UNIVERSITY OF SOUTHERN QUEENSLAND

Ultrasound technology as a pre-treatment for biofouling control in Reverse Osmosis (RO) system

A dissertation submitted by

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Abstract

The scarcity of pristine water resources has been a serious issue worldwide. This issue has resulted in the emergence of alternative water resources represented by seawater and wastewater. To treat these water resources, advanced membrane technologies such as Reverse Osmosis (RO) membrane technology are required. In spite of the capability of the RO technology in desalinating seawater and reclaiming wastewater, the efficiency of this technology is significantly affected by the problem of RO membrane fouling. There are several types of fouling that can occur on RO membrane, biofouling was selected to be the focus of this thesis. In this study, ultrasound technology has been suggested to pre-treat the feedwater of an RO system with the aim of decreasing the formation of biofouling on the membrane.

It has been reported by many studies that the mechanism of deactivating microorganisms through the use of ultrasound lies in the mechanical effects of acoustic cavitation, especially the pressure shock generated from bubble collapse. The intensity of the pressure shock depends on a number of parameters such as ultrasonic intensity, ultrasonic frequency, temperature of the treated sample, pressure of the treated sample and the initial size of the bubble. Therefore, the effect of these parameters on the intensity of collapse pressure of transient bubbles was investigated. A 55 kHz horn type ultrasound batch configuration reactor was used in this study. The experimental ultrasound work, involved sonication, thermosonication and manosonication treatments for *E.coli* deactivation. The effect of different levels of ultrasonic intensity, temperature, pressure and treatment time on the ultrasonic deactivation for *E.coli* suspension was examined in sonication, thermosonication and manosonication experiments.

The experimental results showed that the optimum process parameters of ultrasound treatment for *E. coli* disruption are represented by thermosonication treatment at low sub-lethal temperature of *E. coli* of 45˚C. The feed solution of the RO system was prepared by suspending *E. coli* with a concentration of approximately 4×10^6 CFU/mL in a MacCONKEY broth. A stirred cell UHP 90 with a maximum capacity of 600 ml was used to simulate the RO system in its batch configuration. The efficiency of ultrasound treatment in reducing the formation of biofouling on the RO membrane was assessed based on two criteria: i) the measured amount of permeate flux of the stirred cell and ii) analysis of the developed biofilm on the RO membrane. Straining and epiflourescence microscopy techniques were used in this analysis. The ultrasound treatment was found to be capable of eliminating 10^3 CFU/mL of the existing *E. coli* in the broth-based suspension and resulted in injuring more than 10% of the log survival of *E. coli*. As a consequence, ultrasound was able to recover more than 0.1 $L/m^2.h$ of the permeate flux of the treated feed during fouling treatment lasted for 60 hours. Moreover, the captured microphotographs of the membrane used with untreated and treated feeds revealed that the treated *E. coli* built sparse biofilms on the membrane, while the developed biofilm by untreated *E. coli* covered almost all the membrane area.

The ultrasound and RO experimental results obtained in this study showed that ultrasound technology can be an effective free-chemical method to control the formation of biofouling in a RO membrane system. Further study in optimising the current batch reactor is needed; a flow configuration of the membrane system can be the focus of future work.

CERTIFICATION OF DISSERTATION

I certify that the ideas, experimental work, results, analyses, software and conclusions in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not been previously submitted for any award, except where otherwise acknowledged.

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CHAPTER 1

Introduction and Aims

1.1 Overview

Pristine water resources are becoming scarce worldwide due to population growth, higher standard of living, climate change and an uneven distribution of water resources. Recent studies showed that the estimated number of the people who lack healthy drinking water access in the developing countries has reached around one billion, while the number of the people who lack sufficient water for sanitation has reached two billion (Ridoutt and Pfister, 2010). Therefore, the investigation of non-conventional water resources such as reclaimed wastewater and seawater as potential water resources for different applications has become of utmost importance. The treatment of the emerging non-conventional water resources requires employing advanced water treatment technologies such as Reverse Osmosis (RO) membrane technology (Li et al., 2007; Prihasto et al., 2009; Del Pino and Durham, 1999).

The use of reverse osmosis membrane technology for wastewater reclamation or seawater desalination is hindered by biofouling problems (Chen et al., 2004; Xu et al., 2006). The problem of biofouling in the RO membrane system can be mitigated by applying efficient methods for controlling biofouling (Prihasto et al., 2009). However, the available techniques for controlling biofouling in the RO membrane system have some limitations such as an adverse effect on health public, low efficiency and the deterioration the RO membrane construction materials. Hence, ultrasound technology has been proposed in this thesis to be used as a new anti-

biofouling technique owing to the benign environmental effect of ultrasound (Gogate and Kabadi, 2009) and its capacity to deactivate microorganisms efficiently in various applications.

