcite and that the treatment effect persisted for more than 200 h after the magnetic field was terminated. However, magnetic treatment has been sometimes proven to be ineffective for retarding scale formation [157]. Fathi et al. [158] have demonstrated that a magnetic field can influence the precipitation of calcium carbonate via effects on the associations of ionic species that are involved in nucleation. However, magnetic water treatment studies can be inconsistent, possibly due to the use of non-standardized methods, variations in water composition or differences in the course of the treatment [159]; and the efficiency of magnetic water treatment could also depend on the nature of the pipe materials [160]. Salman et al. [277] have recently reviewed the effectiveness of magnetic treatments for controlling scale deposition, as well as conducting their own controlled experiments. From their review and experiments, these researchers conclude that magnetic treatment could have a positive influence on scale control, albeit more by reduction rather than by prevention. Furthermore, their experiments confirmed that the effectiveness of treatment was dependent on a wide range of parameters including the nature of the magnetic field as well as scale type, water condition and whether conditions were static or flowing.

In terms of the effect of magnetism on microorganisms, Kohno et al. [161] have determined the effects of static magnetic fields (using ferrite magnets) on the growth rates of three species of bacteria, including *Streptococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli*. They found that when *S. mutans* and *S. aureus* cells were cultured under anaerobic conditions in the presence of a static magnetic field, the growth rate and maximum cell count were inhibited compared to the untreated controls, according to the strength of the magnetic field. It was suggested that the effect was growth inhibition rather than bactericidal. However, growth was not inhibited under aerobic conditions, suggesting a role for oxygen and it was suggested that magnetic fields may be involved in promoting reactive oxygen species, such as the hydroxyl radical. No effects were detected for *E. coli* cultures. These authors also demonstrated that the magnetic field had no effect on DNA synthesis. Interestingly, Stansell et al. [162] found that exposure of *Escherichia coli* to static magnetic fields significantly increased antibiotic resistance.

The application of electric fields in water and organic liquids has been studied for many years because of its importance in electrical transmission processes and its practical applications in biology, chemistry, and electrochemistry [163]. Bacterial decontamination using pulsed electric fields (PEF) was reported first by Sale and Hamilton in the late 1960s [164,165] in relation to the bacterial decontamination of food [166].

In a typical PEF process, millisecond-duration electrical pulses (20–80 kV/cm) are applied across a containment zone comprised of metal electrodes that are in direct contact with the liquid to be treated or semi-solid foods that are placed between two electrodes [161,166,167]. Some authors have specified a slightly different range i.e. (15–50 kV/cm) [168]. The PEF system used in food systems is generally composed of a pulse generator, a treatment chamber(s), a cooling system and monitoring devices. In such systems, the containment zone (treatment chamber) is designed in such a way as to provide uniform exposure to foods with a minimum increase in temperature and minimization of electrolysis effects [169]. PEF disinfection has been considered a promising technology for non-thermal disinfection [170,171] and is referred to as cold pasteurization [165] - though some authors argue that a temperature rise occurs in response to the electric current flowing in the liquid food [168]. This process has been tested on juices [172,173], dairy fluids [167], wine [174], liquid whole eggs [175], municipal sludge [176,177], nuisance weeds [178] and microorganisms in packed, cooked chick meal [166]. PEF can also be effective for the inactivation of yeasts and molds [179], even though there is evidence to suggest that the yeast cell membrane is more stable than that of the bacterial cell [176]. Some authors have also studied the effect of electromagnetic fields on the denitrification activity of bacteria [180]. However, there is currently a concern with respect to PEF systems with regards to the potential leaking of electrode material into the liquid being treated [181,182].

In an attempt to control scaling, biological growth, and corrosion of industrial systems other than food, PEF methods have been used across a range of applications such as recirculating lines of commercial cooling towers, chillers, heat exchangers, boilers, evaporative condensers, fluid coolers, fountains [183] and residential hot water systems [150]. Such applications suggest that biofouling prevention using PEF is feasible [170,184]. PEF technology applied to water treatment has been described as being a pulsed, time-varying, induced electric field generated within a PVC pipe that is incorporated into a recirculating water system [183]. That is, wires are wrapped around or positioned near an existing PVC pipe through which the treated/treating water flows. There are no electrodes in touch with the treated water and it is considered that, due to the alternating current, an electromagnetic field is induced - hence it is called an electromagnetic field technology (EMF), rather than a PEF method as described for the food systems.

Systems lacking contact with the treated solution which are subject to a quick variation of coil voltage, in the hertz (Hz) to megahertz (MHz) frequency range, are defined as AC induction systems/methods [150]. Some authors have also used the term EMF in relation to food sterilization [185], but it is not clear whether the field is direct or induced. However, in commercial water treatment, the use of pulse-power, electronic water treatment and electromagnetic technologies are not clearly delineated, but both direct current and induced EMF are considered as pretreatments rather than techniques for cleaning of existing fouling. Some authors even suggest that devices that apply electric fields directly to water could be more effective since the

Table 2
Key differences between PEF and EMF processes.

PEF	EMF
• Application of short (microsecond to millisecond) pulses of a high-intensity electric field (15–50 kV/cm) to liquid or semi-solid foods placed between two electrodes. • The PEF system used in food systems is generally composed of a pulse generator, a treatment chamber(s), a cooling system and monitoring devices. • Metal electrodes that are in direct contact with the treated liquid. • There could be a potential leaking of electrode material into the liquid being treated.	• Wires are wrapped around or positioned near a PVC pipe through which the treated/treating water flows. • Water inside the PVC tube is subject to a quick variation of coil voltage in the hertz [Hz] to megahertz [MHz] frequency range. • The EMF system is composed of a signal generator and a treatment module/reaction chamber. • Due to the alternating current, an electromagnetic field is induced. • System electrodes have no direct contact with the treated solution.

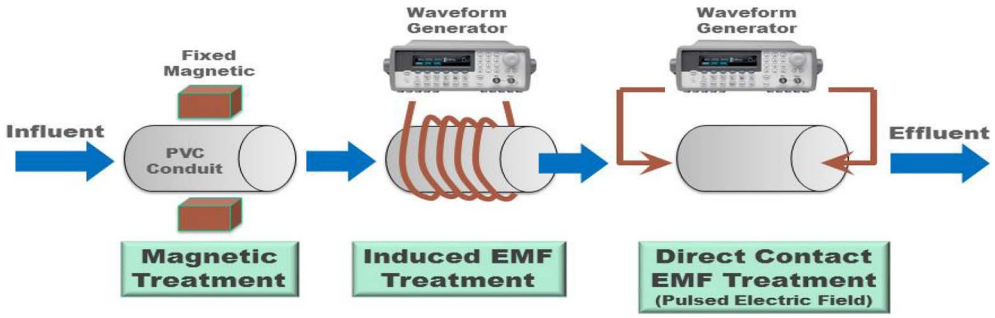

Fig. 3. Magnetic field (MF) and EMF treatment approaches fall into several general categories depending on how the EMF is delivered to the sample. These approaches include: (A) "Magnetic Field" (MF) approaches that employ fixed permanent magnetics or fixed electromagnets to induce a magnetic field in the sample. In MF methods that use electromagnets, the strength and quality of the induced magnetic field can be varied or pulsed; (B) "Induced EMF" approaches that generate an induced EMF (varying in frequency and/or amplitude) in an electrically-isolated sample, e.g., water passing through the interior of a non-conducting PVC pipe, by means of a waveform generator - there is no contact of electrodes with the sample being treated; (C) "Electrode-contact EMF" approaches in which discharge electrodes are in direct physical contact with the sample (e.g. water passing between two electrodes). As in the case of induced EMF methods, a waveform generator is used to deliver an electrical impulse of varying amplitude and/or frequency to the sample.

strength and frequency of the electric field in water could be substantially higher than indirect methods such as permanent magnetics, solenoid coils, and electrostatic devices [186]. Table 2 summarizes the key points that differentiate PEF and EMF processes and Fig. 3 is a schematic representation of these major methods.

Currently, there are a number of commercially available EMF units that are designed for flowing water systems. A typical EMF unit is composed of two main components; the signal generator [187] or driver enclosure [188], and the treatment module [187] or reaction chamber [188]. The common feature is the absence of any electrodes that are directly in contact with the water being treated, but there is a treatment tube associated with a compartment that produces electric pulses [266,270]. According to Pelekani et al.[189], EMF based technology was originally developed in South Africa for application to BWRO and this technology is now owned by GrahamTek (Singapore). Currently, most manufacturers of commercial EMF units are in North America, Canada, Mexico and the United Kingdom (UK) [150]. However, the exact statistics on this are unclear [190].

Huchler [150] has summarized non-chemical water treatment system suppliers, and states that only a few produce AC induction type treatment units and some commercial EMF units are categorized under the electrostatic section in this review. According to this author, a typical electrostatic water treatment system incorporates a cylindrical electrode (placed at the center of an externally grounded cylindrical metal housing) with an insulating coating on the outer surface [153]. The water to be treated flows in the annulus between the housing and the electrode. Whether the electric field is pulsed or not is an operational variable. Therefore, it is controversial why some EMF units have been still listed as electrostatic techniques. As an example, Cho et al.[62] explain that The Dolphin System™ of Clearwater Systems Corporation delivers a combination of high frequency electric pulses and a varying DC electric field to the water when it passes through a solenoid coil.

According to Vidic [191], the mechanism of action for electrostatic treatment systems are essentially identical to that of PEF treatment systems, the primary difference being that electrostatic systems apply a static electric field rather than pulses of energy. They further highlight the absence of published literature indicating that the application of weak static electric fields for a very short exposure time over a relatively large distance is capable of producing any antimicrobial effects.

Whereas Harfst [153] claims in a recent review that the scale prevention mechanisms of electrostatic devices differ from those of EMF devices, little or no scientific evidence was provided to support these assertions. Harfst suggests that in electrostatic devices, water molecules are presumed to be rearranged into an orderly array between the electrodes, thereby producing a "cloud of water molecules" surrounding scale-forming ions in solution which in turn discourages scale formation. He further states that bacteria also are controlled by disruption of the charged surfaces of the cell wall. Although such electrostatic treatment devices were actively marketed in the 1970s, most have since been discontinued.

Another electronic treatment technique has been mentioned by Romo and Pitts [107]. Here, a patented ceramic electrode has been used in order to achieve electrostatic dispersion of mineral and organic colloids to prevent scaling, biofouling, and corrosion in cooling and process water systems. This equipment has also been tested by the same workers on RO membranes and promising results have been reported [192]. Other authors have mentioned the use of an electric field as a way to reduce membrane fouling and as a method that favors separation processes [120]. They surmise that the applied electric field would potentially lift charged particles and release them into the bulk fluid, thus being more of a cleaning method (cleaning of already fouled membranes) rather than electric field pretreatment.

EMF units are reputed to be effective for dispersing colloidal particles [176], control of algal blooms, for scaling control in recirculating cooling water systems [193] and control of corrosion [183]. In most of these studies reduced bacterial counts in the system over an extended period of time is claimed, leading to inhibition of biofilm formation. Laboratory studies indicate that for effective microbial reductions, approximately 15 min of continuous treatment was required [191]. Indeed, biofouling of water-cooling and RO systems share similarities suggesting that similar control methods may be used [84,194,195]. Table 3 compares the control of scaling and biofouling between cooling towers and RO membranes.

In Australia, the SWRO desalination plant at Penneshaw, Kangaroo Island, had some short-term experience with EMF technology [189]. However, the trial EMF device failed due to technical problems and the performance of the device in relation to the prevention of inorganic scale formation could not be properly assessed. The science behind PEF/EMF water treatment is still unclear though it is a topic which has been alluded to by many authors [125,191,194,196,208–211]. Table 4 summarizes some studies that refer to PEF/EMF water treatment.

3.1. PEF/EMF possible modes of action

Colic and Morse [226,227] performed early experiments relating to the magnetic memory of water using RF EMF. They suggest that when water is treated with magnetic or electromagnetic fields, the water structure changes and the changes are retained for hours or days representing a kind of molecular memory. They also state that such modifications take place primarily at the gas/liquid interface (e.g., at the surfaces of gas bubbles in suspension) [228] and no effects are observed if the water is first degassed. Water surrounding nonpolar species is also a primary target of EMF effects [229]. Such perturbed gas/liquid interfaces can be expected to modify hydrogen bonding

Table 3
Fouling management in cooling towers and RO systems [4,71,84,121,130,151,195–214].

Cooling towers	RO membranes
Problems include: corrosion, scaling, organic fouling and biofouling. Scales include: Calcium carbonate deposits in pipes or heat-exchange surfaces, calcium phosphate, magnesium silicate, silicate deposits formed from silica in boiler systems or heated loops, silica. Foulants include: Dirt and silt, sand, corrosion products, natural organics, bacterial colonization on internal surfaces of piping, tube bundles, and cooling tower fills, aluminum phosphates, iron phosphate. Biofouling can be caused by algae. Factors influencing fouling: water characteristics, temperature, flow velocity, microbial growth, nutrients, atmospheric, corrosion, location (amount of light and moisture). Methods of fouling control (in addition to non-chemical control): Scale control • pH adjustment by acid addition Biofouling control 1. Preventing foulants from entering the system • Mechanical changes • Addition of chemicals 2. Remove or reduce the volume of foulants • Side stream filtering • Periodic tower basin cleaning 3. Regular action to minimize deposition Adding chemical dispersants Back flushing exchangers 4. Microbial control • Oxidizing biocides • Non-oxidizing biocides • Biodispersants	Problems include: scaling, organic fouling, particulate fouling and biofouling. Scales include: Calcium carbonate, calcium sulfate, silica, meta silicates, oxides/hydroxides of aluminum, magnesium, iron and manganese. Less common scales: calcium fluoride, barium sulfate, strontium sulfate and cupric sulfide. Foulants include: Fine debris, plankton, detritus, silt pigments, humic acids, microorganism deposits and secretions of bacteria (most common), protozoa, fungi, algae. Factors influencing fouling: microbial growth, membrane surface characteristics and feed water composition. Methods of fouling control (in addition to non-chemical control): Scale control • System recovery, pretreatment, acid feed, anti-scalants, cleaning Biofouling control 1. Pretreatment of feed water • Nutrient reduction • Bacterial adhesion control i. Adding enzymes ii. Adding anti-precipitants • Biofilm denaturing agents • Killing/control of planktonic bacteria 2. Physical cleaning of formed biofilm 3. Operational/engineering approaches 4. Membrane/spacer modifications

networks and could facilitate hydration of ions and interfaces [228]. In this regard, it has been shown that EMF causes an oscillation at the gas-liquid interphase which causes bubble collapse and light emission emanating from the collapsing bubble(s) – referred to as 'sonoluminescence' [228].

Pang et al.[230] described the influence of a magnetic field [232] on the properties of water itself using a range of spectroscopic methods and surface tension measurements. These investigations point to changes in the hydrogen bonding patterns that are associated with water cluster formation. Kim et al.[231] report that the effect of an electric field on feed water resulted in microbial inactivation and coagulation enhancement, although no molecular level explanations were suggested. In a series of carefully controlled experiments, Piyadasa et al. have recently investigated the influence of a PEMF from two commercially available water treatment devices on bacterial (*E. coli*) culturability [266] and calcium carbonate precipitation [270]. In the former case, exposure to a PEMF was shown to be both inhibitory and stimulatory depending upon the experimental conditions such as wave form and time of exposure. In the latter experiments, exposure to only one of these two devices was shown to accelerate the precipitation of $CaCO_3$ and to change the size and morphology of the microcrystals, consistent with enhanced particle coagulation as observed by other researchers [218,222]. Ahmed et al. [273] have recently demonstrated that the growth rate of *S. aureus* is inhibited by extremely low frequency (ELF) pulsed electromagnetic fields (2–500 Hz), to an extent that is frequency dependent.

Zhang et al. [268] examined the effect of an electromagnetic field on the critical flux of sediment formation on RO membranes and observed that applying an electromagnetic field increased the critical flux of sediment formation and decreased the fouling. Vedavyasan [269] investigated the combined effects of turbulence generating flow distributor and electromagnetic fields on biological fouling of RO membranes. According to these observations, the electromagnetic field decreased biological fouling on the membrane surface and decreased the pressure drop across the membrane. In a study where the effect of magnetic treatment on $CaCO_3$ deposition onto a membrane surface during cross flow nanofiltration (NF) was quantified in real-time using in-situ ultrasonic time-domain reflectometry (UTDR) [271], the electromagnetic treatment was reported to improve the membrane performance associated with a change in crystal morphology, facilitating a looser fouling layer. Zhao et al. [275] investigated the effects of a constant high voltage electrostatic field and a variable frequency PEMF on the formation of $CaCO_3$ scale. Their results demonstrate a significant anti-scaling effect dependent on favorable setting of parameters such as flow velocity and frequency. The beneficial effects are claimed to be related to the smaller particle size and looser morphology of the precipitate upon exposure. Miao et al. [276] also reported the exposure of flowing artificial hard water to EMFs of varying frequencies and were able to demonstrate that the extent of $CaCO_3$ precipitation was dependent on the frequency of the pulses, as was the size and morphology of the microcrystals.

3.2. PEF/EMF possible scaling prevention mechanisms

3.2.1. Activation of the suspended particles by charge removal

The modified/changed water discussed above is able to reduce scale deposition onto metallic surfaces [228]. For example, Clearwater Systems Corporation claims [187] that their device activates small-suspended particles in the water by removing their static electric charge. Activated particles then act as seeds for the co-precipitation of dissolved minerals, which subsequently tend to remain in solution rather than precipitate onto equipment surfaces. It is claimed that activated, mineral-coated particles are then removed by various physical means, such as filtration or centrifugal separation [187]. A similar explanation has been suggested by Griswold Water Systems [188]. Whereas some studies conclude that the Dolphin treatment has no observable effect on boiler scaling [209], other studies support this hypothesis [63]. However, it needs clear, acceptable, published scientific evidence as to how the device could remove the electric charge on the particles and lead to such activation. The role of water structure and

Table 4
Reports/reviews/studies referring to PEF/EMF/electronic physical water treatment (the use of PEF/EMF in food systems has been excluded). ***Studies published as journal articles; **studies published as conference proceedings; *studies found as technical/research/government reports; no asterisk – other published documentation.