1.2 Reverse Osmosis and biofouling issues

Reverse Osmosis is defined as the process of pressurizing a solution with different components through a semi-permeable membrane that allows some components such as water to permeate through it while detaining other components such as ions and microorganisms (Kucera, 2010). The term reverse osmosis is given to the process that reverses the natural osmosis process. In the natural osmosis process, the solute (contaminants) should move from the area of high concentration to the area of low concentration to equalize the concentration of the solute at both areas. On contrary, in the reverse osmosis process, the contaminants stay at the area of high concentration owing to the sieving effect of RO membrane leaving the other area almost free from the solute (contaminants).

Fouling is a common term that is used in water treatment and a number of other fields and it refers to the adhesion of undesirable materials to surfaces. Fouling of the RO membrane can cause deterioration in membrane flux, biodegradation of the membrane, an increase in the differential pressure with a consequent increase in the feed pressure, and an increase in the salt passage. This can inevitably result in an increase in the energy requirements with a potential increase in the cost of operation (Khan et al., 2010).

The main foulants of the RO membrane include solid or colloidal particles, organic foulants, inorganic foulants, and microorganisms (Herzberg et al., 2009;

Pontié et al., 2005; Flemming, 2002). The adhesion of microorganisms to the RO membrane is considered as the most serious type of fouling (Lee et al., 2010). During the initial colonization of microorganisms to the membrane surface, the deposited microorganisms secrete Extracellular Polymeric Substance (EPS) forming a matrix of microorganisms and EPS which is commonly called biofilm (Decho, 2000). The subsequent development of biofilm layers on the membrane is known as biofouling (Flemming, 2002).

The EPS in the biofilm matrix is particularly robust structure that can provide the protection to microorganisms form the introduction of biocides and toxin to water (Flemming, 2002). Moreover, the microorganisms present in the matrix of biofilm can seclude organic or inorganic substances from the surrounding environment as nutrients for maintaining their life functions (Decho, 2000; Prihasto et al., 2009). The metabolic activity of the microorganisms in the biofilm matrix may degrade the materials of the membrane (Chua et al., 2003). Given the adverse impacts of biofouling on the performance of RO membrane, efficient techniques are required to control the adhesion of microorganisms to the membrane that can extend the life time of the membrane and reduce the cost associated with cleaning and replacing the membrane.

1.3 Techniques for controlling biofouling in the RO membrane system

The state-of-the-art methods for controlling biofouling in the RO membrane system as reported by Louie et al. (2006), Prihasto et al. (2009), Hori and Matsumoto (2010) and Flemming (2011), include membrane surface modification, biochemical techniques, membrane filtration and disinfection techniques. Some of the techniques

of controlling biofouling can be applied at the stage of membrane manufacture such as membrane surface modification techniques, whereas the other techniques such as biochemical, membrane filtration and disinfection techniques can be implemented in the water treatment scheme.

1.3.1 Membrane surface modification

Membrane surface modification can be defined as the process of improving some of the membrane surface characteristics such as surface roughness, surface charge and membrane hydrophilicity against fouling. There are number of surface modification techniques including modified membrane synthesis, physical coating and grafting (Louie et al., 2006). Grafting is defined as the process of binding monomers onto polymer chain covalently (Bhattacharya and Misra, 2004).

Although, applying membrane surface modification methods can reduce the adhesion of microorganisms to membrane surfaces (Roosjen et al., 2006; Kingshott et al., 2003), these methods have some limitations. For example, microorganisms have the ability to develop suitable adhesion mechanisms for various environmental conditions (Hori and Matsumoto, 2010), which means they are able to overcome the new conditions of the modified surface. Additionally, modifying the characteristics of the membrane surface against microbial adhesion may attract other foulants, such as natural organic matter, to adhere to the membrane (Louie et al., 2006).

1.3.2 Biochemical techniques

The robust structure of biofilm can be degraded by using biochemical substances such as enzymes, bacteriophage and signalling molecules (Flemming, 2011). Bacteriophage can be defined as viruses that infect the bacteria (Mayer, 2010).

Signalling molecules are specific biomolecules that regulate different biological activities for microorganisms such as cell-cell communication activity in the bacterial communities of the biofilm (Davies and Marques, 2009). These techniques are usually applied for removing a formed biofilm on surfaces.

Applying biochemical techniques for removing the established biofilm on a surface is limited by some drawbacks such as high cost associated with producing the biochemical substance and low efficiency in detaching the adhered microorganisms from substrates (Richards and Cloete, 2010; Flemming, 2011).

1.3.3 Membrane filtration techniques

Membrane filtration is the process of removing a wide range of the contaminants including microorganisms from upstream of the RO membrane using types of membranes such as micro-filtration (MF), ultra-filtration (UF) (Zheng et al., 2009) and nano-filtration (NF) membranes (Hilal et al., 2004). The pore size of these types of membranes is in the order NF< UF< MF, and according to their pores' size, they are capable of retaining microorganisms with size bigger than the pores' size. These techniques were found to be effective in reducing the concentration of microorganisms in the feed water of the RO membrane system (Durham et al., 2001; Wolf et al., 2005). However, membrane filtration techniques suffer form the problem of fouling that negatively affect their performance as pre-treatment techniques (Kang et al., 2004; Subramani and Hoek, 2008; Hörsch et al., 2005).