Main focus and/or claims	Ref
A laboratory based study that shows a commercially available pulsed-power system, based on an induced electric field via Faraday's law could control fouling on heat transfer surfaces. The authors suggest that due to pulsed-power treatment, precipitation of calcium carbonate was altered from a surface nucleating scale to a non-adherent bulk solution powder.	[62]***
This industry report proposes underlying mechanisms of pulsed-power treatment for physical water treatment.	[63]
A simple language report on pulse-power water treatment systems including technology description (system components, installation, preventing scale, removal of existing scale, microbial control, corrosion protection).	[68]
An industry report about 'electronic' water treatment' that is used to treat scaling. States that electronic water treatment has evolved from magnetic water treatment and explains that electronic water treatment is possible by creating an oscillating field of energy with low frequency radio waves.	[71]
Conference paper discussing the use of commercially available pulsed power equipment (same device used in [62]) for condenser water treatment. Includes photos of equipment installation in the recirculating loop.	[125]**
Discusses implementation of pulsed power systems for *Legionella* control in cooling towers, as alternatives to chlorine.	[130]
Five NCDs (magnetic, pulsed electric field, electrostatic, ultrasonic and hydrodynamic cavitation) have been scientifically evaluated for efficacy in reducing planktonic and sessile microbial populations in modeled cooling water systems. The pulsed electric field device was the same device used by [62,125] above. None of the NCDs demonstrated significant biological control under tested conditions.	[149]***
Literature review on non-chemical water treatment systems. This includes a survey of different categories of such systems, including manufacturer's details.	[150]**
Reviews a model device with solenoid coils inserted into a cylindrical kernel inside a pipe, with respect to magnetic flux density generated. Also includes schemas of magnetic, electromagnetic devices and simulations of magnetic flux distribution inside a pipe wall.	[151]***
Publication by American Society of Heating, Refrigerating, and Air-Conditioning Engineers. Use of pulse-powered physical water treatment to control scaling, biological growth and corrosion across a range of fields, pros and cons.	[183]*
Evaluates EMF for scale control in a brackish groundwater membrane desalination system in Australia. Authors suggest that, in the presence of EMF, the required dose of anti-scalants was low. Findings supported with Scanning Electron Microscopy images that show calcium carbonate accumulation on the membrane surface and feed spacer. The treatment system was adapted from GrahamTek Technology discussed elsewhere [210].	[189]*
Discusses biological control in cooling water systems using a few nonchemical treatment devices including pulsed power treatment. Investigators state that effective microbial control in cooling water systems may not be achieved using a non-chemical device as the sole method of water treatment. Employed a commercial water treatment pulsed power device used in [62,125,149].	[191]*
A pulsed power system and a hydrodynamic cavitation device have been evaluated against conventional chemical treatment for cooling tower operation facilities. Both of the NCDs have delivered better results and showed a clear cost savings advantage. Employed the commercial water treatment pulsed power device used in [62,125,149,190].	[193]**
A book chapter that discusses alternatives to chorine in cooling water systems. One paragraph describes electrical methods (electric fields with high-energy electric shocks or low-level currents have been shown to prevent fouling settlement) and another short paragraph on magnetic fields. Also states that magnetic systems are being sold and are, purportedly, able to control scaling in pipes and disturb Mollusca.	[198]**
A study which quantitatively compares the effectiveness of pulsed-power water treatment with traditional chlorine water treatment on the microbial content and formation of biofilm in cooling tower systems. Employed the commercial water treatment pulsed power device used in [62,125,149,190,192]. Pulsed-power treatment has apparently generated an antimicrobial effect.	[207]**
Case history report of the use of a nonchemical pulsed power device on boilers and cooling towers. Employed a commercial water treatment pulsed power device used in [62,125,149,190,192,206]. However, in this study, the pulsed power device has no reported effect on scale control.	[208]
An paper that proposes fouling mechanisms and theories underlyimg physical water treatment. Includes several useful sketches, calculations and diagrams that explain mineral fouling in heat exchangers.	[209]***
A conference presentation on non-chemical water treatment for cooling towers which discusses principles of operation. Discusses commercial PEMF devices including pulsed power device used in [62,125,149,190,192,206,207] and others.	[210]**
A research report by GrahamTek Technologies, explaining electromagnetic field (EMF) effects and proposing possible underlying mechanisms for the effects of EMF on RO performance.	[211]*
A field study of non-chemical water conditioning in cooling tower water using commercially available EMF devices without using conditioning chemicals. Does not reveal the manufacturers of the EMF device employed.	[212]*
Categorizes NCD devices into magnetic, electromagnetic, electrostatic, catalytic and mechanical. The characteristics and market acceptance of each category are discussed based on several case history reports. Also states names of suppliers/manufactures of the commercial devices in each category. Includes the commercial PEMF device used in [62,125,149,190,192,206,207,209].	[213]**
Use of magnetic/electronic water treatment devices for treating well water hardness in New Hampshire. States that this method has low capital and operational cost and is non-chemical - but the effectiveness is disputed.	[215]*
Scale inhibition effects of alternating electromagnetic fields claims to be verified. Solubility of calcium carbonate shows an optimum increase when the frequency of the electromagnetic field is at 1 kHz.	[216]**
The effect of a modulated electromagnetic field (MEF) on fouling in a double pipe heat exchanger (DPHE) has been investigated. Authors suggest that an increase in the water velocity could decrease in the MEF efficiency.	[217]***
An investigation of the electromagnetic antifouling technology for scale prevention. Includes a schematic diagram of an electromagnetic antifouling unit and discusses possible mechanisms of scaling prevention.	[218]***
Research that proposes a new method of placing the solenoid coil in an electronic descaling (ED) apparatus. This method has demonstrated an enhanced descaling effect while effectively inhibiting the formation of scale at slow flow conditions.	[219]***
Discusses possible mechanisms of the electronic anti-fouling technology in relation to the scaling control in heat exchanger tubes. Includes a schematic diagram of controlled precipitation through electronic anti-fouling technology.	[220]***
A successful case study that used a laboratory constructed 'electronic unit' to treat scaling ed solution due to treatment.	[222]***
A field study using pulsed electric field devices for acceptable control of planktonic microbes in bulk water of cooling water systems.	[223]**
A field study of the control of scaling, total bacteria, and corrosion in a cooling tower environment using pulsed power water treatment. Describes the operating principle of a specified commercial electromagnetic anti-fouling technology for scale control. Includes a schematic diagram of the electromagnetic antifouling treatment set-up and installation of such device. The device is the commercial PEMF device used in [62,125,149,191,193,207,208,210,213].	[224]**
A variable frequency electromagnetic field has been successfully trialed in controlling deposits in geothermal water installations.	[225]***
A review of the magnetic amelioration of scale formation that discusses the controversy surrounding manufacturers' claims in the light of scientific research. Where positive results are evident, the various mechanisms that have been presented to account for the observed effects are described and discussed.	[244]***

clustering of water and hydrated ions, combined with hydration shell changes and influences on colloidal suspensions, is of relevance for both scaling studies [270] and biological effects [274].

Kim et al.[231] also studied the effects of electric field treatment for the reduction of microfiltration membrane fouling, microbial inactivation, and coagulation enhancement. However, they have reported reduction of active bacterial numbers, splitting of particles into smaller sizes and an increase of particle zeta potential with an increase in

electric field intensity. They believe that those observations indicate that electric fields could enhance particle coagulation processes, but any possible mechanisms remain unanswered. Knez and Pohar [233] is another detailed study of the influence of MF on polymorph composition of $CaCO_3$. They conclude that MF can have a significant influence on the morphology of the $CaCO_3$ crystals formed by leaving the zeta potential without any significant difference. On the other hand, in the study of Fathi et al. [158] $CaCO_3$ was precipitated while being passed through a stationary magnetic field which consists of a series of 5 pairs of permanent magnets where the field strength of about 0.16 T. They have noted that, in the absence of MF, the total amount of precipitated $CaCO_3$ was independent from the flow rate. Also, in the presence of the MF, the total amount of precipitate was significantly increased and there is an optimal treatment time that can results larger total amount of precipitated calcium carbonate. Fathi et al. [158] further suggest that nucleation induction time will be low in the presence of MF which then increases the nucleation rate.

3.2.2. Crystal collision frequency

Using scanning electron microscopy and dynamic light scattering technology Xing et al. [218] analyzed changes occurring in $CaCO_3$ scale, with and without EMF treatment. They reported that without EMF treatment, $CaCO_3$ scaling occurred as dense, sticky aragonite, which was difficult to remove. However, with EMF treatment, the $CaCO_3$ existed as clusters of small, loosely connected, hexagonal-shaped calcite, which was easy to remove. They also stated that EMF increased crystal collision frequency, which implies that the particle growth was supported mainly by an agglomeration mechanism rather than nucleation growth. This is in agreement with results reported by Xing et al. [222] in which EMF technology was found to precipitate crystals in solution as calcite.

Jianguo et al. [216] demonstrated that an alternating electromagnetic field can change the solubility of $CaCO_3$ when the electromagnetic frequency is at 1 kHz, resulting in scale inhibition by reducing the surface tension of the solution.

3.3. PEF/EMF possible mechanisms of biofilm and biofouling prevention

3.3.1. Electroporation

PEF and EMF units may work by a number of different modes of action. Most PEF anti-microbial effects appear to be related to changes in the outer and inner (i.e. cytoplasmic) membranes of bacteria [157]. Electroporation of cell membranes is one of the hypotheses [153] put forward by manufacturers to explain how these systems may prevent biofilm formation [171,191] and the same explanation is used in relation to the cold pasteurization of food [234]. With an applied electric field, a voltage difference across a microbial membrane is created [235] and, if the voltage exceeds a certain threshold (the voltage threshold for electroporation of the outer membrane is generally assumed to be about 1 V) [236], transmembrane pores can open [184,197] causing an ion imbalance and metabolic stress to the microbe. When the PEF is removed, microorganisms can repair the pores [165] but these may become irreparable at high field strengths, leading to irreversible cellular inactivation [178,184,237].

The electroporation effects depend on the field intensity, the pulse width and rate, the size of the bacteria [238], the species [176], treatment time and temperature, and the characteristics of the treatment substrate [179]. Any microorganism that survives PEF treatments may still be sub lethally injured and may, therefore, have the potential to re-grow under more favorable circumstances [239]. Short electric field pulses of about 25 kHz are known to cause electroporation [168] and/or influence the microorganism's genetic material, i.e. fragmentation or degradation of DNA with a field of 50 MHz [234] and/or lead to an ion imbalance [240]. On the other hand, some authors argue that ultra-short pulses with durations of ~50 ns, can cause cell death by damaging intracellular structures even without the rupturing of the cell membranes [236]. Racyte et al.[182] also summarize effects of such fields on microbial life. Torgomyan et al. [240] reported electromagnetic irradiation of one-hour duration with 51.8, 53, 70.6, and 73 GHz frequencies elicited effects on *E. coli* growth and cell morphology, and led to physicochemical changes in the liquid growth medium. They also reported that the electromagnetic irradiation treatment caused fundamental changes in cell membrane structure and function leading to the disruption of specific membrane-associated metabolic processes. Campli et al. [241] reported that exposure of *Helicobacter pylori* biofilms to extremely low-frequency electromagnetic fields (ELF-EMF) induced phenotypic changes on adhering bacteria and decreased cellular co-adhesion which destabilized the biofilms. The study of Cellini et al. [242] reveals that an exposure to extremely low EMF (50 Hz) for 20–120 min can cause significant change of *E. coli* morphotype suggesting a probable alteration during cell division process. Bacteria can express such morphotype alteration as response to stress which can also be linked with membrane damage [243].

3.3.2. Encapsulation

According to some EMF unit manufacturers, bacterial control is thought to be mediated by mineral encapsulation of single cells [187], although little scientific evidence is available to support such a mechanism. According to this hypothesis, the surface charge of particulate matter (including suspended microbes) is modified by the applied EMF causing the cells to act as the nucleating sites for mineral crystal growth [125]. Bacteria are engulfed and entrapped by these materials and this process is referred to as encapsulation [130]. This hypothesis and the electroporation hypothesis discussed above have been alluded to in the Envirometrics Staff Paper [130]. It is believed that due to such encapsulation, bacteria will be unable to reproduce and hence, population growth is repressed [187]. The precise mechanism of encapsulation and kinetics of the process have not been determined.

3.3.3. Free radicals

Electromagnetic fields are characterized by their frequency or wavelength, with the latter being inversely proportional to the frequency [244]. The electromagnetic spectrum can be divided into ionizing radiation, which has energy sufficient to break chemical bonds and form ions and non-ionizing radiation, which is too weak to break chemical bonds [244]. Baker and Judd [245] reviewed some literature from the 1970s on the effects of anti-scale magnetic treatment of water and emphasized that such treatment may result in multiple effects on water chemistry and water behavior. Some of these effects include changes in surface tension, electrical conductivity, gas (oxygen) solubility, formation of reactive oxygen species (e.g. hydrogen peroxide) and ability to support microbial growth. Similarly, Vallée et al. [246] also reported that the properties of water may be influenced by electric and magnetic fields, and it has been suggested that electromagnetic signals can produce reactive oxygen or hydrogen species, such as stabilized atomic hydrogen [221,228]. This is supported by the study of Koza et al. [247] and Lin et al. [248] who showed hydrogen evolution under the influence of a magnetic field.

Biological systems are especially vulnerable to reactive oxygen species (ROS) [249]. ROS includes a number of reactive molecules and free radicals derived from molecular oxygen [250] including superoxide, hydrogen peroxide, hydroxyl radicals, which can cause oxidative stress in cells. The biological targets for these highly reactive oxygen species are DNA, RNA, proteins and lipids [251]. In particular, the hydroxyl radical (·OH) has the potential to oxidize and disrupt the cell wall and membrane, diffuse into the cell where it may inactivate enzymes, damage intracellular components and interfere with protein synthesis and DNA structure [251,252,253]. It is also considered to have a sporicidal effect [125]. Oxidative stress can cause physiological changes in bacteria, which can result in alterations in phenotypes and metabolic inactivation [78]. Free radicals may also alter the natural

charge of EPS [91].

3.4. Other effects

PEF is reported as causing minimal temperature increase [168,241] and is generally considered to be a non-thermal treatment. However, some researchers maintain that the low amount of heat that is generated could have a synergistic effect with the PEF [176]. High electric fields may directly modify the functional groups of membrane proteins or may indirectly induce cell fusion through heating [254]. However, it is still not clear whether cellular inactivation occurs because of localized rapid rupture of a portion of the cell membrane or because of chemical stress associated with molecular transport phenomena [255]. Although experiments on bacteria such as *E.coli*, have been performed since the early 1960s over a wide range of electric field strengths, direct comparison of pulse durations and pulse repetition frequencies are difficult to make due to lack of standardized experimental protocols and variability in process parameters [179]. Failures of PEF in biological growth inhibition are also reported [191], and pulsed power treatment has required long contact durations for effective microbial reductions with significant effects not appearing until nearly 15 min of continuous treatment [191]. Duda et al.[149] compared chemical control measures with nonchemical device treatment for controlling biological activity in a model cooling tower system and reported the latter have little effect on biological growth. Furthermore, the effect of pulsed electric fields on different stages of biofilm formation can differ [246]. Rabinovitch [256] demonstrated that if chloride ions are present in water treated by pulsed-power technologies, (e.g. sea water) [257], then free chlorine can be generated, which is a powerful antimicrobial agent [257]. This suggests that pulsed-power treatment systems invoke both physical and chemical processes [191].

Giladi et al.[258] investigated bacterial growth inhibition by high-frequency, low-intensity electric fields generated by insulated electrodes. Since the electrodes were insulated, the electric fields were not believed to be associated with electrolysis or the production of free radicals or other ROS. In addition, due to the low intensity of the applied fields (0.5 to 4 V/cm), electroporation, which occurs at field intensities in the range of 1000 V/cm, was unlikely. Furthermore, in spite of continuous control of the medium temperature, which presumably eliminated thermal effects, growth of planktonic *Staphylococcus aureus* and *Pseudomonas aeruginosa* cells was nevertheless inhibited. The authors concluded that the observed antimicrobial effects were probably the result of non-homogeneous electric fields generated near the bridge separating daughter cells undergoing cell division.

EMF unit manufacturers such as Clear Water Systems Corporation [187] and Griswold Water Systems [188] indicate that algal slime layers in cooling tower water can be eliminated through nutritional limitation, a process in which algae are presumably shocked by starvation resulting from inactivation or removal of bacteria due to EMF treatment. To make such claims, there should be some acceptable published evidence showing the relationship between the algal removal and bacterial numbers since algae are photosynthetic and do not depend on bacteria, but such scientific verification is lacking.

4. Conclusions and future perspectives

It has not been fully scientifically demonstrated that the electromagnetic exposure generated by commercially available water treatment devices are powerful enough to produce strong antimicrobial or anti-scaling effects. However, manufacturers continue to market NCDs despite such a general lack of peer-reviewed laboratory data, proof-of-principle studies, clear mechanistic explanations and documented field studies [150]. Also, standardized operating procedures are frequently unclear and important parameters, such as the operating time needed for scale reduction and/or reduced bacterial counts, are often not reported. Indeed, favorable claims reported by investigators are generally based on un-substantiated claims, visual inspections alone and testimonials from “satisfied” end users [153]. Therefore it could be argued that the paucity of demonstrated applications and understanding of the mechanisms that may be involved in this technology could be contributing to a lack of uptake/interest in such devices for fouling control in large-scale desalination facilities.

Based on the more acceptable evidence from the limited number of peer reviewed scientific studies summarized in this review, it may be concluded that EMF might control scaling via altering nucleation and precipitation [62,151,209,218,219,220] and might also delay concentration polarization [259]. However, it is difficult to make comparisons between such studies due to the different experimental conditions. Therefore, future studies require standard operating procedures and controlled laboratory conditions. Such future studies also must be supported by credible instrumental techniques such as X-ray diffraction for crystal morphology investigations and/or Electrospray Mass Spectrometry for the delineation of potential clustering effects in solution that might impinge on nucleation and precipitation.

With regards to bacterial control [260], there are few credible studies that support the commercial claims and, in fact, some studies report growth stimulation by EMF exposure [261–265], including our own recent work, Piyadasa et al.[266]. Also the effects of PEF/EMF may actually become less pronounced after exceeding a certain number of EMF cycles or pulses [267].

With an enhanced understanding of the use and underlying mechanisms of action of PEF/EMF processes in treating RO feed waters, and considering installation and operational costs [193], it could be possible to develop improved strategies to minimize biofouling and scaling in desalination. Thus, additional reproducible studies are needed to explore and elucidate the fundamental scientific basis for the purported antimicrobial and anti-scaling effects of EMF technologies [167,248]. Progress in this and in related areas would be expected to catalyze a more widespread application of non-chemical EMF technologies to membrane-based desalination processes.

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Chapter 3 – PEMF exposure-system design

3.1 Overview

This chapter discusses the acquisition and characterization of two commercially available PEMF devices that are suitable for bench/pilot scale, controlled, laboratory testing with respect to antimicrobial and anti-scaling effects. The experimental designs are outlined for experiments conducted under static and flow conditions. The actual antimicrobial and anti-scaling experiments are discussed in detail in Chapters 4 and 5 respectively.

3.2 Commercial PEMF devices

The use of such commercial PEMF devices is more common in the cooling tower industry than for biofouling and scaling control issues associated with RO membrane systems. Currently, most manufacturers of commercial PEMF units are located in North America, Mexico and the UK (Huchler 2002). Some of these are listed in Table 3.1.

A typical PEMF unit is composed of two main components; the signal generator (Clearwater Systems Corporation 2008) or driver enclosure (Griswold Water Systems 2011) and the treatment module (Clearwater Systems Corporation 2008) or reaction chamber (Griswold Water Systems 2011), though the detailed designs between different manufacturuers might vary. After an analysis of the available documentation, it was clear that manufacturers tend to make their claims based on uncontrolled laboratory and field conditions and the specifications/manuals omit important technical details such as frequency information and power parameters - and proper Standard Operating Procedures (SOPs) are either not available or unclear. It has been suggested that because the magnetic field component of such devices is

relatively weak, the water must be re-circulated through the system to receive adequate exposure (Bisbee 2003). In other words, it has been suggested that flow conditions are required.