1.3.4 Water disinfection methods

The use of disinfection as pre-treatment for the RO membrane system is an effective way to inhibit the adhesion of microorganisms to the membrane (Hori and

Matsumoto, 2010). Disinfection techniques have the potential to deactivate the microorganisms before the attachment takes place. The water disinfection process includes the use of a wide spectrum of treatments ranging from conventional treatments such as chemical and thermal treatments, to non-conventional treatments such as ultraviolet (UV) light treatments, electrical treatments, mechanical treatments and ultrasound treatments.

Chemical treatments for water disinfection include the addition of chemical germicidal agents to the water that have the potential to deactivate the existing microorganisms in the water. In spite of the advantages of chemical methods, such low cost and the capacity to deactivate a wide range of microorganisms effectively, these techniques have some shortcomings related to Disinfection by-Products (DBPs) production and mutagenic agents, attacking the materials of the RO membrane and mass transfer limitation (Gogate, 2007; Hulsmans et al., 2010; Kim et al., 2009).

Traditional *thermal treatments* of water are regarded as an inefficient choice owing to its high energy requirements. Therefore, utilizing solar energy for water treatment (Davies et al., 2009) has emerged as an alternative to traditional thermal treatment. Solar disinfection is regarded as a low-cost water disinfection technique, nevertheless its efficiency depends on the geographical location and the climate conditions which restrict its feasibility (Davies et al., 2009).

Ultraviolet light treatment is an alternative treatment technique, that widely used for disinfecting potable water, process water and wastewater (Schwartz et al., 2003). In spite of this, the use of UV light as a disinfectant has some limitations such as low performance in the light scattering (Parker and Darby, 1995) and absorbing solutions (Harris et al., 1987).

The use of *electrical treatments* in deactivating microorganisms harnesses the electrical energy to destroy the cell of microorganism (Guyot et al., 2007; Aronsson et al., 2001). The most common electrical techniques for water disinfection are electro-chemical techniques (Kerwick et al., 2005) and Pulsed Electric Field (PEF) (Guyot et al., 2007). The use of electrical techniques for water disinfection has some drawbacks such as producing mutagenic components in the treated suspension (Reyns et al., 2004) and cathode fouling (Zhe et al., 2008).

Mechanical treatments for water disinfection include the methods that use various forms of mechanical energy as a tool for disrupting microorganisms in the water. The common mechanical methods for microorganisms disruption include High Pressure Homogenization (HPH) (Wuytack et al., 2002), Hydrodynamic Cavitation (HC) (Arrojo and Benito, 2008), bead mill (Melendres et al., 1992) and Micro-fluidizer (Geciova et al., 2002) . Although these methods can be exploited to inactivate microorganisms in an environmentally friendly way, the use of these methods for deactivating microorganisms is accompanied by some disadvantages. For example, the application of HC and HPH can cause erosion of the materials of hydraulic equipments which is not desirable from the engineering point of view (Gogate and Kabadi, 2009).

1.4 Ultrasound technology for microorganisms deactivation

The term ultrasound waves implies the application of sound waves with a frequency higher than the upper limit of human hearing (more than 16 kHz) (Gibson et al., 2008). The typical limit for the frequency that is used in ultrasound application ranges between 20 kHz and 500 MHz (Thompson and Doraiswamy, 1999).

Ultrasound technology for microorganisms deactivation can be applied in different ways; sonication, the combination of sonication with pressure (manosonication), temperature (thermosonication) or pressure and temperature (manothermosonication) (Piyasena et al., 2003). The use of ultrasound technology for water disinfection is very valuable application, due to the environmentally friendly effect of ultrasound and its ability to deactivate and disintegrate clusters of the pathogenic microorganisms (Joyce et al., 2003; Gogate and Kabadi, 2009). The potency of ultrasound for disintegrating microorganisms lies in the simultaneous effects of acoustic cavitation.

Acoustic cavitation is defined as the process of generation, growing and subsequent collapse of the bubbles as a response for the passage of ultrasound waves through a liquid body (Vichare et al., 2000; Gogate, 2007). The implosion and the oscillation of bubbles can generate three effects: mechanical, thermal and chemical (Gogate, 2007). Mechanical effects are represented by the generation of high pressure shock (collapse pressure), shear stresses and turbulences that level up as a result of liquid circulation (Gogate and Kabadi, 2009). Thermal effects are evident through the generation of localized high temperature spots, whereas the chemical effects appear as the pyrolysis of water producing free radicals (Gogate and Kabadi, 2009).

Ultrasound technology has been reported as an effective technique for rupturing microorganisms in different applications. Such applications include water disinfection (Mason et al., 2003; Hulsmans et al., 2009), biological material extraction (Gogate and Kabadi, 2009), biodiesel production (Scragg et al., 2003) and improving an aerobic digestion of waste activated sludge (Wang et al., 1999). The problems associated with ultrasound technology such as high cost of operation can

be offset by the attractive traits of ultrasound represented by the effective and chemical-free disruption of microorganisms. However, there is no reported data on the use of ultrasound for controlling biofouling in membrane systems. Thus, the use of ultrasound for reducing biofouling in the RO membrane system was investigated in this thesis.