Table 3.1: Some manufacturers of commercial PEMF devices.

Manufacturer	Website
Dolphin Water Care	http://www.dolphinwatercare.com/about
Griswold Water Systems	http://griswoldwatersystems.com/gws-products/WAVE™ /
Evapco *Pulse*~Pure® Water Treatment System	http://www.evapco.com/products/pulse_pure_water_treatment_sytem
Scale-free Systems	http://www.scalefreeintl.com/
Scalewatcher	http://www.scalewatcher.co.uk/limescale-remover.html
Environmental Treatment Concepts Ltd	http://www.electronicdescaler.com/Our-Solution/98/
Water Imp	https://www.waterimp.co.uk

Intellectual property (IP) may also hinder the release of the technical details of PEMF devices that are critical for the scientific evaluation of their effectiveness (Dresty 2012). For example, it is known that there may be two or more coils wrapped around the treatment pipe of a PEMF device (Cho *et al.* 2005) and the number and thickness of such coils are often claimed as part of the manufacturer's IP (Dresty 2012). On the other hand, 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 outcomes.

Two PEMF devices were sourced and purchased from different commercial suppliers in the U.S.A. As summarized in Table 3.1, the 'Dolphin' device was purchased from Clearwater Technologies (http://www.dolphinwatercare.com) and the WAVE™ device was purchased from Griswold Water Systems (http://griswoldwatersystems.com). Both devices share common features; namely a signal generator housing the power and control components and a treatment module that is connected to the signal generator via an "umbilical" cable. (Fig 3.2a). For the purposes of this study these two devices are designated Device D and Device G respectively.

3.2.1 The 'Dolphin' PEMF (Device D)

Clearwater Systems Corporation is the manufacturer of the Dolphin PEMF device. According to the website, http://www.dolphinwatercare.com/, the research and development of the technology behind the Dolphin device goes back to 1990. In 1998 the commercial production of this device started with the Dolphin model 1000 and installed in Alcoa World Head Quarters (Patton 2008). The Dolphin 2000 model followed in 2002, and then in 2006 the Dolphin 3000 model was commercialized. Dolphin devices are claimed to control microorganisms, scale and

corrosion and, amongst the various models, the Dolphin 3000 is claimed to have more advanced operational enhancements, including biological control, four times more than the Dolphin 2000.

Dolphin devices are mainly being sold in North America, Europe, Australia, Russia, Southeast Asia, some parts of China and the Arabian Peninsula. Clearwater Systems claim that the device is known to have over 5000 installations all over the world, including the serving of cooling towers, HVAC and process chillers, ammonia condensers, process heat exchangers, fluid coolers and hot water systems. The Dolphin device purchased for this project was the G 3010 PVC (serial 00394) module that uses 230 V, 50/60Hz single phase, with an internal diameter of 1 inch.

Field characteristics

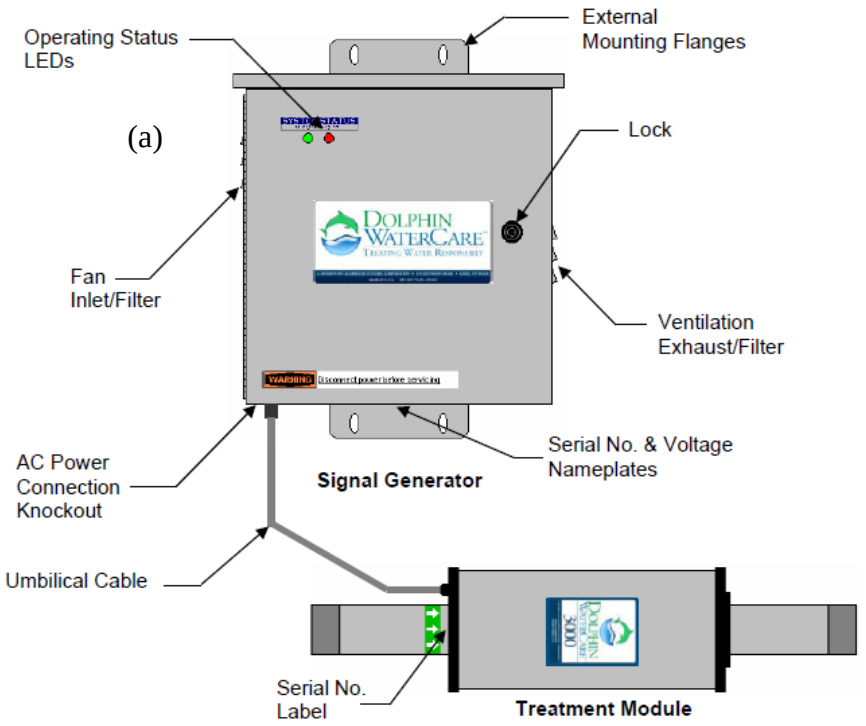
Clearwater Systems Corporation explains in their manual (Clearwater Systems Corporation 2008) that the Dolphin 3000 series are able to impart pulsed, high frequency electric fields into flowing water that varies 60 times per second - which is different to magnetic devices that produce a linear and fixed field that does not change direction with respect to the water as it passes through a solenoid coil. They also state that these devices induce a magnetic field of the same frequency but in a direction around the circumference of the pipe. During each cycle, the field strength varies from zero to a maximum value and then back to zero. Halfway through each cycle the field is pulsed, causing a ringing effect on the water (Clearwater Systems Corporation 2008) and this ringing has a natural frequency which based on the geometry of the coil and the capacitance of the circuitry. Over a time of about 3 milliseconds, this field dampens to a few percent of its original intensity. The transient dampening causes harmonics of the natural frequency, resulting in measurable frequencies up to the megahertz range depending on the scale of the device.

Flow pipe features

A case history report of the Dolphin NCD on boilers and cooling towers by Keister (2003) stated that they inspected a Dolphin device for their study and claim that it appeared to be nothing more than an insulated coil wound around a pipe spool. Cho *et al.* (2005) state that there are multiple coils outside of the flow pipe. They further explain that with a diagram that there are 3 individual coils around the flow pipe and the center coil has approximately twice the field strength of outer coils. Figure 3.1 illustrates the Dolphin PEMF device and the waveform.

Real world installations

In real world situations where Dolphin devices are used with cooling towers, they are installed in different positions as shown in Figure 3.2. The preferred location for installing the reaction chamber is between the discharge side of the condenser water circulation pump and the chiller (Bisbee 2003). However, the recommended installation depends on the particular application (Pisano 2011).



(b)

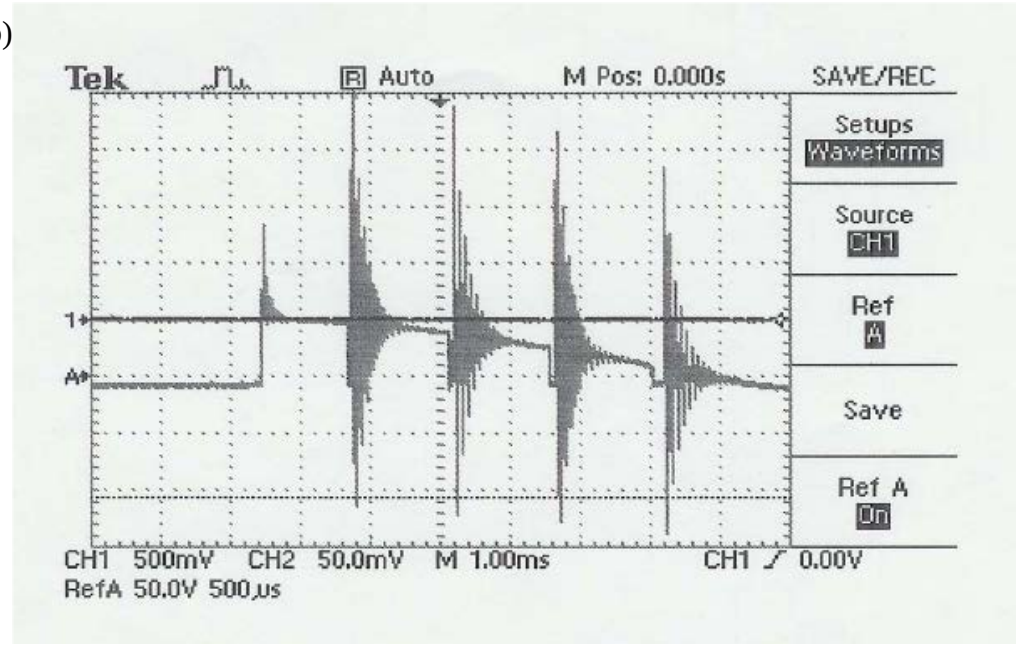


Figure 3.1: Schematic diagram of the Dolphin device (a) and characteristic waveform (b) as indicated in manufacture's documents (Clearwater Systems Corporation 2008).

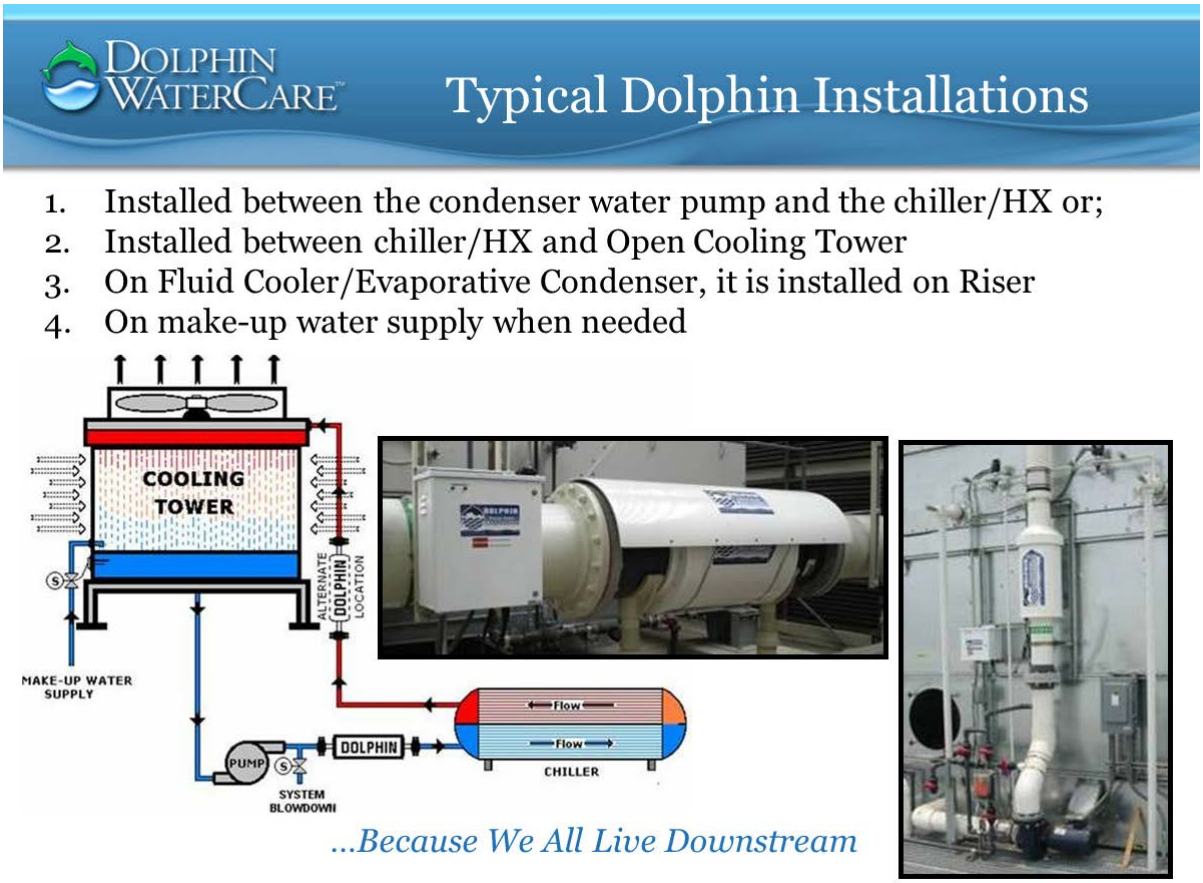


Figure 3.2: Typical installation diagram of a Dolphin Device.

Table 3.2 summarizes existing studies employed using Dolphin PEMF devices. More details can be found in the published review (Piyadasa *et al.* 2017).

Table 3.2: Summary of literature referring to Dolphin devices employed in this study.

Main focus and/or claims	**Ref**
A laboratory-based study using the Dolphin device. The authors suggest that, due to pulsed-power treatment, precipitation of calcium carbonate was altered from a surface nucleating scale to a non-adherent bulk solution powder.	(Cho *et al.* 2005)***

Main focus and/or claims	Ref
These authors conclude that the Dolphin device does not control corrosion in a cooling water set-up. Make up water was not softened, there was no evident effect on scale formation and it was expensive to use compared to normal chemical control methods, contrary to the manufacturers' claims.	(Keister 2003)
A conference paper that discussed the use of pulsed power equipment for condenser water treatment. Includes photos of installation in the recirculating loop. They claim that Dolphin pulsed-power treatment appeared to maintain a low level of planktonic microorganisms, a large decrease in biofilm formation and an improvement in the water quality attributes of clarity and odor.	(Cho *et al.* 2005)**
Five NCDs (magnetic, pulsed electric field, electrostatic, ultrasonic, and hydrodynamic cavitation), including a Dolphin device, have been scientifically evaluated for efficacy in reducing planktonic and sessile microbial populations in modeled cooling water systems. None of the NCDs demonstrated significant biological control under tested conditions.	(Duda *et al.* 2011)***
A study about biological control in cooling water systems using a few NCDs including the Dolphin device. Investigators state that effective microbial control in cooling water systems may not be achieved using a NCD as the sole method of water treatment.	(Vidic *et al.* 2010)*

Main focus and/or claims	Ref
A Dolphin device and a hydrodynamic cavitation device have been evaluated against conventional chemical treatment for a cooling tower facility. Both of the NCDs are reported to deliver better results and showed a clear cost savings advantage.	(Kitzman *et al.* 2003)**
A study where Pulsed-power treatment is reported to have generated more calcium carbonate formed than in the control in the form of bulk-solution powder but no scale formation on surface. The formed powder could be removed from solution.	(Opheim 2000)**
A case history report on the use of using such nonchemical pulsed power device on boilers and cooling tower. In this study, the Dolphin pulsed power device was reported to have no effect on scale control.	(Keister 2003)
A conference presentation on non-chemical water treatment for cooling towers that discusses principles of operation.	(Pisano 2011)**
Categorizes NCD devices into magnetic, electromagnetic, electrostatic, catalytic and mechanical devices. The characteristics and the market acceptance of each category are discussed based on several case history reports. States the names of suppliers/manufactures of commercial devices in each category, including Dolphin.	(Keister 2004)**

Main focus and/or claims	Ref
A field study of controlling scaling, total bacteria, and corrosion in a cooling tower environment using pulsed power water treatment.	(Alley *et al.* 2008)**
Reduced water clarity is caused by small particles (less than 10 microns) suspended in the water. The Dolphin System manages these particles, inducing coagulation and easier removal.[1]	(Clearwater Systems Corporation 2013)

From: (Piyadasa *et al.* 2017). *** Studies published as journal articles, ** Studies published as conference proceedings, * Studies found as technical/research/Government reports

3.2.2 WAVE™ PEMF (Device G)

Griswold Water Systems is the manufacturer of the WAVE™ PEMF device (Device G), https://griswoldwatersystems.com/.

Field characteristics

Griswold Water Systems explain in their manual (Griswold Water Systems 2011) that the WAVE™ device uses a voltage of 85-264 VAC, 47- 63 Hz and less than 150 Watts power consumption.

Flow pipe features

There are four independent coils in the Griswold PEMF device and the unit is self-tuning in that it seeks out the natural resonant frequency based on coil and capacitor size (Griswold Water Systems 2009; Griswold Water Systems 2011). Frequency is chosen based on coil size to provide specially targeted proprietary signal output (Dresty 2012), however it does not permit or need tuning (Dresty 2012). According to the company's website, the WAVE™ device generates "uniquely effective electric fields in the flowing water". The unit behaves as

[1] This is consistent with our own experiments.

a standard solenoid coil and the magnetic component of the field is in the same direction as fluid flow and the electrical component is perpendicular to the coil. The maximum field intensity will be inside the coil (Griswold Water Systems 2011). It is advised that the coil should not be placed next to metal objects, such as metal supports or sides of metal lab benches and magnetic flow meters should be kept at least 4 feet away from the reaction chamber to prevent interference (Dresty 2012). Figure 3.3, taken from the manufacturer's documentation, proposes an anti-scale mechanism of action for Device G. They suggest that exposure to Device G reduces zeta potential of particles which then make them agglomerate and result bigger clumps.

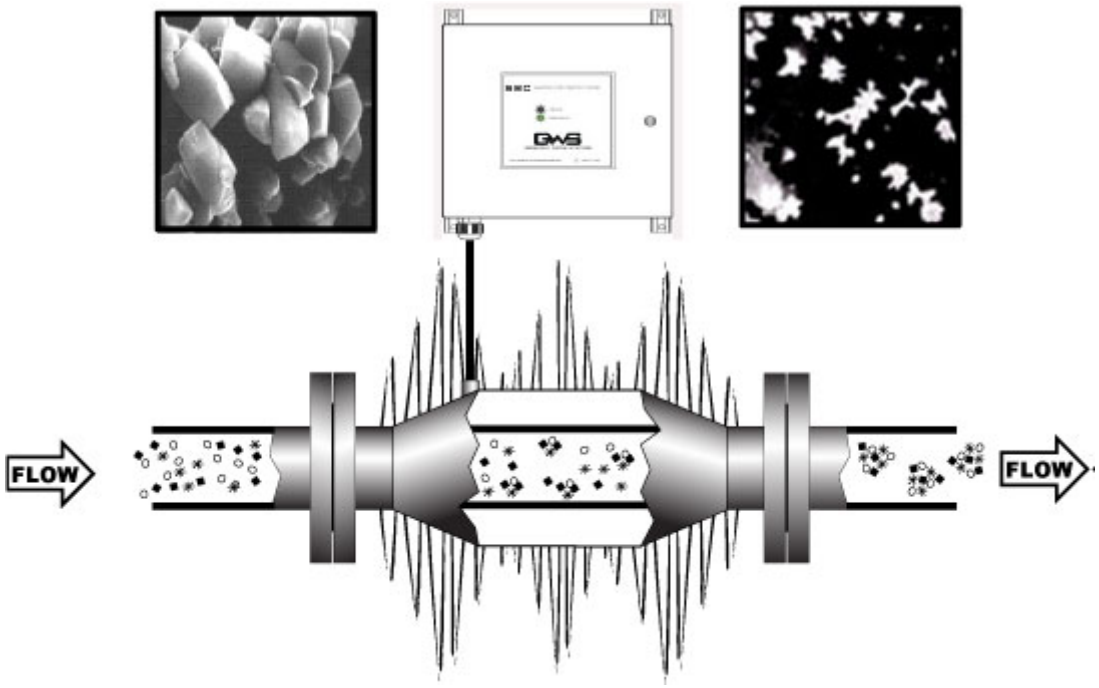


Figure 3.3: Schematic of the proposed mechanism of the WAVE^{TM} PEMF device working in scale reduction - as indicated in manufacturer's documents (Griswold Water Systems 2011).