1.5 Rationale of this study

Biofilm consists of two main components: micro-colonies of microorganisms and water filled channels (Danese et al., 2000). The micro-colonies of the microorganisms consist of microorganisms and EPS. Since EPS is produced by the live microorganisms on the membrane (Hori and Matsumoto, 2010), reducing the number of the live microorganisms in the upstream of the RO membrane, can decrease the formation of biofouling on the membrane. In view of the shortcomings of the common anti-biofouling techniques, ultrasound technology has been proposed in this study as a free chemical pre-treatment technique for reducing the formation of biofouling on the RO membrane.

The deactivation of microorganisms under the effect of ultrasound treatment is attributed to the mechanical, thermal and chemical effects of acoustic cavitation (Hulsmans et al., 2010). However, many studies have pointed out that the mechanical effects of ultrasound play the major role in rupturing microorganisms in ultrasound treatment, while thermal and chemical effects play only supporting role (Mason et al., 2003; Gibson et al., 2008; Hulsmans et al., 2009; Koda et al., 2009; Piyasena et al., 2003). A recent study conducted by Yusaf (2011) showed that, among the mechanical effects of ultrasound, the pressure shock generated from bubbles collapse *(or as it is referred to in this thesis as collapse pressure)* is the most responsible

effect for microorganism disruption in ultrasound treatment. The intensity of the collapse pressure depends on the process parameters of ultrasound treatments such as ultrasonic power, ultrasonic frequency (Gogate and Pandit, 2000), pressure of the treated solution, temperature of the treated solution (Thompson and Doraiswamy, 1999) and initial size of the bubble (Gogate and Pandit, 2000). Therefore, it has become imperative to evaluate the effects of ultrasonic process parameters on the collapse pressure theoretically to identify the optimum process parameters that yield high cavitational effects.

The intensity of collapse pressure is affected by bubble growth (Gogate and Pandit, 2000). The collapse pressure is directly proportional to the maximum radius of the bubble and inversely to the minimum radius of the bubble. The effect of the process parameters of ultrasound treatments on collapse pressure was evaluated through determining the effects of the parameters on bubble growth investigated by the theory.

In addition to the theoretical analysis of the effect of ultrasonic process parameters on the evolution and collapse of bubbles, experimental work that involved using sonication, thermosonication and manosonication for deactivating microorganisms in water was conducted. The experimental study provided information about the effect of the ultrasonic process parameters on the microorganisms disruption. Since, ultrasonic cellular disruption is caused mainly by collapse pressure, the parameters that theoretically result in high collapse pressure should give high cellular disruption of microorganisms. Together, the theoretical and the experimental results of ultrasound treatment were applied to identify the optimum process parameters of ultrasound treatment that was used as an anti-biofouling technique for the RO membrane system.

The efficiency of ultrasound treatment in decreasing the formation of biofouling on the RO membrane was assessed by measuring the permeate fluxes and numerating the adhered *E. coli* on the membrane of the treated and untreated suspensions.

1.6 Objectives

The overall aim of this study is to identify the shortcomings of the common biofouling controlling techniques and investigate the efficiency of ultrasound technology as a new biofouling controlling technique for the RO membrane system. This can be achieved through the following objectives:

1. Review the available anti-biofouling techniques emphasizing the drawbacks associated with using these techniques and referring to the importance of ultrasound technology as a pre-treatment technique for controlling biofouling in the RO membrane system.
2. Theoretically, investigate the effect of the process parameters of ultrasound treatment on collapse pressure.
3. Identify the optimum process parameters of sonication, manosonication and thermosonication in deactivating microorganisms through experimentations.
4. Evaluate the performance of the optimum ultrasound treatment as a pre-treatment technique for reducing the formation of biofouling on the RO membrane.

1.7 Scopes of this study

This thesis focuses on investigating the performance of ultrasound technology in reducing the formation of biofouling on the RO membrane. *Escherichia coli* (*E.coli*) was selected as the sample microorganism in this work owing to its widespread use as an indicator for the microbial contamination in most water resources (Szewzyk et al., 2000) and its ability to adhere and develop biofilm on the RO membrane (Hori and Matsumoto, 2010; Danese et al., 2000). The theoretical part of this study addressed the collapse of a single transient bubble under the effect of different ultrasonic treatment parameters including ultrasonic intensity, frequency, temperature of the treated water, pressure of the treated water and the initial size of the bubble. Ultrasound treatments using sonication, manosonication and thermosonication treatments were used in this study, while manothermosonication treatment was outside the scope of this study. The experiments of ultrasonic deactivation of *E. coli* and RO fouling experiments were conducted using batch configuration system, so flow configuration experiments were outside the scope of this study.

1.8 Outline of the thesis

This thesis has been laid out in six chapters:

Chapter1 gives background of this study along with the objectives, and the scope of this study.

Chapter 2 gives an overview of the RO technology, the problem of biofouling and the reported biofouling controlling techniques in the literature. Extensive details about the mechanisms of the available techniques for controlling biofouling in

mitigating the negative effect of biofouling on the membrane performance and the advantages and disadvantages of each technique are presented.

Chapter 3 introduces and discusses the theoretical analysis of a single transient bubble collapse under the effect of different ultrasonic intensities, frequencies, temperatures, pressures of the treated water and initial bubble radius. A MATLAB routine was developed to study the effect of the aforementioned ultrasonic process parameters on collapse pressure of a single transient bubble in water.

Chapter 4 illustrates the experimental work of using sonication, thermosonication and manosonication treatments for deactivating *E. coli*. Description of the experimental set-ups of sonication, thermosonication and manosonication treatments was demonstrated in this chapter. Optimization of the experimental set-ups was performed prior to conducting the experiments. Additionally, the results of deactivating *E. coli* using sonication, thermosonication and manosonication treatments were presented and discussed.

Chapter 5 provides details about the test procedure of biofouling experiments and the results obtained by using the optimum ultrasound treatment for reducing the formation of biofouling in the RO unit. A control experiment procedure was followed in biofouling experiments, using nano-pure water and MacCONKEY broth as reference feeds in RO unit and treated and untreated suspension of *E. coli* as the comparable feeds.

Chapter 6 presents the conclusions and recommendations for future work.

CHAPTER 2

A review of problems and controlling methods of biofouling

2.1 The aim of the chapter

This chapter provides an overview of RO technology, biofouling problems and the pre-treatment techniques that can be used for controlling biofouling in the RO membrane system. The role of pre-treatment in improving the performance of RO technology is highlighted in this chapter. The mechanisms, influential factors and theories of biofouling are also covered in this review. The major part of this review focuses on presenting the common techniques that can be used to disinfect the feed water of the RO system whilst pointing out the shortcomings associated with these techniques. Detailed information on the use of ultrasound as an alternative disinfection method is concerned in this chapter.

2.2 RO technology

The earliest observation of a semi permeable membrane took place when the French cleric Abbe Nollet discovered the phenomenon of osmosis in 1748 (Cheryan and Munir, 1998; Kucera, 2010). While the first documentation of water desalination using the osmosis phenomenon dates back to 1948 when Dr. Gerald Hassle, a researcher from the University of California Los Angeles (UCLA), used an air film surrounded by two cellophane membranes for water desalination (Glater, 1998). Since 1948, many efforts have been made to improve the performance of RO technology in the water treatment process, a brief summary of these efforts is presented in Table 2.1.

Table 2.1: Timeline for the developments of RO membrane and filter manufacturing (Kucera, 2010)

Timeline	Events
1940-1950	**1948**-Hassler studied osmotic properties of cellophane membranes at University of California, Los Angeles.
1950-1960	**1955**-The first reported use of the term Reverse Osmosis. **1955**-Professer Charles from the University of Florida proposed the use of osmotic membrane for demineralization. **1959**-Berton and Reid at the University of Florida demonstrated the desalination capability of cellulose acetate film.
1960-1970	**1960**-Leob and Sourirajan at (UCLA) developed asymmetric cellulose acetate membrane. **1963**-First practical spiral wound module developed by General Atomics. **1965**-First commercial brackish water facility established at Coalinga, USA. **1965**- Lonsdale et al. (1965) developed the solution-diffusion transport model that describes the transportation of solution through a membrane. **1967**-First commercially successful hollow fibre module developed by Du Pont. **1968**-First multi-leaf spiral wound module developed by Fluid Systems.
1970-1980	**1971**-Richter-Hoehn at DuPont patented the aromatic polyamide membrane. **1972**-Cadotte developed the interfacial composite membrane **1974**-First commercial seawater RO facility established at Bermuda, the British overseas territory.
1990-2000	**1994**-TriSep corporation introduced the low fouling membrane. **1995**-Hydranautics introduced the energy saving polyamide membrane.
2000-2010	**2002**-Koch membrane systems introduced the first 18-inch diameter "Mega Magnum" module. **2006**-The thin-film nano-composite membrane developed at UCLA.

Table 2.1 illustrates the developments in the manufacture of RO membranes and filters in the aim of enhancing the efficiency of RO technology in water treatment. However, the performance of RO technology can also be enhanced by applying adequately pre-treat the upstream of the RO membrane system (Fritzmann et al., 2007).

Many studies were conducted aiming to minimize fouling problems in the RO membrane system and enhance its performance by improving the quality of the RO feed water. Kucera (2010) reported that an efficient RO pre-treatment can improve the permeate flow-rate of the membrane as shown in Figure 2.1. It can be seen from Figure 2.1, that permeates flow rate of the RO system with marginal or inadequate pre-treatment continuously deteriorate with the operating time of the system. In contrast, the permeate flow rate of the RO system with ideal pre-treatment decreases only at the early stage of the operating time, and then settle at a certain level as the operating time increases. Prihasto et al. (2009) reported that pre-treating the feed water of the RO membrane system can maintain the performance of the membrane at a satisfactory level and increase the working lifetime of the membrane.