Table 3.3 summarizes the comparative fouling control claims for the two PEMF devices used in this study as drawn from the manufacturer's documentation.

Table 3.3: Fouling control claims for the two PEMF devices used in these studies as indicated in the manufacturer's documentation (Clearwater Systems Corporation 2008; Griswold Water Systems 2011).

	The Dolphin System – Device D	**WAVE™ System – Device G**
Bacterial Control	Theory 1: This system is claimed to activate "suspended particles" (they do not explain the term suspended particles further) by removing the static electric charge on their surface - leading to powder formation. The bacteria in the water are attracted to the powder by dispersive forces and become entrapped. This phenomenon prevents the reproduction of bacteria and the entrapped bacteria eventually die. Theory 2: The high frequency, pulsing action of the electric fields of the Dolphin create small "pores" in the outer membrane of the bacteria weakening them and limiting their reproductive capability.	No clear mechanistic theories were found on this website. The WAVE™ system is claimed to successfully control bacteria in non-evaporative closed loops. For effective bacteria control, it is recommended to get as much recirculation as possible and to turn over a system's volume 3 times per hour. WAVE™ treatment is claimed to reduce the total bacterial population to less than 1,000 CFU/mL. However, the manufacturers recommend that bacterial testing should not begin until the system has been running for at least 4 consecutive weeks of stable operation (Griswold Water Systems 2011).

	The Dolphin System – Device D	WAVE™ System – Device G
Biofilm or Slime Control	The Dolphin System eliminates the slime layer through a process called 'nutrient limitation', which is not clearly explained.	The WAVE™ is claimed to be very effective against existing and potential biofilm formation. After the device has been in stable operation for several weeks, it is suggested that there should be no clear slimy feeling biofilm.
Corrosion Control	Exposing the water to a variable electromagnetic energy causes minerals to 'clump together' (nucleate)[2] rather than depositing onto the equipment surfaces (Bisbee 2003).	Hard water is required for the WAVE™ System to provide any corrosion protection. The induced electrical reaction zones is said to create "seed crystals" in the flowing water (Clearwater Systems Corporation 2013).

[2] Consistent with our own published studies.

3.3 Development of test apparatus

The laboratory systems that were designed to quantify the effect of PEMF were developed in the following two stages:

1. **Static mode treatment system:** The idea behind the development of a static mode system was to deliver maximum contact time for the water being treated with the field generated. Here the samples being treated were not flowing and remained static and were treated for a specified time period.

2. **Flow mode treatment system**: The idea behind the development of a flow mode treatment system was to allow the liquid to undergo short but repeated exposure and recirculate, as per suggestions in some reports (Bisbee 2003). In this set-up the liquid media that was exposed to PEMF was pumped through the treatment chamber using an external pump and a tubing system from a reservoir.

Figure 3.4 (a) represents the basic components of a device - employed under ‘Dry conditions’, Figure 3.4 (b) represents the Static system - ‘Wet conditions’, and Figure 3.4 (c) represents the Flow system - ‘Wet conditions’. Figure 3.5 represents the overall experimental plan involving the developed treatment systems.

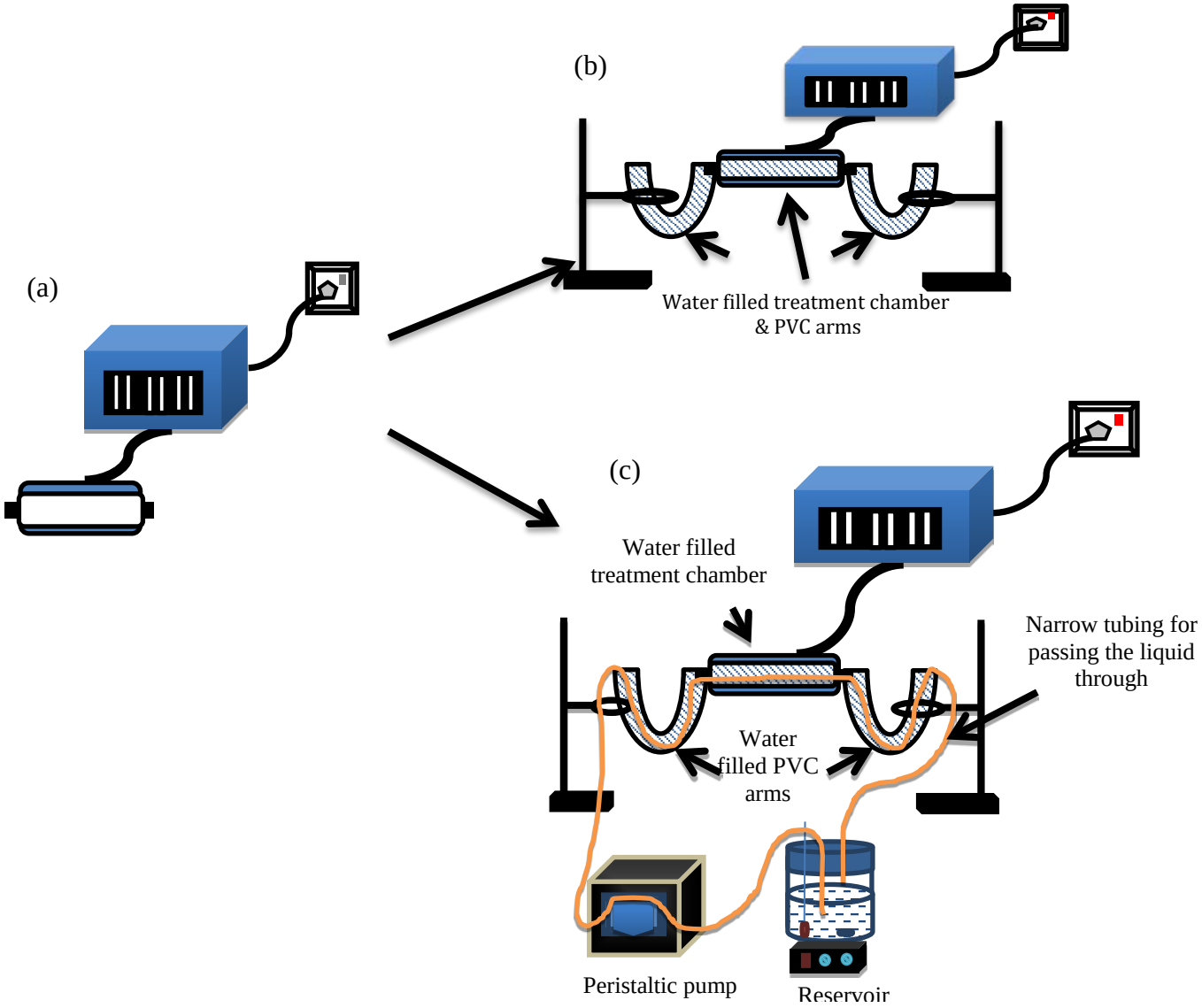


Figure 3.4: Treatment systems employed throughout this study

(a) Basic components of device - employed under 'Dry conditions' (b) Static system - 'Wet conditions' (c) Flow system - 'Wet conditions'.

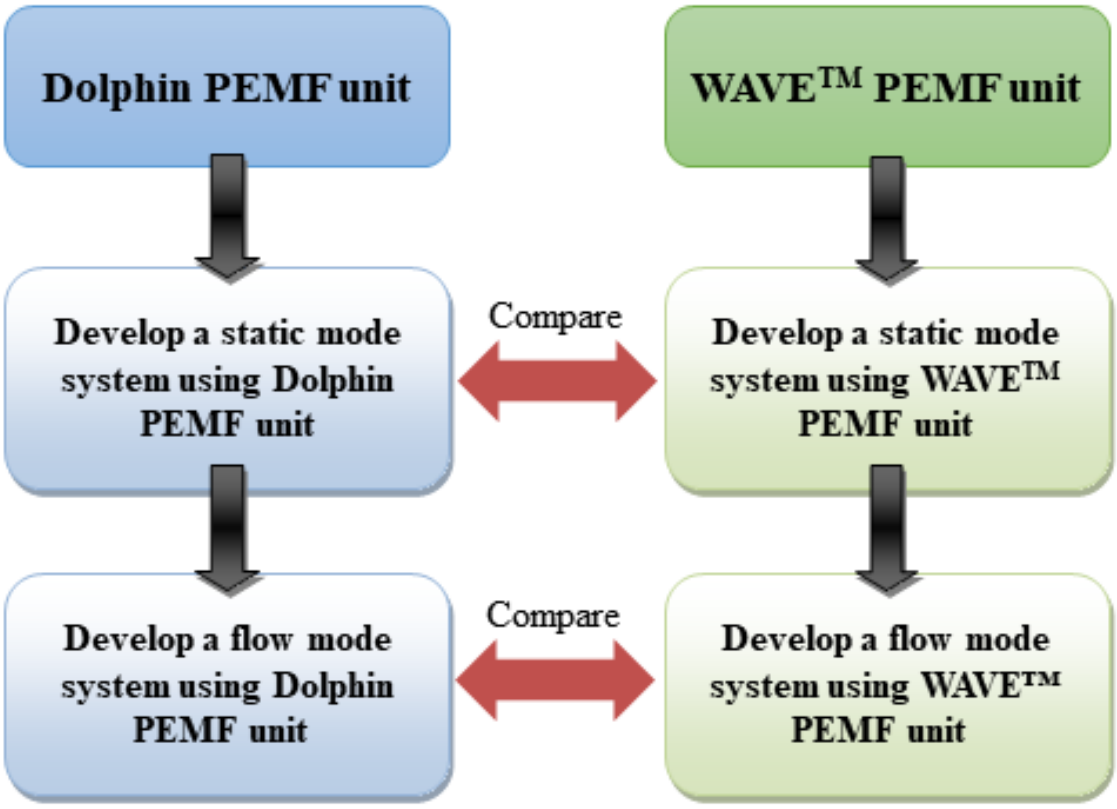


Figure 3.5: The experimental plan involving the developed treatment systems.

3.3.1 Development of the static mode treatment system

The static mode laboratory system was composed of a commercially available PEMF device, either Dolphin or WAVE™**,** with two PVC tube arms supported by ring stands to make a continuous U-shaped system, Figure 3.4 (b). Thus, two lengths of approximately 2.54 cm diameter PVC tubing were attached to both ends of the treatment modules of each device, using commercially available O rings'. The arms were removable. This set-up was used to initiate initial experiments on PEMF effects on bacterial viability and/or culturability, precipitation of calcium carbonate, formation of radicals and the effects of PEMF on water/liquid structure. There were two methods tested i.e. (1) flooding the treatment chamber with the liquid to be treated and (2) enclosing the liquid inside sterile tubes and placing the tubes inside the treatment

chamber. In the latter method, the treatment chamber was employed either under wet conditions (where the PVC arms and the treatment chamber were filled with water for cooling effect) or dry conditions (Figure 3.4 (a) where the set-up was used without water filled PVC arms hence using a dry treatment chamber.

System flooded with the test solution

According to PEMF device manufacturers fully flooded systems have yielded excellent performance in the field (Dresty 2012). Therefore, in the initial experiments, a fully flooded system was employed with the static mode treatment, where the system was filled with 600 mL of the test solution. This method involved exposing treated liquid and was only employed for preliminary bacterial tests and was not employed in calcium carbonate precipitation tests. In direct effect testing, 'contamination' and insufficient mixing was an issue especially with the experiments where bacteria were involved (where the treatment chamber and PVC arms had to be filled with bacterial culture).

When the whole system was filled with a test solution (i.e. the solution was poured into the PVC arms for direct effect testing) and the samples were required to collect from the middle of the treatment chamber, a sample was taken using a syringe attached to a long narrow tube that runs down to the middle of the treatment chamber, Figure 3.6.

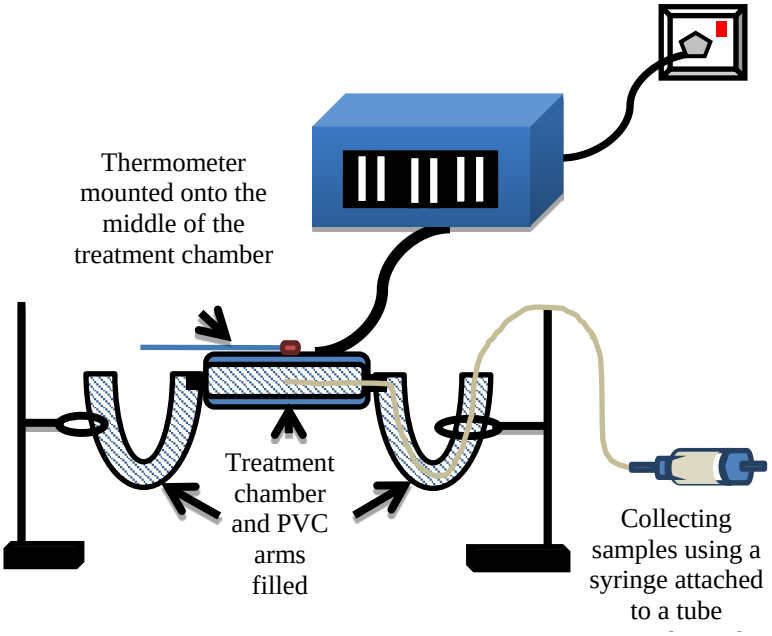


Figure 3.6: Schematic of sampling with the system flooded with test solution.

Sampling when the test solutions were contained in tubes

Here, the samples were contained in 10 mL sterile screw cap graduated tubes (Techno Plas, St Mary's, South Australia 5042). During the manual placing of the sample tubes inside the treatment chamber, the stabilized PEMF units were disconnected from power for a moment (the device was switched OFF for a short period of time, assuming the heat loss to be minimal and with minimal interruption to thermal and electronic stabilization). Then, 'thermally stabilized water' of the PVC arms were emptied into a container by manually lifting the whole apparatus. The temperature of the water in the container could then be measured. For sampling, one side of the PVC arms was removed and the sample tubes were pushed through into the middle of the treatment chamber Figure 3.7. Then the removed PVC arm was reattached and the same water was used to refill the system. This was done as quickly as possible to minimize any heat loss from the water and the device was switched back ON.

When screw capped tubes were placed inside the treatment chamber, it left no room for the temperature probe. Therefore, the temperature inside the treatment chamber was measured indirectly by measuring the temperature of the 'stabilized' water emptied into a container.

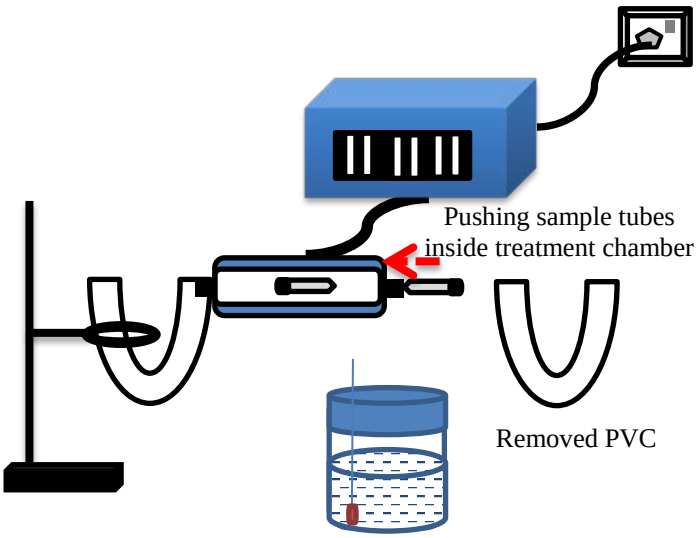


Figure 3.7: Placement of sample tubes inside the treatment chamber in static mode system.

Table 3.4 compares the challenges of the static and flow mode exposure set-ups.

Table 3.4: Comparison of the challenges of the static and flow mode exposure set-ups.

Challenge	Static mode exposure set-up	Flow mode exposure set-up
1. Experiment set-up	The set-up employed was either under wet conditions, where the PVC arms were filled with water to have a cooling effect, or dry conditions without cooling water.	In the flow mode treatment system, solution or culture pumped from the reservoir was released back to the reservoir. The reservoir was a 2L container and the total liquid volume was set to 1L. To minimize particle or cell deposition, the reservoir was kept stirring throughout the whole experiment duration and covered with aluminum foil to minimize effect from light. The reservoir was essentially left at room temperature conditions.
2. Set-up sanitation	Static mode system sanitization was carried out by pouring approximately 500 mL of 70% ethanol into the U-shaped system, and the system was gently rocked as a whole, which was labour intensive and involved risky manual handling. Then the ethanol was emptied in to a container (by tilting the whole system into the container). Then the washing repeated twice with 500 mL of MilliQ water in order to flush any excess ethanol in the system. This was performed before and after the experiments.	The reservoir was replaced with 1L of 70% ethanol and allowed to recirculate for 10-15 minutes. Then the reservoir was replaced with 1L of MilliQ water and recirculated for another 10-15 minutes. The thinner diameter tube was autoclaved, 121°C for 15 minutes.

Challenge	Static mode exposure set-up	Flow mode exposure set-up
3. Heating of the devices	As described in the above section, the core temperature of the treatment chambers can increase above room temperature during treatment. To minimize such heating the PVC arms were filled with water except in the experiments where dry conditions were essential.	The PVC arms were filled with water and a thinner diameter flexible tube was passed through the treatment chamber from the reservoir via a peristaltic pump while the PVC arms were filled with water. No change was made with the stabilization period, which was considered as 4 hours. However, manufactures of PEMF devices assume that a smaller diameter tube can diminish the signal and heating is negligible and the devices are kept ON at all times (Griswold Water Systems 2011; Dresty 2012).
4. Control experiment set-up	Suitably calibrated water baths were employed as controls for the wet condition testing and dry ovens for the dry conditions.	Setting up suitable controls for the flow treatment set-up was more challenging than setting up controls for the static testing. In the flow system, the 'treated' liquid (which flows through the system and reticulated back into the reservoir) was passing through a heated chamber (in addition to the PEMF) therefore the 'treated reservoir' had slightly high temperature. Also, in addition to the stirring effect from the stirring, there was turbulence due to the pumping.