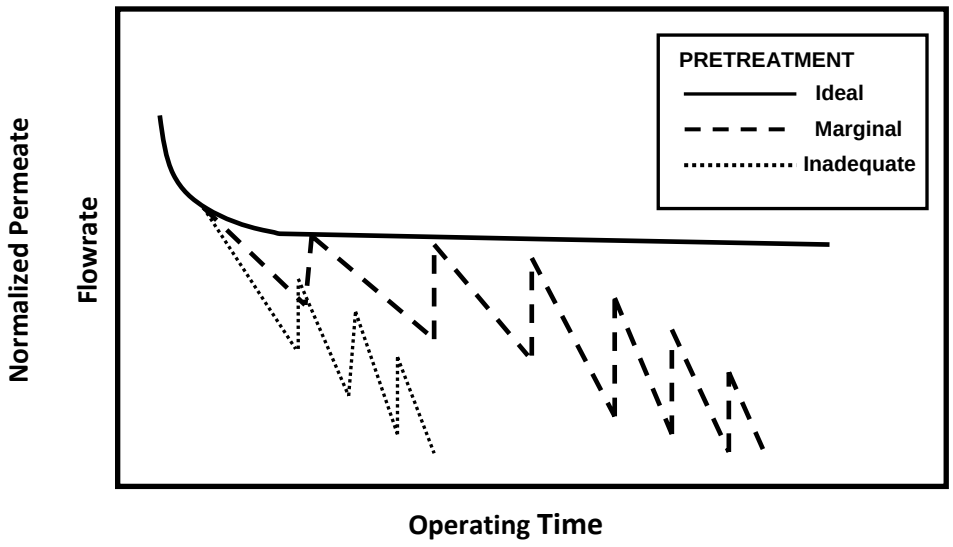


Figure 2.1: Performance of the RO system as a function of the quality of feed water pre-treatment (Kucera, 2010)

The pre-treatment technique of the RO system varies with the characterizations of the contaminants existing in the feed water of the RO system (Greenlee et al., 2009). Since the focus of this study is centred on the microorganisms as the targeted contaminants, it is necessary to understand the mechanisms of biofouling in the RO membrane. Understanding the mechanisms of biofouling promotes the choice of pre-treatment technique to reduce the formation of biofouling on the RO membrane.

2.3 Biofouling problems in the RO membrane system

Biofouling is defined as an accumulation of microorganisms accompanied by agglomeration of extracellular materials on the membrane surface (Hori and Matsumoto, 2010). Biofouling is not confined to the RO membrane, as it can be found in all filtration technologies as shown in Figure 2.2.

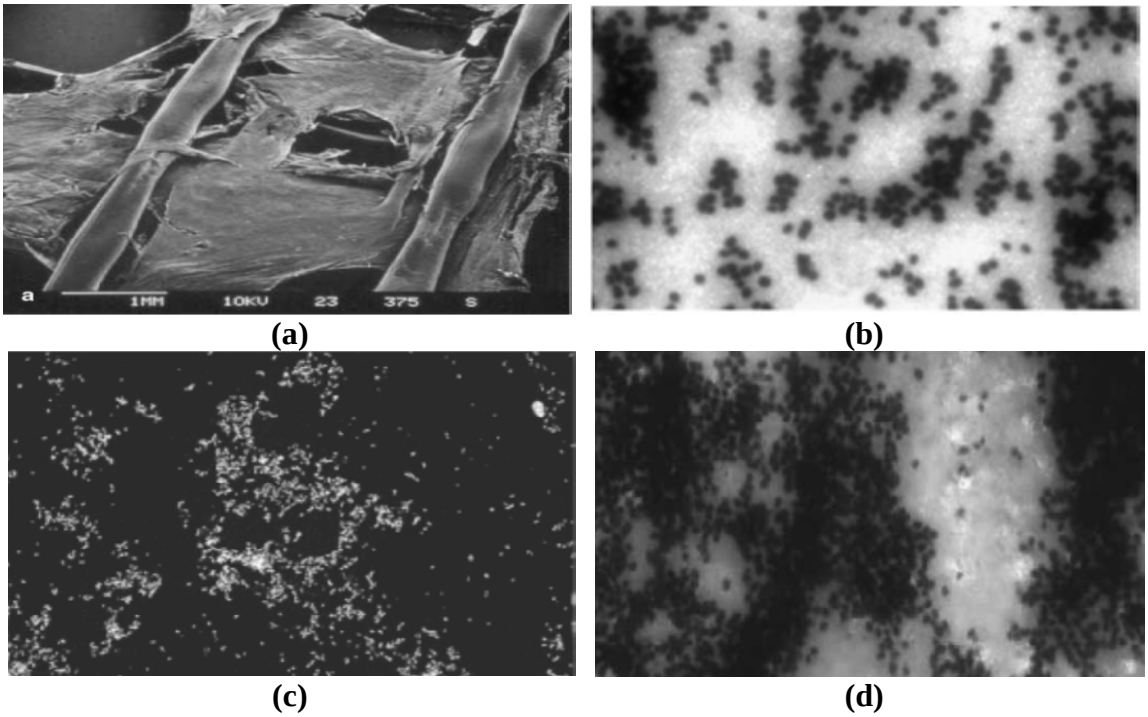


Figure 2.2: Biofouling on a) RO (Flemming, 2002), b) NF(Subramani and Hoek, 2008), c) UF (Hörsch et al., 2005) and d) MF (Kang et al., 2004)

The formation of biofouling is a complicated process and to understand this process, many aspects must be considered:

1) The types of surfaces on which biofouling occur.
2) The driving forces behind microbial adhesion.
3) The theories that describe the interactions between microorganisms and surfaces.
4) The factors that affect the microbial adhesion.