Challenge	Static mode exposure set-up	Flow mode exposure set-up
		Therefore, there were two effects had to be taken into account. To clear the effects due to the turbulence, separate control tests were performed without the field, which means without switching the field ON. However, then there was no heating effect. Therefore again, the control setups were employed depending on the objective and the practicability. As an example, in a 'control flow' the reservoir was slightly heated on a hotplate (at the lowest level possible) in addition to stirring. Temperature changes of the reservoirs were monitored using inserted thermometers.
5. Sampling	Sample containment plastic tubes were manually placed inside the treatment chamber of a stabilized (wet conditions) un-stabilized (dry conditions) PEMF device. After the required duration, tubes were taken out manually.	Samples were directly obtained from the reservoir at the preferred sampling times while the system was still running. Sampling could be performed at any time and described in individual chapters. However, the samples were prone to contamination (in the bacterial experiments) as compared to the static system as the sampling was carried out in the open air.

Challenge	Static mode exposure set-up	Flow mode exposure set-up
6. Replicates	When the samples were contained in 10 mL tubes during static exposure, there was no room inside the treatment chamber for more than two tubes at a time. Therefore, when bacteria cultures were exposing to PEMF (healthy and AgNP exposed), only one tube per culture could be placed inside the treatment tube. Therefore, the replicates were 'replicate platings' instead of replicate exposures. Also, due to the small diameter of the treatment chamber it did not allow us to directly expose biofilm grown in a mictotitre plate to static PEMF, as done in (Segatore *et al.* 2012). For the static PEMF, exposure of parent solutions of $CaCO_3$, for precipitation studies (Chapter 5), only one tube of each solution could be placed inside each treatment chamber at a time. Replicate readings could not be taken as the precipitation was taking place quickly. Therefore, the replicates were independent repeated experiments.	

Challenge	Static mode exposure set-up	Flow mode exposure set-up
7. Experiment duration	Up to 7 hours with wet conditions (including the 4 hour stabilization period) and up to 3 hours in dry conditions. No stabilization was employed under dry conditions to minimize any device failure.	Maximum experiment duration was up to 8 hours (including the 4 hour stabilization period).
8. Ambient temperature	Essentially the ambient temperature was set to 20-25°C using an electronic controller system. Though the laboratory temperature was set around 20-25°C, the actual ambient temperature might have varied within that range rather than a set single value due to reasons such as number of occupants in the laboratory, use of other equipment in the laboratory such as Bunsen burners etc.	Essentially ambient temperature was set as with the static mode treatment system.

Figure 3.8 shows the temperature measurement during the static mode system operation of Dolphin PEMF device. It was observed that the surface temperature of the treatment chamber increased to above 45°C.

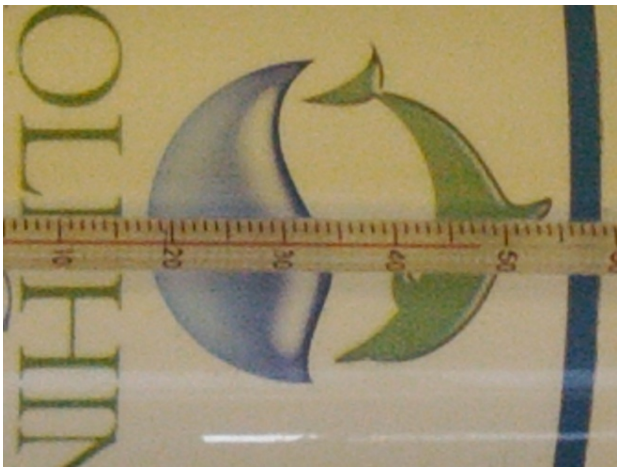


Figure 3.8: Temperature observations during the static mode system operation of Dolphin PEMF device.

3.3.2 Development of flow mode treatment system

The flow mode treatment system was employed after the static mode treatment system. The flow mode system flow mode system was composed of a PEMF device, tubing, a peristaltic pump and reservoir (see Figure 3.4 (c)). The specifications of the various components are given in Table 3.5.

By the time flow mode system was developed many of the methods (for bacteria and calcium carbonate tests) were finalized and were ready to repeat with the flow mode system, with some modifications.

Table 3.5: Components and specification of the flow mode system.

	Specifications	Supplier
Peristaltic pump	Masterflex L/S Precision Variable-Speed Drive with Remote Input, 6 to 600 rpm, 90 to 260 VAC, Masterflex L/S Easy-Load II pump head flow rate of 0.36-3400 mL/min.	Cole Parmer Internal Sales John Morris Scientific, Service plus solutions, 61-63 Victoria Avenue, Chatswood NSW 2067.
Reservoir	2 L polypropylene container with screw cap lid. Always 1 L of liquid (bacteria culture or $CaCO_3$) was used for experiments. In bacteria experiments, the container was covered in aluminum foil.	Cospak Pty Ltd, Victoria, Australia.

3.4 Summary

Both devices thermally stabilized after 4 hours. Therefore, in the later experiments the devices were left ON for 4 hours prior to the experiment and the samples were introduced after 4 hours into the stabilized system, being manually placed inside the treatment chamber. However, if the dry conditions were used (Chapter 3 and 4) no stabilization period was employed to prevent the device form over-heating and dry conditions were used only under static conditions and performed as one-off trials. Essentially, the maximum experiment duration was limited to 12 hours (including the 4 hour stabilization period). The control experiments were planned according to the different objectives and the nature of the system utilized; described in relevant chapters.

Chapter 4 – Effects of PEMF on bacterial cultivability

4.1 Overview

Chapter 4 presents the effects of PEMF in on bacterial culturability. This chapter consists of two peer reviewed journal articles. The effects of PEMF in on bacterial culturability under static and flow conditions are discussed separately in this chapter as two sections.

4.2 Section 1

The paper (Paper 2) entitled “The effect of electromagnetic fields, from two commercially available water-treatment devices, on bacterial culturability’’ by Chathuri Piyadasa, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart, Harry F. Ridgway, Con Pelekani and John D. Orbell was published in the Journal of *Water Science and Technology*, (2016) **73(6)**, 1371-1376. The declaration of co-authorship for this paper is below, followed by the paper itself.

4.3 Section 2

The paper (Paper 3) entitled “Antimicrobial effects of pulsed electromagnetic fields from commercially available water treatment devices – controlled studies under static and flow conditions’’ by Chathuri Piyadasa, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart, Harry F. Ridgway, Con Pelekani, and John D. Orbell was submitted to the Journal of Chemical Technology and Biotechnology in July 2017. This paper was submitted as a follow up for the first paper and is the subject of Section 2 of this Chapter.

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GRADUATE RESEARCH CENTRE

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book: The effect of electromagnetic fields, from two commercially available water-treatment devices, on bacterial culturability.

Surname: Piyadasa First name: Chathuri

College: College Candidate's Contribution (%): 71

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Accepted and in press: ☐ Date:

Published: ☑ Date: 8/12/2015

2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

[redacted] 26/06/17

Signature Date

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and

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5. The original data will be held for at least five years from the date indicated below and is stored at the following location(s):

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Chathuri Piyadasa	71	Performed experiments, analyzed data, prepared major part of manuscript	[redacted]	26/06/17
Thomas R. Yeager	8	Suggestions to experiments , Revision of manuscript	[redacted]	26/06/17
Stephen R. Gray	4	Revision of manuscript	[redacted]	27/6/2017
Matthew B. Stewart	1	Suggestions to experiments	[redacted]	11/7/17
Harry F. Ridgway	5	Suggestions to experiments, graphical inputs, Revision of manuscript	[redacted]	12/07/2017
Con Pelekani	1	Suggestions to experiments	[redacted]	17/7/17
John D. Orbell	10	Suggestions to experiments , Revision of manuscript	[redacted]	18/7/17

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

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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.

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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; Königs *et al.* 2015). 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

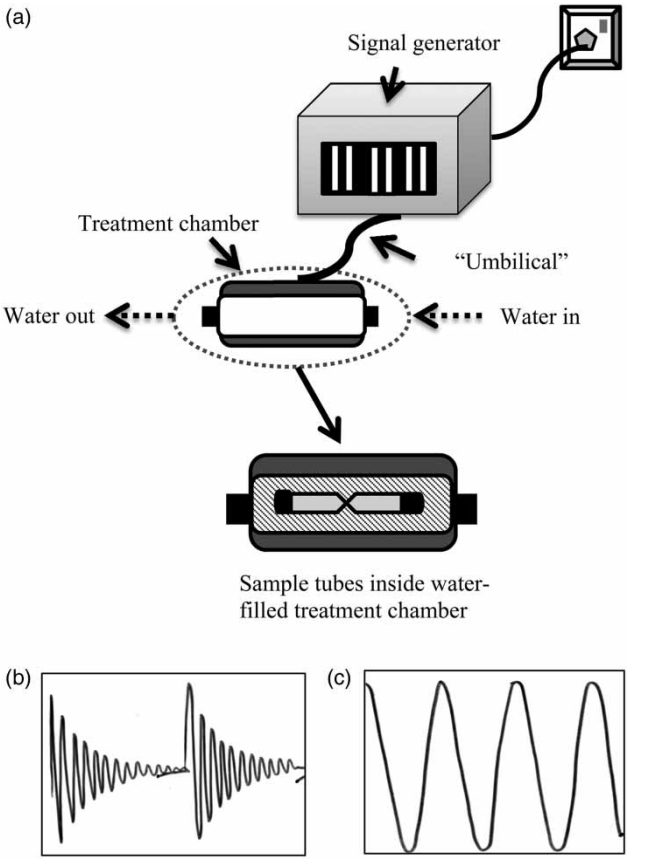

Figure 1 | (a) Main elements common to the two PEMF devices used in this study. Bacterial suspensions to be exposed to PEMF were placed in sealed plastic tubes inside the water-filled treatment chamber. In the current study, the treatment chamber was operated in a static mode, i.e. without any flow. 'Umbilical' refers to an electrical cable connecting the signal (waveform and power) generator to the treatment chamber. Schematic representations of waveforms for (b) Device-D and (c) Device-G adapted from their oscillograms (Trio 15 MHz, CS-1560AII).

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 $35 \pm 2\,^{\circ}C$ 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 $OD_{600nm} \sim 1.0$ 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 $4.0–6.0 \times 10^5$ 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

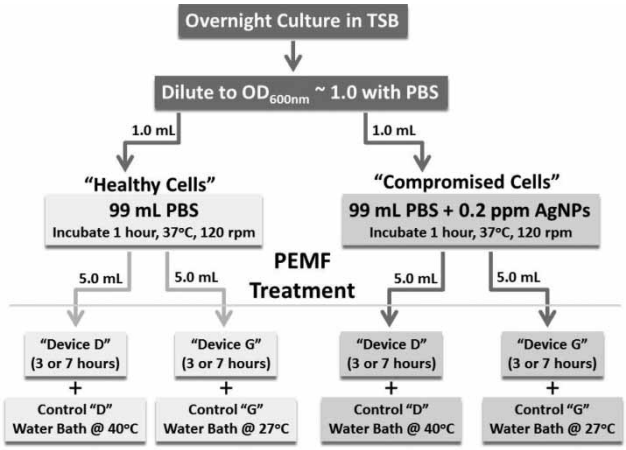

Figure 2 | Schematic of the method. After the 1 hour pre-incubation period, 5 mL of both 'healthy' and 'compromised' suspensions were introduced to two 10 mL sterile screw-cap graduated tubes which were then placed inside the treatment chamber of each temperature-stabilized PEMF device and exposed to PEMF under static conditions for either 3 or 7 hours. 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.

(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. The Device-D PEMF unit and the Device-D water-bath control were operated at ~40 °C, while the Device-G PEMF unit and its corresponding water-bath control were operated ~27 °C.

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%)

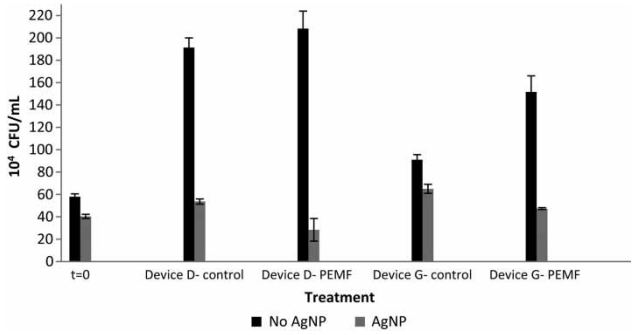

Figure 3 | Enumeration of healthy and AgNP-compromised *E. coli* populations (expressed as CFU/mL) following exposure for 3 hours to PEMF Device-D or Device-G and their respective non-PEMF temperature pre-equilibrated water-bath controls. Error bars are standard errors for three replicates. *Notes:* (i) bars at $t = 0$ represent the 'establishment stage' after ~1 hr of growth, at which time the bacteria were introduced into the experiments; (ii) the difference between the observed magnitudes for the D and G controls is due to the different temperatures.

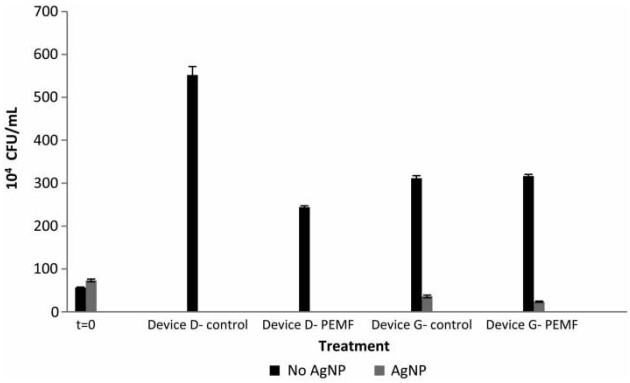

Figure 4 | Enumeration of healthy and AgNP-compromised *E. coli* populations (expressed as CFU/mL) following exposure for 7 hours to PEMF Device-D or Device-G and their respective non-PEMF temperature pre-equilibrated water-bath controls. Error bars are standard errors for three replicates. *Notes:* (i) bars at $t = 0$ represent the 'establishment stage' after ~1 hour of growth, at which time the bacteria were introduced into the experiments; (ii) the difference between the observed magnitudes for the D and G controls is due to the different temperatures.

decline in cell numbers compared to their respective non-PEMF water-bath controls.

When the PEMF exposure time was extended to 7 hours, Figure 4, the healthy cell population for Device-D exhibited a marked reduction in cell viability compared to its corresponding non-PEMF water-bath control. Similarly, the growth enhancement noted previously for the healthy cell population that was exposed for 3 hours in Device-G was significantly diminished compared to the non-PEMF water-bath control. The AgNP-compromised *E. coli* population exposed to PEMF in Device-D for 7 hours was found to be below the plating detection level (~10 CFU/mL). This result was also observed for the corresponding Device-D water-bath control. However, for Device-G, the AgNP-compromised population exposed for 7 hours to PEMF continued to exhibit a noticeable reduction in CFU counts compared to its non-PEMF water-bath control.

In relation to a growth-inhibitory effect becoming more evident after a longer exposure time, for both devices, previous investigations have also demonstrated that static magnetic fields can result in decreased cell viability with longer exposure times (Ji *et al.* 2009).

With regards to the somewhat surprising statistically significant growth *enhancement* of healthy *E. coli* exposed to PEMF for 3 hours, previous studies have reported similar growth stimulation by EMF exposure (Nascimento *et al.* 2003; Gaafar *et al.* 2006; Segatore *et al.* 2012; Aslanimehr *et al.* 2013).

It has also been shown that after exceeding a certain number of EMF cycles or pulses, the subsequent impact of 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-inhibition 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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1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book:	Antimicrobial effects of pulsed electromagnetic fields from commercially available water treatment devices - controlled studies under static and flow conditions
Surname:	Piyadasa
First name:	Chathuri
College:	College of Engineering & Science
Candidate's Contribution (%):	71

Status:

Accepted and in press:	☐	Date:	
Published:	☑	Date:	22/09/17

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[redacted]	09/01/18
Signature	Date

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Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Chathuri Piyadasa	71	Performed experiments, analyzed data, prepared major part of manuscript	[redacted]	09/01/18
Thomas R Yeager	8	Suggestions to experiments, revision of manuscript	[redacted]	24/01/18
Stephen R Gray	4	Revision of manuscript	[redacted]	9/1/18
Matthew B Stewart	1	Suggestions to experiments		
Harry F Ridgway	5	Suggestions to experiments, revision of manuscript	[redacted]	17 Jan 2018
Con Pelekani	1	Suggestions to experiments		
John D Orbell	10	Suggestions to experiments, revision of manuscript	[redacted]	9/1/18

Piyadasa, C., Yeager, T.R., Gray, S.R., Stewart, M.B., Ridgway, H.F., Pelekani, C. and Orbell, J.D.(2018) Antimicrobial effects of pulsed electromagnetic fields from commercially available water treatment devices – controlled studies under static and flow conditions. Journal of Chemical Technology and Biotechnology, 93 (3). 871 - 877.

4.8 Auxiliary studies on the leakage of DNA material from PEMF exposed *E. coli* cells

Intracellular material such as DNA could be leaked into the external environment because of weakened cellular membranes (Virto *et al.* 2005). Consequently, it was surmised that if PEMF exposure weakens the cellular membrane, excessive DNA might be detected in the surrounding medium. To test this idea, preliminary experiments were initiated in order to foster future research directions. These initial outcomes are included in this thesis for reference, although time constraints did not allow for follow up investigation. To quantify intracellular DNA material released from the bacterial cells due to such potential membrane damage, possible DNA leaking was detected as absorbance at 260 nm according to the method of (Virto *et al.* 2005). Thus 1 mL samples were centrifuged at 14,000 rpm for 10 minutes and the UV absorbance of 1 µL of the supernatant was measured at 260 nm with a DeNovix DS-11+Spectrophotometer. The ssDNA and dsDNA were individually recorded as ng/µL and the total DNA leakage was calculated from the sum of the ssDNA and dsDNA values. Three separate readings were made for each sample.

Figure 4.1 is a graphical representation of DNA leakage from samples exposed to two PEMF devices in the presence and absence of 0.2 ppm AgNP, compared to controls.

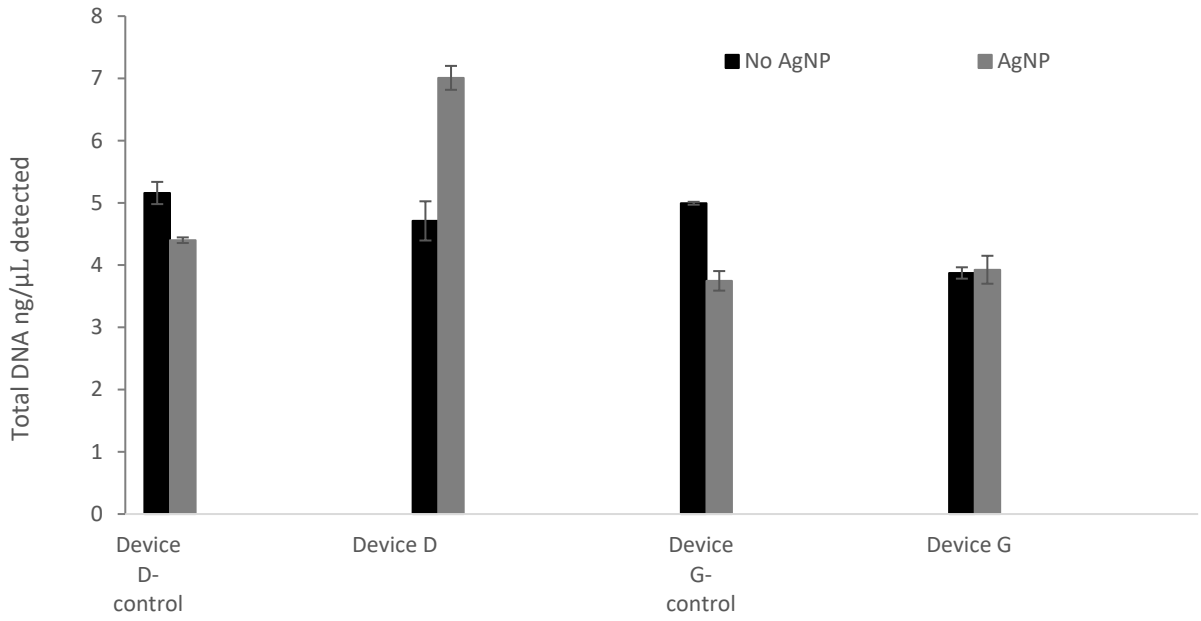

Figure 4.1: DNA leakage from *E. coli* cultured in the presence and absence of AgNPs for 1 hour and then exposed to (a) Device D PEMF unit and water bath for 7 hours (b) Device G PEMF unit and water bath for 7 hours; error bars are standard errors for three replicates readings.

AgNP treated samples are expected to damage the cell membranes expecting resulting in more such DNA release. Figure 4.2 shows the particle size distribution report of the AgNPs used in this study with an average diameter of 5.5 nm. It is apparent from Figure 4.1 that AgNP combined with the PEMF from Device D promotes the highest DNA release, although this is not apparent for Device G. For the *E. coli* that is not compromised by AgNPs there is no significant increase in DNA release for Device D but there is a significant decline in DNA release for Device G.

Both Devices D and G have been found in our work to elicit a stimulatory response in *E. coli* (Papers 2 & 3), although prolonged exposure to Device D results in an inhibitory adaptive

response up to the 7 h exposure time. It would appear from this data that Device G has more of a stimulatory effect than Device D. This corresponds to a greater decline in the surrounding DNA for Device G exposure, Figure 4.1, and perhaps suggests a strengthening of the cell membrane upon PEMF stimulation. This is consistent with what happens when the AgNP-compromised *E. coli* is exposed to Devices D and G. Here, we observe inhibition in both cases (Paper 2). Therefore, we might expect a release of DNA due to membrane damage and this is observed and is found to be much more significant for Device D than for G (Figure 4.1) suggesting a membrane protective effect for Device G (that is more stimulatory). A fully detailed analysis is beyond the scope of this thesis and was limited due to time restrictions. However, this intriguing work is continuing in our research group.

Size Distribution Report by Volume

v2.0

Malvern

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Sample Details

Sample Name: Colloidal Silver 14ppm 1
SOP Name: silver colorless.sop
General Notes:

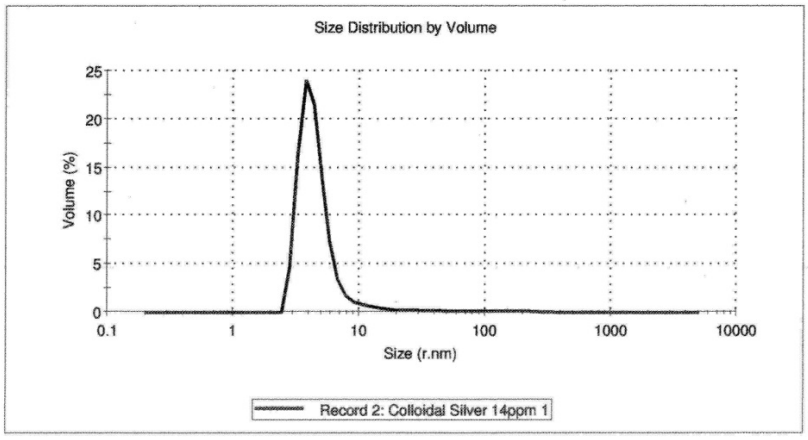
File Name:	Silver NP0.3 mM.dts	**Dispersant Name:**	Water
Record Number:	2	**Dispersant RI:**	1.330
Material RI:	0.99	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.00	**Measurement Date and Time:**	Thursday, 6 March 2014 ...

System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	175.6	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	6

Results

			Diam. (nm)	% Volume	Width (nm)
Z-Average (r.nm):	56.14	**Peak 1:**	188.2	1.3	81.03
PdI:	0.443	**Peak 2:**	5.496	98.7	6.925
Intercept:	0.879	**Peak 3:**	0.000	0.0	0.000
Result quality	Refer to quality report				



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Zetasizer Ver. 6.01
Serial Number : MAL1037108

File name: Silver NP0.3 mM.dts
Record Number: 2
06 Mar 2014 3:26:39 PM

Figure 4.2: Particle size distribution of AgNPs.

Chapter 5 – The effect of PEMF on $CaCO_3$ precipitation

5.1 Overview

Chapter 5 presents the effects of PEMF in on calcium carbonate precipitation. The rate and profile of calcium carbonate precipitation in the presence and absence of PEMF exposure of the parent calcium nitrate and sodium carbonate solutions was tracked by UV absorption at 350 nm and by turbidity measurements. The size and morphology of the corresponding crystalline precipitates were also assessed using light microscopy and SEM.

The paper (Paper 4) entitled “The influence of electromagnetic fields from two commercially available water-treatment devices on calcium carbonate precipitation” by Chathuri Piyadasa, Thomas R. Yeager, Stephen R. Gray, Matthew B. Stewart, Harry F. Ridgway, Con Pelekani and John D. Orbell was published in *Environmental Science: Water Research & Technology*, (2017), **3**, 566-572. The declaration of co-authorship for this paper is below, followed by the paper itself.

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1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book:	The influence of electromagnetic fields from two commercially available water-treatment devices on calcium carbonate precipitation
Surname:	Piyadasa
First name:	Chathuri
College:	College of Engineering & Science
Candidate's Contribution (%):	70%

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[redacted] 26/06/17

Signature Date

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In the case of the above publication, the following authors contributed to the work as follows:

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Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
C. Piyadasa	70	Performed experiments, analyzed data, prepared major part of manuscript	[redacted]	26/06/17
T. R. Yeager	8	Suggestions to experiments, Revision of manuscript	[redacted]	26/06/17
S. R. Gray	5	Suggestions to experiments	[redacted]	27/6/2017
M. B. Stewart	1	Suggestions to experiments	[redacted]	14/7/2017
H.F. Ridgway	5	Suggestions to experiments	[redacted]	12/07/2017
C. Pelekani	1	Suggestions to experiments	[redacted]	12/7/17
J. D. Orbell	10	Suggestions to experiments, Revision of manuscript	[redacted]	18/7/17

Piyadasa, C., Yeager, T.R., Gray, S.R., Stewart, M.B., Ridgway, H.F., Pelekani, C. and Orbell, J.D. (2017) The influence of electromagnetic fields from two commercially available water-treatment devices on calcium carbonate precipitation. Environmental Science: Water Research and Technology, (3). 566 - 572.

5.4 Auxiliary studies on the effect of the PEMF exposure of *individual* parent solutions

Given that the pre-exposure of both the parent Na_2CO_3 and $Ca(NO_3)_2$ solutions to the PEMF of Device D, prior to mixing, had a significant influence on the rate of $CaCO_3$ precipitation and the resulting $CaCO_3$ crystal morphology, as descibed in Paper 4, there is obviously more work warranted in this area. Given that there was no noticeable effect from the PEMF of Device G, it is clear that a range of parameters and conditions, could affect the precipitation characetistics in different ways. Therefore, the experiments described below were initiated in order to promote further enquiry. Consequently these preliminary results they have been included in this thesis. Time constraints did not allow further development in this study.

5.4.1 Pre-exposed Na_2CO_3 and un-exposed $Ca(NO_3)_2$ solutions

In the previous section, *both* parent solutions were pre-exposed to the PEMF of Devices D and G and compared to their respective controls. Here, Na_2CO_3 *alone* was pre-exposed to PEMF under 'dry conditions' for 30 minutes and mixed with un-exposed $Ca(NO_3)_2$ at room temperature. Since this is under 'dry conditions', the maximum exposure time was limited to 30 minutes to prevent any possible device failure due to overheating. The control for Devices D and G respectively consisted of: Na_2CO_3 heated to either 40°C (oven) or 27°C (oven) mixed with $Ca(NO_3)_2$ at RT.

Note that this, and subsequent experiments, were not performed in replicate nor supported by either UV absorbance or precipitation studies. The resulting crystal morphologies were visually examined using SEM. The SEM images of the crystalline $CaCO_3$ precipitate, Figures 5.1 (a) and (b), suggest that pre-exposure of Na_2CO_3 alone to Device D PEMF results in significant

differences in crystal morphology with ‘clustered rods’ dominating upon exposure, suggesting an influence on crystal growth and morphology. On the other hand, with Device G PEMF treatment, the crystals appear more cuboidal with little to no evidence of rods, Figures 5.1 (c) and (d). Thus exposure of the parent Na_2CO_3 *alone* appears to affect the susequent $CaCO_3$ in different ways for Devices D and G. Notably, when both parent solutions were exposed (see Paper 4), the field from Device D promoted cuboidal crystals and for Device G there was evidence for plate-like morphology. In this case there was no discernible morphology in the controls.

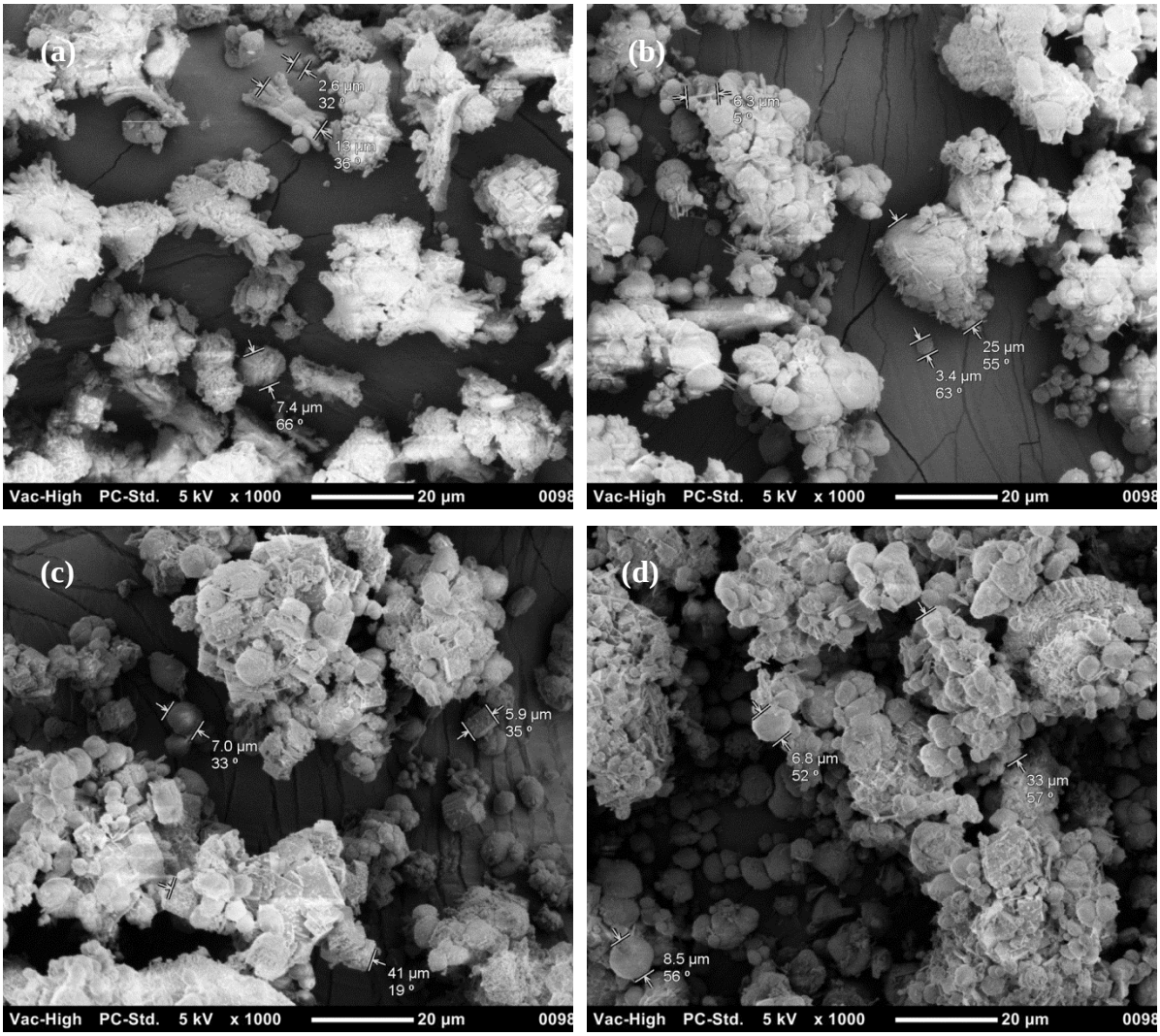

Figure 5.1: SEM images of dried precipitate mixtures of pre-exposed (30 min) Na_2CO_3 mixed with un-exposed $Ca(NO_3)_2.4H_2O$ at room temperature, (a) Device D PEMF (b) Device D Control (c) Device G PEMF (d) Device G Control.

5.4.2 Pre-exposed $Ca(NO_3)_2$ and un-exposed Na_2CO_3 solutions

Here, the $Ca(NO_3)_2$ parent solution *alone* was pre-exposed to Devices D and G PEMF under 'dry conditions' for 30 minutes and mixed with un-exposed Na_2CO_3 at room temperature. The control for Devices D and G respectively consisted of: $Ca(NO_3)_2$ heated to either 40°C (oven) or 27°C (oven) mixed with Na_2CO_3 at RT. Figure 5.2 shows the SEM images of the crystalline $CaCO_3$ precipitates formed under the respective treatments compared to the controls. These SEM images show that exposure to Device D PEMF results in more plate-like crystals (and possibly rods), whereas the control is mainly cuboidal (Figures 5.2 (a) and (b)). Upon exposure to Device G PEMF, the crystals also appear to to be plate-like with some rods whereas the control is also primarlily cuboidal (Figures 5.2 (c) and (d)). Thus exposure of the parent $Ca(NO_3)_2.4H_2O$ *alone* appears to affect the susequent $CaCO_3$ in a similar way for both Devices D and G.

5.4.3 PEMF exposure of reaction mixture *during* the precipitation of $CaCO_3$

Aqueous solutions of 1M Na_2CO_3 and $Ca(NO_3)_2.4H_2O$ were mixed together at room temperature and the mixture was immediately placed into each of the stabilized PEMF devices under dry conditions for 30 minutes, allowing respective Device D and Device G PEMF exposure during the crystallization process. In this case the controls were the reaction mixture placed in the respective Device D and G ovens at 40°C and 27°C.

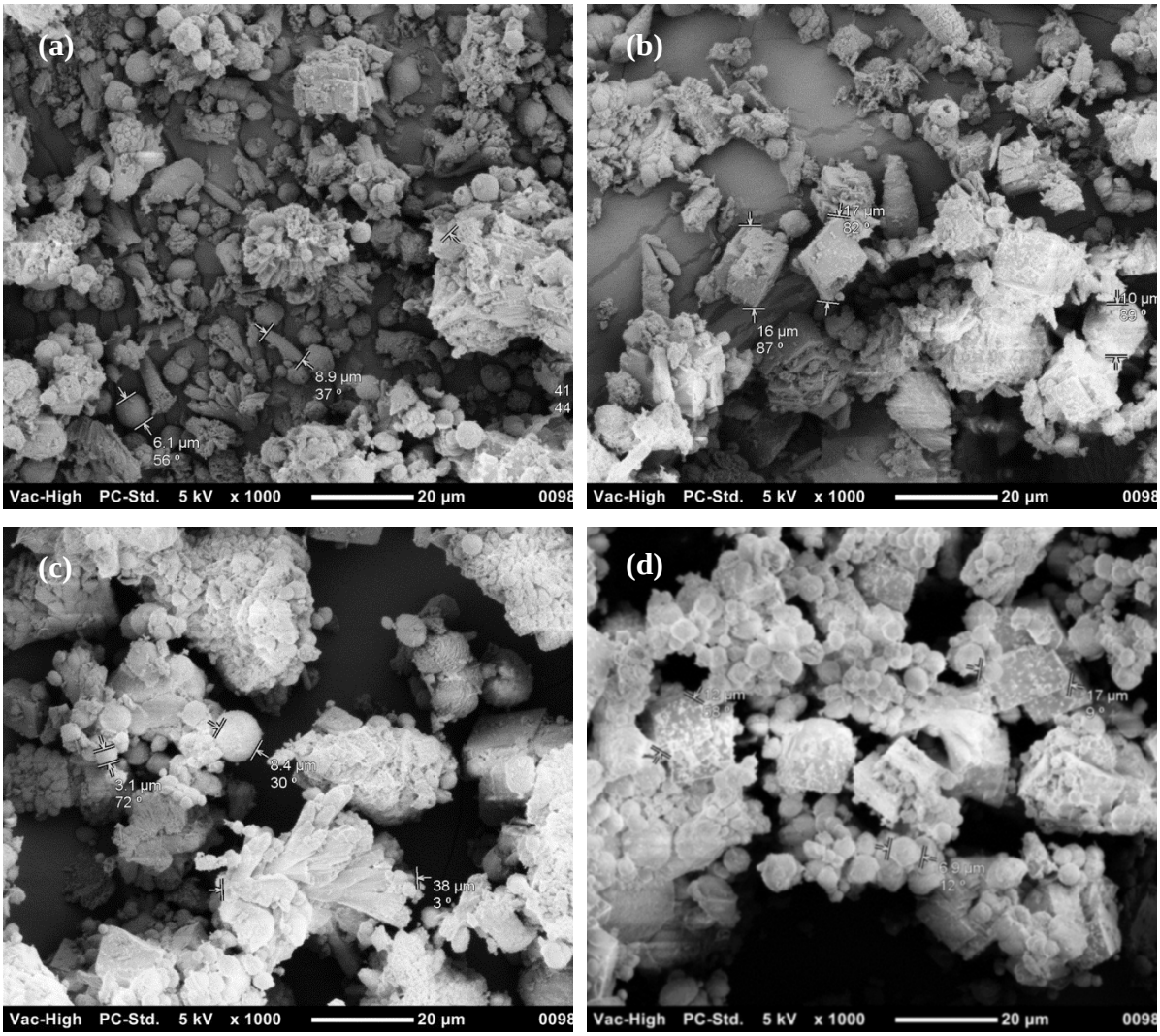

Figure 5.2: SEM images of dried precipitate mixtures of pre-exposed (30 min) $Ca(NO_3)_2.4H_2O$ with un-exposed Na_2CO_3 at room temperature, (a) Device D PEMF (b) Device D Control (c) Device G PEMF (d) Device G Control.