2.3.1 Types of substrate

The surface on which the deposition of microorganisms occurs is called substrate. The adhesion of microorganisms can take place on pristine surfaces or on surfaces covered with conditioning film. Conditioning film is defined as a layer of proteinous contents that covers a surface which provides receptor sites for the microbial adhesion (Gristina, 1987). The mechanisms through which the microbial adhesion develops on these two surfaces are different (Busscher et al., 2010). The microbial adhesion onto pristine surfaces is controlled mainly by the macroscopic properties of the interacting surfaces such as surface charge and surface hydrophilicity, and this type of interaction is called non-specific interaction (Busscher and Weerkamp, 1987; Malte, 1999).

The microbial deposition on surfaces covered with conditioning film could involve the microscopic interaction "*ligand-receptor bond*" which is commonly called the specific interaction (Busscher et al., 2010). Receptor is defined as protein molecules present on the plasma membrane of the cytoplasm of the cell and it receives specific types of signals from the surrounding environment (Cuatrecasas, 1974). The substance that binds with the receptor is called ligand. The locations

where the ligand-receptor binding occurs is called binding sites. In reality, when immersing any surface in contaminated water, the surface will be covered by a conditioning film (Flemming, 2011) and this case is applicable to the RO membrane surface.

2.3.2 Driving forces of the microbial adhesion

The adhesion of microorganisms to membrane systems takes place under the effect of number of driving forces as illustrated in Figure 2.3.

1) *Hydrodynamic forces* resulted from the convective and the diffusive natures of water flow passing an RO membrane. These forces are responsible of bringing the microorganisms and the other contaminants existing in water to the vicinity of the membrane (Brant and Childress, 2002; Kucera, 2010). When the microorganisms get close to the membrane surface, the adhesion of the microorganisms to the membrane will be facilitated by the following three interactions.
2) *The physio-chemical interactions* between the surface of the membrane and microorganisms (non-specific interactions) (Busscher and Weerkamp, 1987). The adhesion of microorganisms to a surface comprises two stages: initial and secondary adhesion. The initial adhesion of microorganisms to a membrane surface is facilitated by the physio-chemical interactions, while the secondary adhesion is mediated by the cohesion between the adhered microorganisms and the suspended microorganisms in the solution (Brant and Childress, 2002). The physio-chemical interactions that occur between the membrane surface and microorganisms include Lifshitz-van der Waals, Lewis acid-base and

electrostatic double layer (Kang et al., 2007). Lifshitz-van der Waals interaction is a long-range interaction, whereas Lewis acid-base and electrostatic double layer interactions are short-range interactions (Busscher and Weerkamp, 1987; Brant and Childress, 2002).

3) *ligand-receptor* interactions between the receptors of the cell membrane and the binding sites of the conditioning film layer such as polarized bonds, charged groups or OH groups (Flemming, 2011)

4) *The adhesive interaction that results from the sticky nature of the EPS and the appendages of some microorganisms* (Hori and Matsumoto, 2010; Flemming, 2011). The appendages and EPS can help the microorganisms to anchor themselves to the membrane surface. The appendages of microorganisms have the ability to remove the water film between the microorganisms and the membrane surface to allow the cells to adhere to the surface directly.