Here, the SEM images of the crystalline precipitates suggest that placing the mixed parent solutions (the actual reacting mixture) inside Device D causes significant morphological with needle morphology dominating whereas these are lacking in the control that is more cuboidal/plate-like Figures 5.3 (a) and (b). For Device G, the analogous experiment results in cuboidal morphology dominating, Figures 5.3 (c) and (d). Again, the control tended to be more cuboidal and plate-like – as expected since for these experiments the controls are identical. Therefore, it can be concluded that exposure to both Device D and G PEMF (while precipitation

is actually taking place) has significant influences on the crystal morphology. Again, this is quite different from when both parent solutions were exposed, Paper 4, where the field from Device D promoted cuboidal crystals and for Device G there was evidence for plate-like morphology.

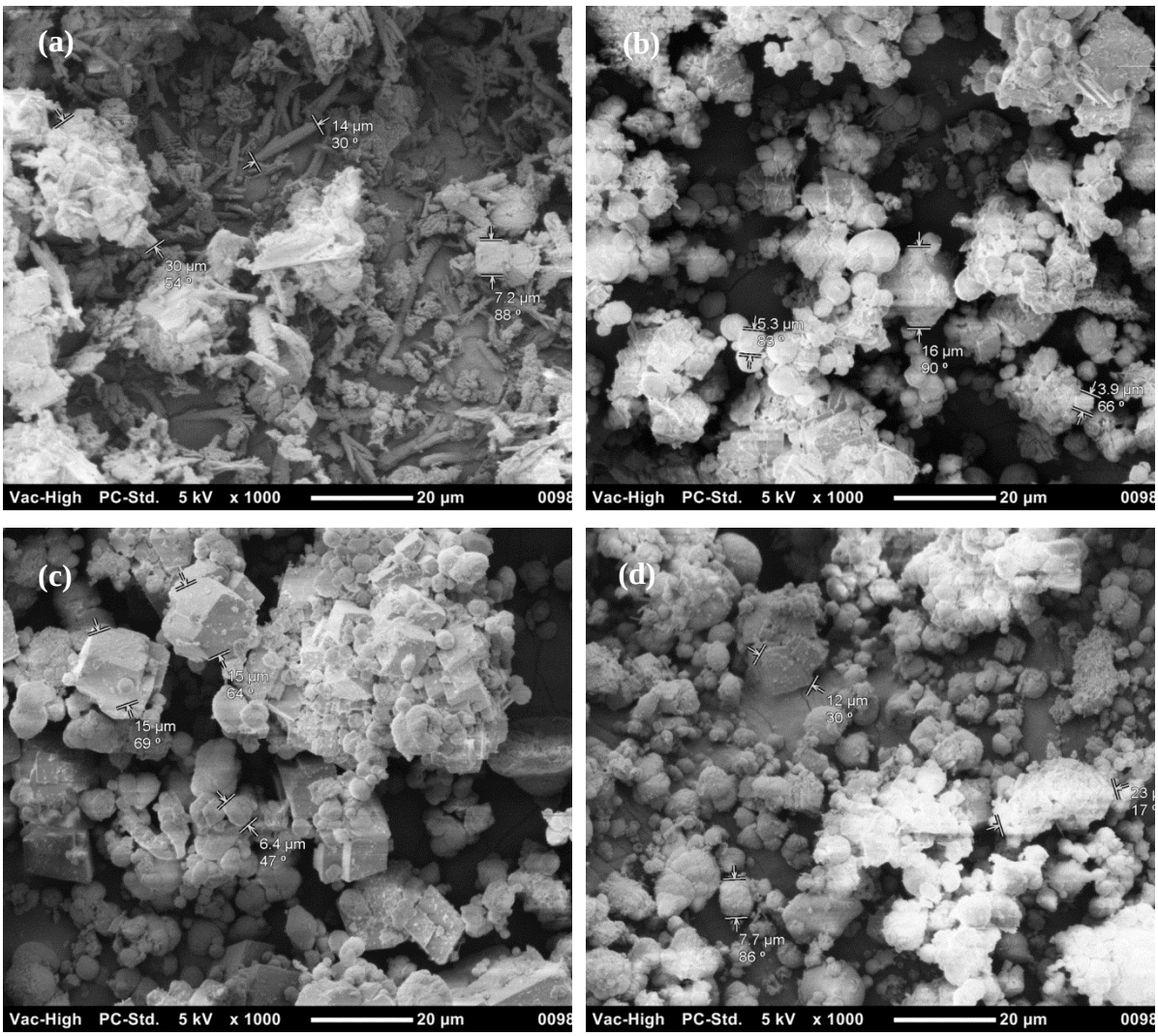

Figure 5.3: SEM images of dried precipitate mixtures

of from un-exposed solutions mixed and then placed in ovens for 30 minutes

(a) Device D PEMF (b) Device D Control (c) Device G PEMF (d) Device G Control.

5.5 Discussion

The results of the above experiments are summarized in Tables 5.1 and 5.2

Table 5.1: Summary of crystal morphology of $CaCO_3$ due to different exposures.

Exposure	Device	PEMF exposure	Controls
Both Na_2CO_3 and $Ca(NO_3)_2$ exposed separately	D	Cuboidal	NDM
	G	Plates	NDM
Only Na_2CO_3 exposed	D	Rods	NDM
	G	Cubes	NDM
Only $Ca(NO_3)_2$ exposed	D	Plates/few rods	Cubes
	G	Plates/rods	Cubes
Reacting mixture was exposed	D	Needles	Cuboidal/Plates
	G	Cuboidal	Cuboidal/Plates

Note: NDM = No Discernible Morphology

Table 5.2: Summary of morphology type promoted by each PEMF device.

Morphology	Device D	Device G
Cuboidal	√	√√
Needles	√	x
Plates	√	√√
Rods	√√	√

The general observations from the data in the above Tables are that:

- Device G appears to promote cuboidal and plate morphology.
- Device D appears to promote a wider range of morphologies including cuboidal, needles, plates and rods.

These diverse outcomes are a function of the different characteristics of the two devices such as different waveforms) and different experimental conditions and highlight the complexity of defining the effects and mechanism of PEMF exposure on various physical, chemical and biological processes. This reinforces our argument for highly controlled and systematic enquiry into this area.

5.6 Some further comments

There are contradictory statements are found in the literature regarding the nature of the scale formed under electric/magnetic/EMF treatment. Some state that rhombohedral 'calcite' is produced; which is softer than needle-like aragonite and believed to be more easily removable (Cho *et al.* 1997; Xiaokai *et al.* 2005; Tijing *et al.* 2009) whereas others state that, under such treatment, 'aragonite' is produced (which is softer than calcite) (Coey and Cass 2000; Knez and Pohar 2005). Using SEM and dynamic light scattering technology Xiaokai *et al.* (2005) analyzed changes occurring in $CaCO_3$ scale, with and without EMF treatment. They reported that without EMF treatment, $CaCO_3$ scaling occurred as dense, sticky aragonite, which was difficult to remove. With EMF treatment, the $CaCO_3$ existed as clusters of small, loosely connected, hexagonal-shaped calcite, which was easy to remove. They also stated that EMF increased crystal collision frequency, which implies that the particle growth was supported mainly by an agglomeration mechanism rather than nucleation growth. This is in agreement

with results reported by Xiao-kai *et al.* (2006) in which EMF technology was found to precipitate crystals in solution as calcite. Coey and Cass (2000) state that the treatment effect persisted for more than 200 hours after the magnetic field was terminated.

However, magnetic treatment has been sometimes proven to be ineffective for retarding scale formation (Lipus *et al.* 2012). Available magnetic water treatment studies can be inconsistent as a result of a number of factors, including use of non-standardized methods, variations in water composition, differences in the course of the treatment (Szkatula *et al.* 2002) and use of different pipe materials, which has been shown to affect the efficiency of magnetic water treatment (Gabrielli *et al.* 2001). Changes in crystal structure may reduce scaling by facilitating deposition rather than adherence but this will be a function of the system design as hydrodynamic shear effects will alter from system to system, as will the presence of biofilms and other causes of scale adhesion. Such an explanation might also elucidate why EMF is able to prevent scaling in some systems but not in all systems.

5.7 Preliminary Electrospray Mass Spectroscopy (EMS) studies of PEMF exposed and un-exposed 'parent' solutions

The experiments described below were also initiated in order to promote further enquiry and prleiminary results have been included in this thesis. Again, time constraints and instrument decommissioning did not allow further deveopment of these ideas in this thesis.

As stated previously, our experiments have demonstrated that PEMF exposure of the 5 mM Na_2CO_3 and $Ca(NO_3)_2$ 'parent' solutions, at least for one device (Device D), has a significant effect on both the precipitation rate (presumably due to particle aggregation) and the crystal

morphology of $CaCO_3$ - following subsequent mixing. Auxiliary studies, *vide supra*, have also shown that variations on PEMF exposure for both devices had a variety of effects on the crystal morphology.

It is our contention that both of these factors (precipitation rate and crystal morphology) are expected to be influenced by nucleation processes that involve aqueous cationic and anionic clustering in solution.

Such clusters may be conveniently detected by the technique of electrospray mass spectrometry (EMS) (Diomides 2005). Therefore, we considered it appropriate to use this technique to examine the cationic clustering profiles of the above parent solutions in the presence and absence of the PEMFs of both devices.

Thus 5 mM Na_2CO_3 and $Ca(NO_3)_2$ aqueous solutions were enclosed in plastic tubes and exposed to each PEMF Device D and G for 10 minutes. 1:10 dilutions were made by mixing in 0.1% formic acid in methanol and injected into the EMS (LX P00211, LCQ Deca XP Mass Spectrometer System, ThermoFinnigan, USA) at room temperature. For controls, the same experiments were repeated without PEMF exposure. Unfortunately, these experiments could not be taken further due to the untimely decommissioning of the instrument. However, some intriguing results did emerge that are considered to be worthwhile for inclusion into this thesis and that suggest further enquiry.

Figures 5.4 – 5.8 clearly demonstrate that exposure to PEMF can cause significant changes to the solution clustering profiles, which is consistent with the observed differences in crystal morphologies and precipitation profiles upon exposure to Device D PEMF. In general, when

the solutions were not exposed to PEMF, larger clusters were apparent. When the solutions were exposed to the PEMF, the larger clusters appeared to break down. Therefore, it can be argued that, if the clusters are pre-cursors to nucleation and crystal growth, then this provides a possible explanation for why there are differences in crystal growth, precipitation rate and morphology. Unfortunately, these results are not conclusive at this stage due to the EMS instrument becoming unavailable, although this is an obvious avenue for future enquiry and will be continued in our laboratories.

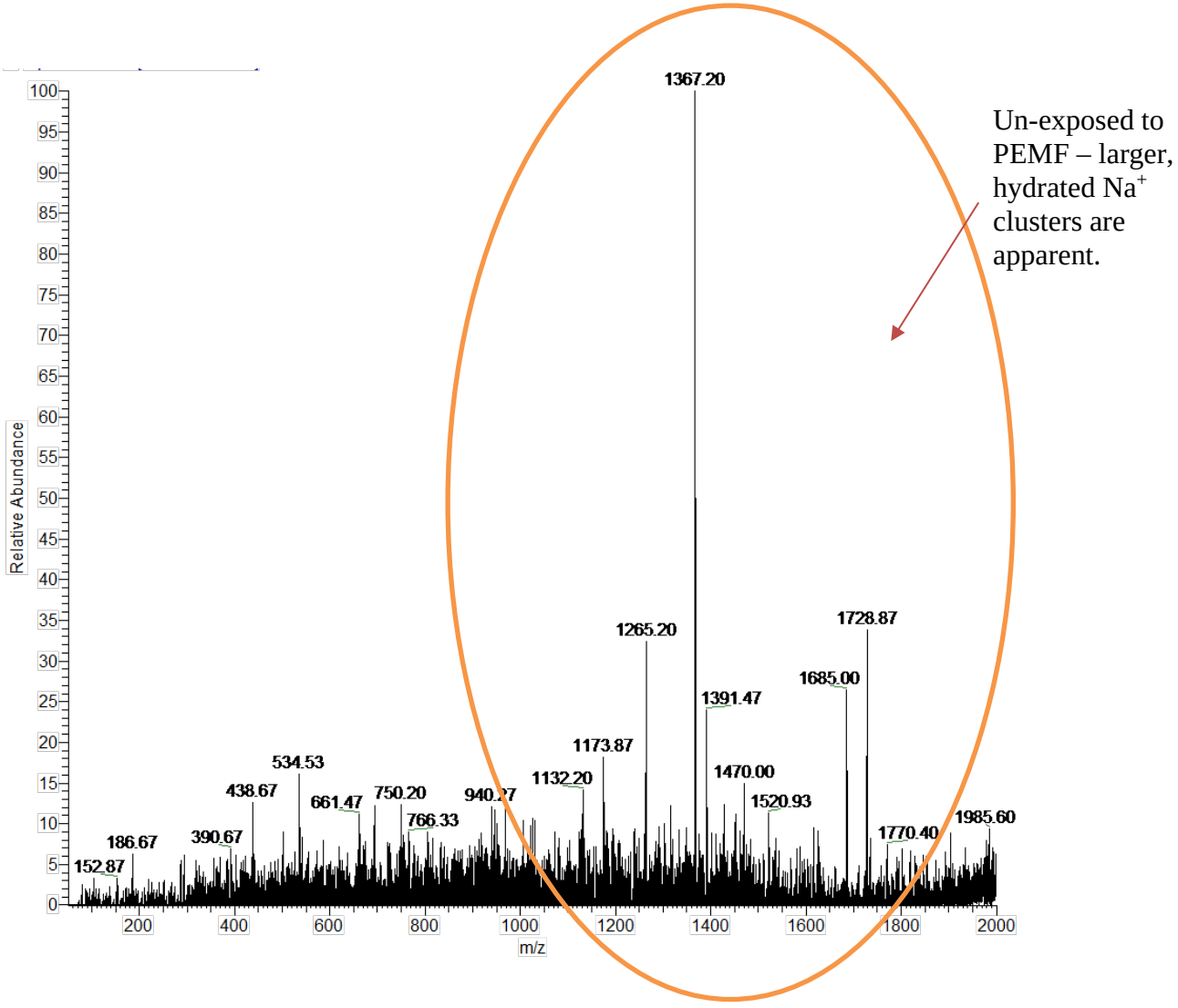

Figure 5.4: Electrospray spectrum of un-exposed Na_2CO_3 solution in positive ion mode.

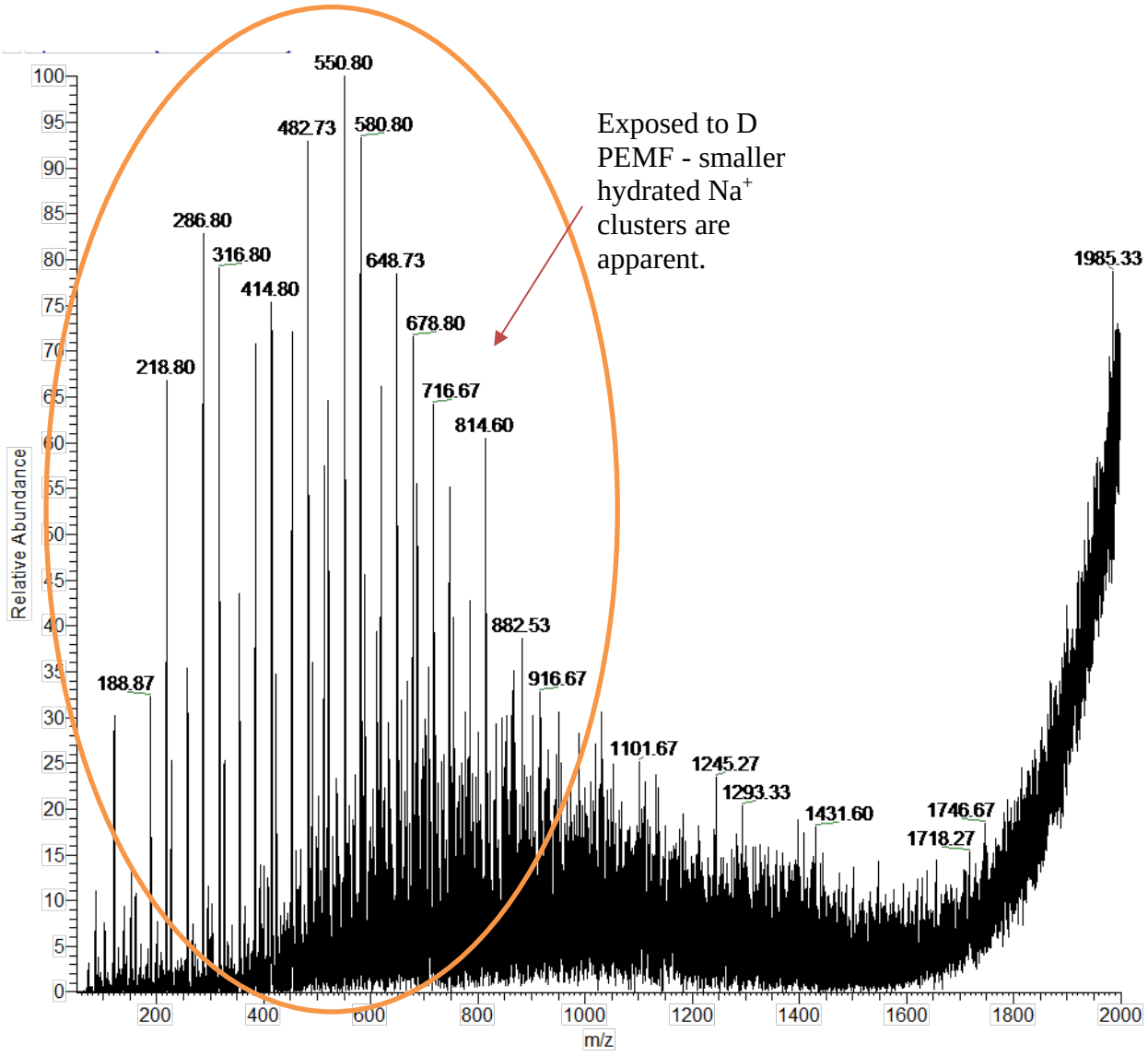

Figure 5.5: Electrospray spectrum of Na_2CO_3 solution exposed to Device D for 10 minutes in positive ion mode. Larger clusters appear to have been broken up by the PEMF, with smaller clusters favoured.

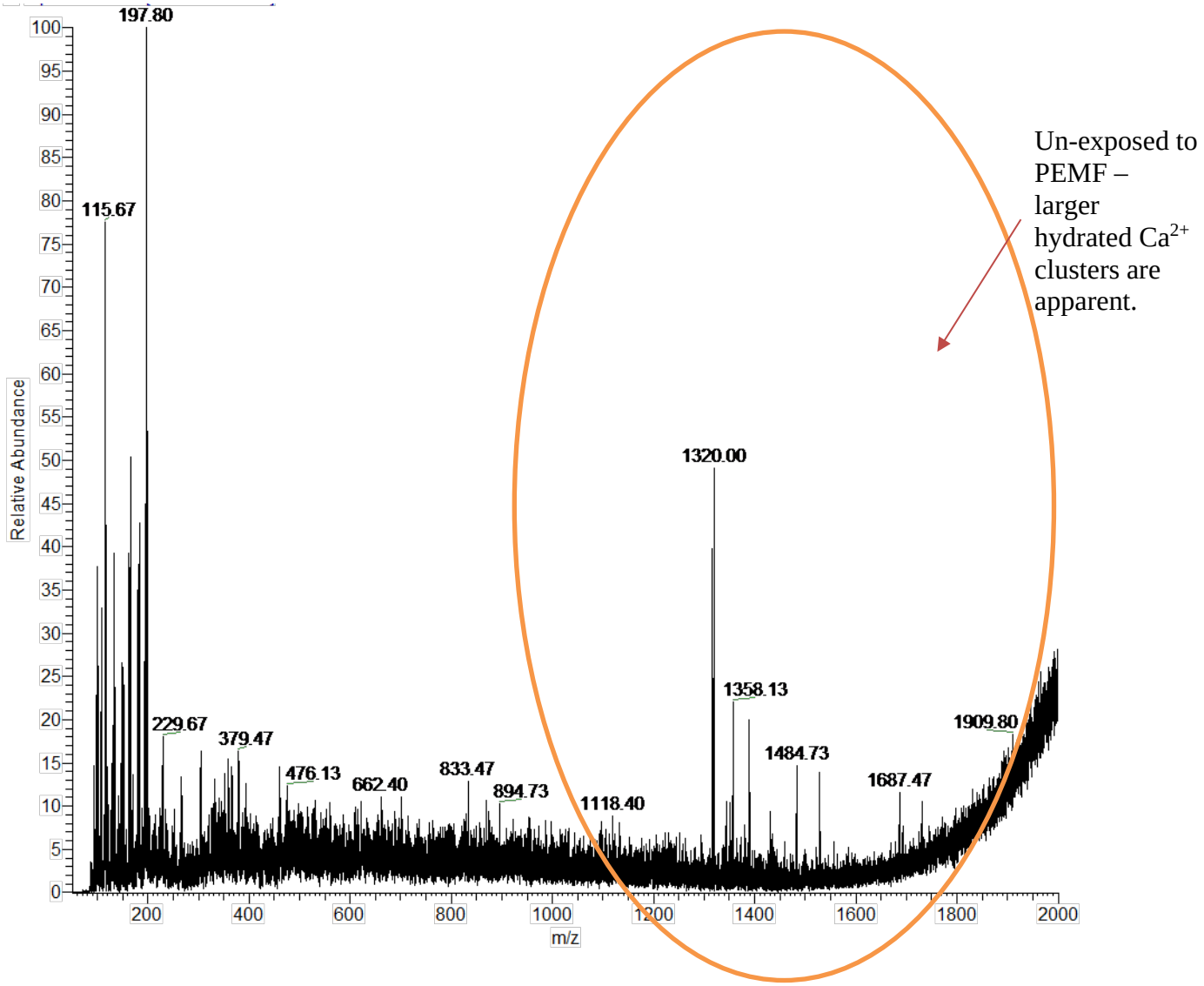

Figure 5.6: Electrospray spectrum of un-exposed $Ca(NO_3)_2$ solution in positive ion mode.

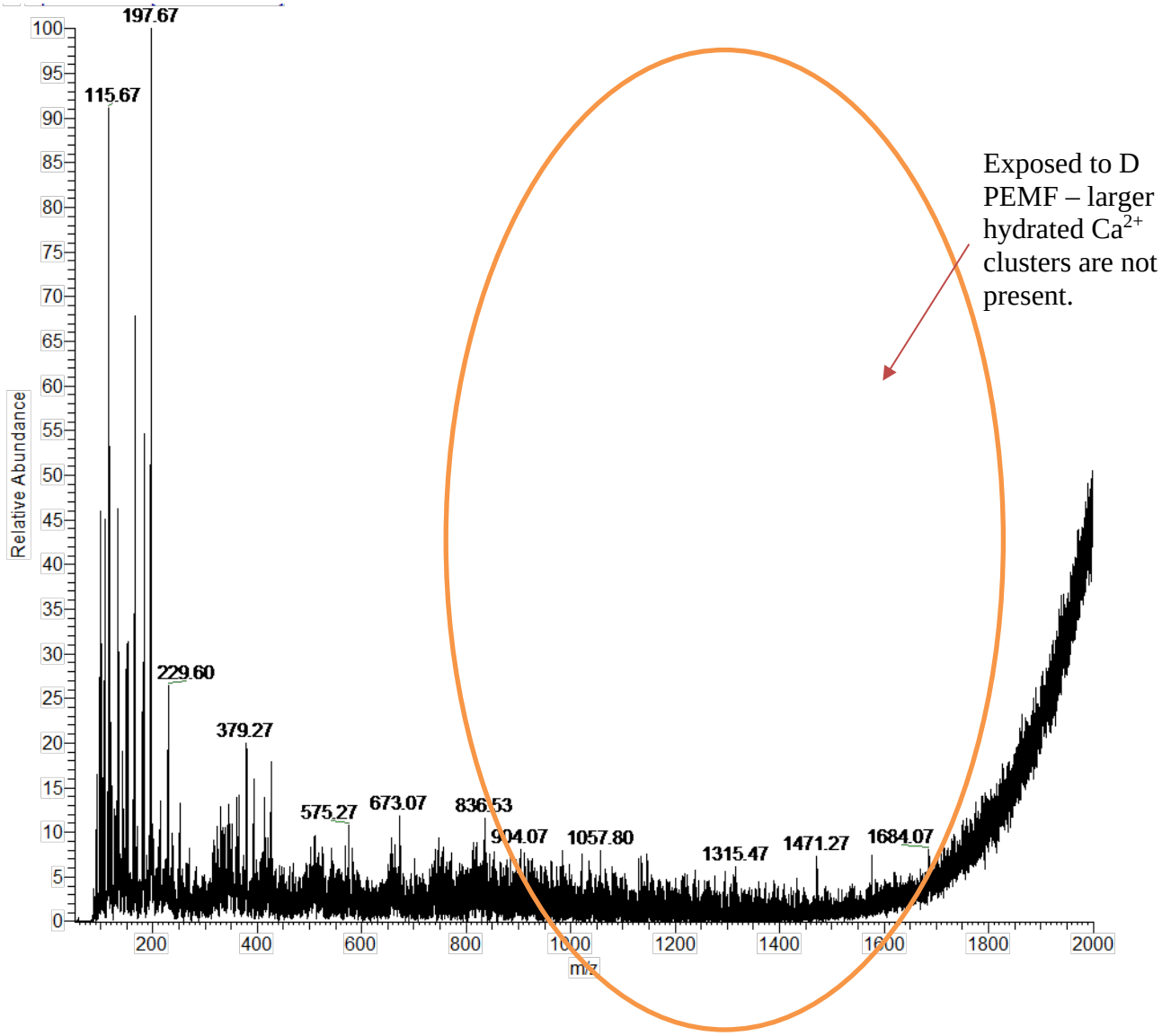

Figure 5.7: Electrospray spectrum of $Ca(NO_3)_2$ solution exposed to Device D for 10 minutes in positive ion mode. Larger clusters appear to have been broken up by the PEMF.

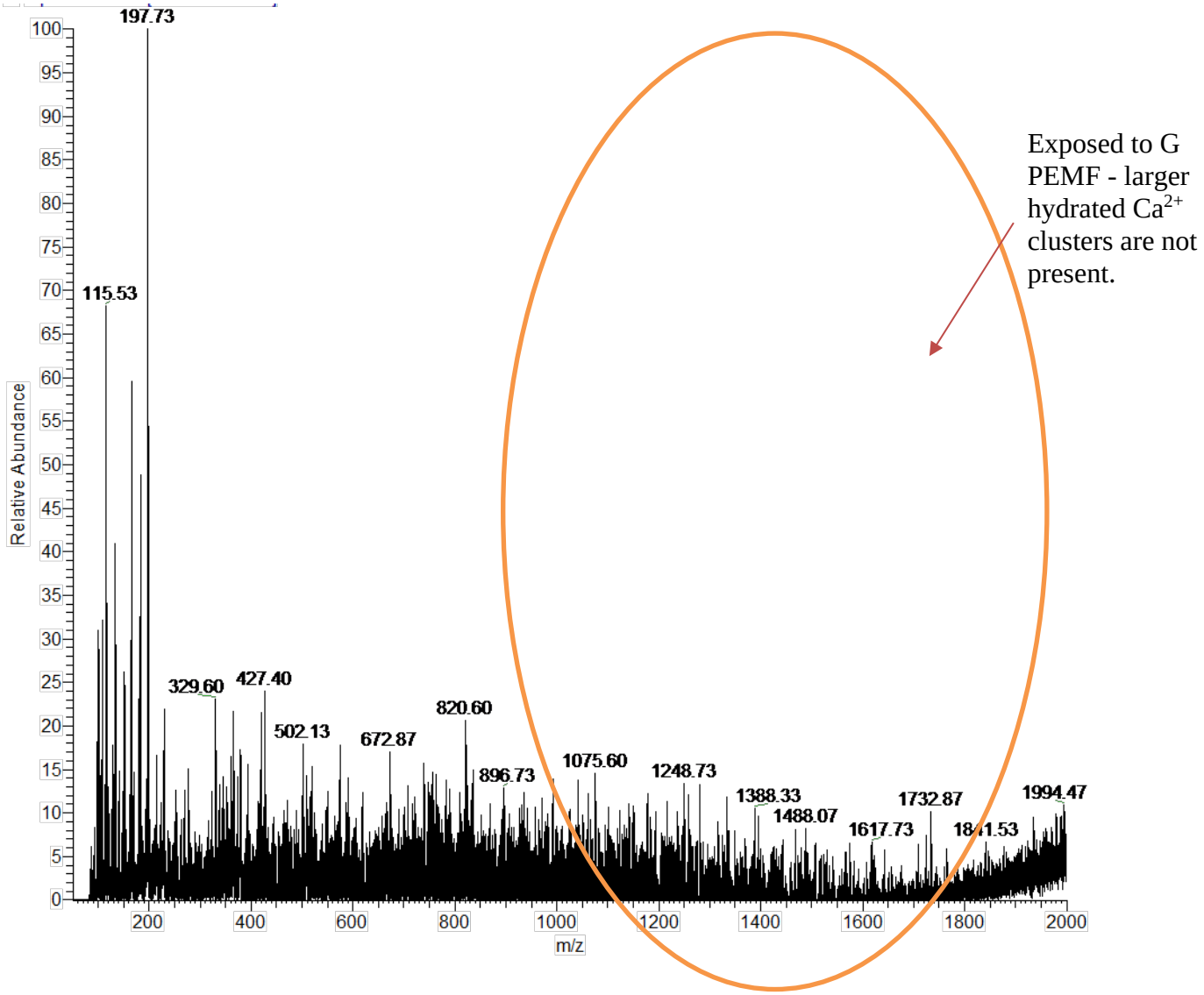

Figure 5.8: Electrospray spectrum of $Ca(NO_3)_2$ solution exposed to Device G for 10 minutes in positive ion mode. Larger clusters appear to have been broken up by the PEMF.

Chapter 6 – Conclusions and recommendations

6.1 General conclusions

This project was undertaken in an attempt to establish a scientific basis for the claims made by the manufacturers of two different commercially available PEMF Devices D and G that, purportedly, control biofilm formation (i.e. are anti-microbial) and/or scaling (i.e. can influence the precipitation and deposition of inorganic material (such as $CaCO_3$) in industrial applications. In the experimental design for the investigation of both of these areas, an emphasis has been placed on strict controls and replicability. No attempts were made to vary the specifications of the devices as supplied to us by the manufacturers themselves.

The project was broadly divided into two parts. Firstly, "Bacterial Studies" has sought to establish whether the generated PEMF from Device D or G has any effect at all (i.e. no effect, inhibitory or stimulatory) on two different kinds of common microorganism (*E. coli and P. pseudomonas*), under both static and flow conditions - and for a range of exposure times. The extent of any observable effect(s) has also been established. Secondly, "Precipitation Studies" has sought to establish whether the generated PEMF from Device D or G has any effect at all on the precipitation characteristics of $CaCO_3$, and if so, to what extent.

These experiments have demonstrated that there are, indeed, measurable effects on bacterial culturability and on $CaCO_3$ precipitation that are induced by PEMFs under different conditions. However, such effects are highly variable and much more complex than might be assumed, being influenced by a wide range of interdependent parameters Some more specific conclusions are as follows:

6.2 Bacterial studies

Static PEMF exposure of 'healthy' and Ag-NP compromised *E. coli* cells: Although relatively minor, the observed growth-inhibition effects for 3 h PEMF-exposed AgNP-treated (i.e. physiologically compromised) cell populations, compared to their non-PEMF controls, were statistically significant for both devices. The observed growth response of healthy *E. coli* cells exposed to PEMF energy for a 7 h 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. These observed growth-inhibitory effects, albeit small, are consistent with the hypothesis that PEMF exposure under controlled conditions may result in a decrease in cellular viability and culturability. However, it is also apparent that under certain conditions bacterial growth is actually *stimulated* by a PEMF. These initial studies prompted more extensive scientific investigations involving an additional microorganism, *P. fluorescence,* and with experiments extended to flow conditions.

Static and flow PEMF exposure of *E. coli* and *P. fluorescence* cells: The outcomes of these "follow-on" experiments supported our initial findings (*vide supra*) and demonstrated that the effects of PEMF exposure (inhibitory or stimulatory) were dependent on a range of characteristics and were consistent with the findings of other researchers whereby negative (growth inhibitory) or positive (growth stimulatory) adaptive responses of different microorganisms, upon exposure to magnetic or electromagnetic fields, are observed under various conditions. Thus, via our 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 type of

microorganism, flow rate and exposure time - and possibly other factors, such as pH and the presence of other chemical species. 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 - as discussed below.

6.3 $CaCO_3$ precipitation studies

Effects of static PEMF on $CaCO_3$ Precipitation profiles: In this study, two commercially available devices that, ostensibly, control scaling in water systems were tested in five-fold replicate, under controlled laboratory conditions, for their respective abilities to influence $CaCO_3$ precipitation. These represented, carefully controlled, proof-of-principle experiments that were conducted to establish a scientific basis for the manufacturers' claims. Thus, the effects of PEMF pre-exposure of 'parent' Na_2CO_3 and $Ca(NO_3)_2 \cdot 4H_2O$ aqueous solutions were investigated with respect to the $CaCO_3$ precipitation profiles and precipitate characteristics, upon subsequent mixing. The precipitation profiles were tracked by turbidity and UV absorbance experiments and the precipitate characteristics (e.g. crystal morphology) were also examined using SEM. $CaCO_3$ precipitation is dependent on a wide range of variables, including the pH. However, apart from ensuring that the temperature remains constant between the experiments and the controls, no attempts were made to measure, adjust or vary any other variables in these experiments, including the pH. Nevertheless, the dependence of pH and various additives will need to be addressed if a focus on more representative 'feed solutions' is to be considered. Therefore, this study sets the stage for such experiments into the future and for the potential optimization of such effects.

Thus, under the conditions of our experiment, one of the commercial devices but not the other was found to have a significant influence on the $CaCO_3$ precipitation profile and was also found to have an effect on the morphology of the crystalline precipitate. We have previously reported that these two devices have comparable frequencies of ~100 kHz but quite different waveforms and it is possible that this could be a reason why one device appears to have an effect but not the other. It is notable that we have also demonstrated that these two devices have quite different effects on microbial culturability (*vide supra*).

The work presented here is supportive of an influence on $CaCO_3$ precipitation for one of the devices and this is broadly consistent with the results of other researchers in this field. However, it is difficult at this stage to say how this translates to the actual control of scaling for specific equipment under operational conditions.

6.4 Recommendations

Up to now, there has been very little scientifically based evidence or fundamental research to support or refute the claims of manufacturers of commercially available PEMF water treatment systems, such as those studied here, with respect to anti-microbial or anti-scaling effects.

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

It is suggested from these studies that the magnitude and complexity of investigating this area of research, as demonstrated in this thesis, 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.

as those conducted here, is required. Given the number of interdependent parameters possible, this will constitute a substantial long-term scientific venture. 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, it is not considered 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. Controlled experiments under such conditions are an obvious avenue for further enquiry, as is more scientific investigation into NCDs in general in regard to proof of principle experiments.

More specifically, the following is recommended for future studies, although these are not exhaustive:

- The design, construction and implementation of a laboratory-based (P)EMF generating device where all specifications are well-defined and parameters such as frequency, waveform, power, intensity etc. may be chosen in a systematic way for various experimentation purposes. Such a device may then be applied to the study of bacterial culturability and scaling under strictly controlled conditions so as to isolate the effect of the individual parameters. Such a project is obviously a huge undertaking but the outcomes of the present project suggests that this is the best way forward.

- More attention needs to be paid to the mechanisms of inhibitory or stimulatory effects on various microorganisms. In this regard, we initiated some experiments on DNA leakage from *E. coli* under the influence of a PEMF (*vide supra*) - this work is continuing (publication in preparation).

- More attention also needs to be paid to the mechanisms whereby exposure to a PEMF influences the crystallization of various inorganic compounds such as $CaCO_3$. Again, experiments in this regard have been initiated (*vide supra*) whereby the effect of a PEMF on ion clusters in aqueous solution has been investigated using electrospray mass spectroscopy. Our preliminary results suggest that such clustering is indeed affected and could well influence nucleation, precipitation characteristics and crystal morphology. This could have wider implications for the control of crystallization in general. This work also needs to be extended to the use of X-Ray Diffraction (XRD) for the precise characterization of the precipitate – rather than just SEM as was used in this project (*vide supra*)

- More complex aqueous solutions need to be studied where the pH, ionic strength and the presence of other solutes such as Natural Organic Matter (NOMs) - including humic/fulvic acids, proteins and polysaccharides, are included so as to better represent "real" feed water.

- The PEMF generating device would require integration with other experimental equipment that would allow a wide range of flow conditions, temperatures and exposure times to be systematically studied. The apparatus designed for the current project has set the stage for such an experimental design, especially with respect to temperature control.

- The PEMF generating device could be 'married' to a membrane test rig whereby, for example, RO membrane systems could be operated with and without PEMF pre-treatment of the feed. Water flux, rejection, temperature and system operating pressures

could be data-logged for a wide range of well-defined PEMF settings and membranes from the PEMF treated and untreated flow trains could be removed at intervals and autopsied to determine if there are differences in the chemical and microbiological makeup of fouling substances that accumulate on the membrane surfaces, and for signs of membrane degradation.

- Studies on bacterial adhesion to surfaces is desirable. For example, a suspension of bacteria could be recirculated in a test loop and at appropriate intervals and the number and distribution of attached bacteria could be quantified by direct microscopy in MFCs. In addition, coupon samples could be removed at intervals from Robbins devices to estimate viable and total bacterial numbers by ATP and DNA analysis. The rate of cellular attachment would be determined from the slopes of each metric as a function of time.

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APPENDICES

Appendices are provided on a CD