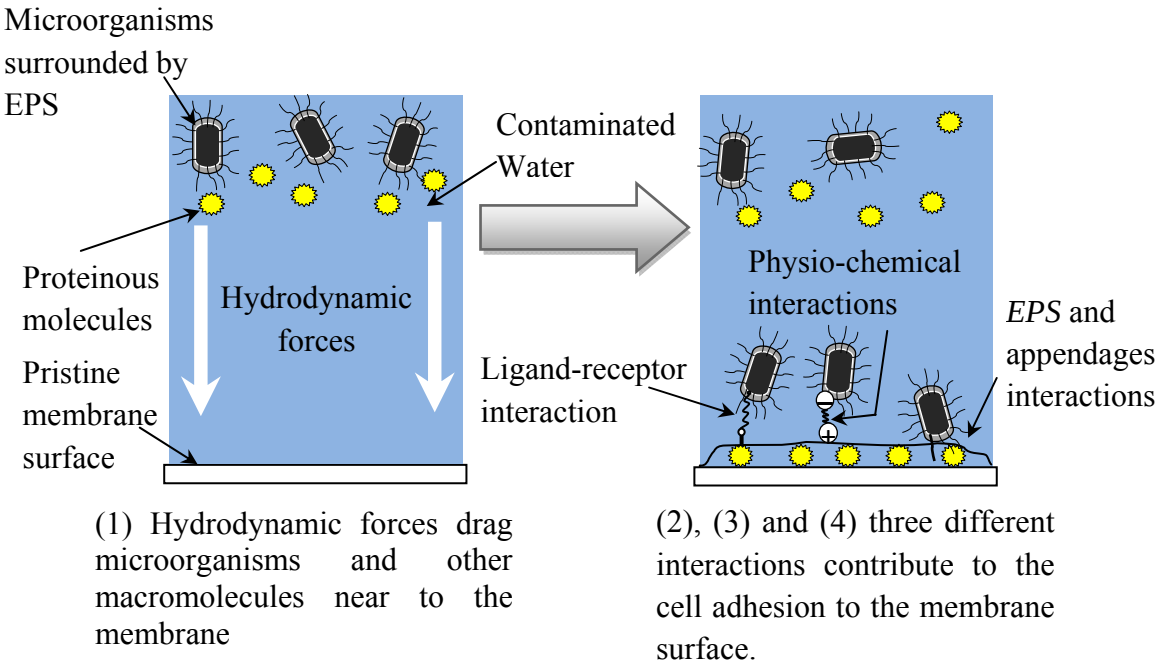


Figure 2.3: Schematic illustrates the driving forces of the microbial adhesion to membrane surface

2.3.3 Theories of microbial adhesion

The theories proposed in the literature (Busscher and Weerkamp, 1987; Van Oss, 1993; Ong et al., 1999; Brant and Childress, 2002; Kang and Choi, 2005; Busscher et al., 2010; Hori and Matsumoto, 2010) to describe the effects of the physio-chemical interactions on the microbial adhesion are the thermodynamic approach, the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory and the extended-DLVO. Hori and Matsumoto (2010) reported that the DLVO theory was originally established to describe the interaction between colloidal particles and surfaces. Because the bacteria size is comparable to the colloidal particles' size, the DLVO theory was applied to predict the occurrence of biofouling on membrane surfaces (Hori and Matsumoto, 2010).

The total free energy for the interacting surfaces in the thermodynamic approach is a combination of the Lifshitz-van der Waals and Lewis acid-base free energies (Busscher and Weerkamp, 1987; Hori and Matsumoto, 2010). In DLVO theory, the total interaction energy is the combination of Lifshitz-van der Waals and electrostatic double layer energies. The DLVO theory was developed by Van Oss (1993) to include the Lewis acid-base interaction in addition to the two interactions involved in the theory to explain the adhesion of colloidal particles or microorganisms to surfaces (Hori and Matsumoto, 2010). These three theories suggest that the adhesion of microorganisms to membrane surface can happen when the total free energy for the interaction (energy of adhesion) between the microorganisms and the membrane surface is negative (the interaction between the membrane surface and microorganisms is attractive). Alternatively, the adhesion is not promoted when the interaction energy is positive (i.e. the interaction is repulsive) (Brant and Childress, 2002).

2.3.4 The influential factors of microbial adhesion

The adhesion of microorganisms to a membrane surface is affected by a number of factors (Busscher and Weerkamp, 1987; Kang and Choi, 2005; Herzberg et al., 2009; Flemming, 2011):

1) Mass transport conditions of the liquid.
2) Ionic strength of the solution.
3) Normality (pH) of the solution.
4) Characteristic of interacting surfaces.
5) Concentration of the nutrients in the bulk solution.
6) The concentration of the microorganisms in the solution.

Mass transport conditions of the liquid play a crucial role in the microbial adhesion, as these conditions can influence the rate at which the microorganisms accumulate at the surface of the membrane (Adamczyk and Van De Ven, 1981). Additionally, mass transport conditions of the flowing liquid can affect the shear forces generated at the membrane surface which in turn can affect the stability of the adhering microorganisms (Busscher and Van Der Mei, 2006). Although maintaining high shear forces in the adjacent area of the membrane can impair the growth of biofilm, high shear forces may result in thinner layer of biofilm on the membrane but mechanically stable (Characklis, 1990).

The change of the *ionic strength* of the solution can alter the electrostatic double layer interaction between the microorganisms and the membrane from attraction to repulsion. Hori and Matsumoto's review (2010) pointed out that decreasing the ionic strength of the solution can lead to an increase in the repulsive

energy of the electrostatic double layers interaction between microorganisms and the membrane surface.

The *normality of the solution (pH)* is another factor that can have an influence on the electrostatic double layer interaction between the membrane and the microorganisms through its effect on the surface charge of the microorganisms' membrane. Brant and Childress (2002) found that the membrane charge changed slightly with the pH change of the solution, as opposed to the change of the colloids' charge which was substantially higher within the same pH rang.

The *characteristics of the interacting surfaces* play an important role in the process of microbial adhesion. Such characteristics include hydrophobicity, hydrophilicity and surface roughness. Hydrophobicity and hydrophilicity of the membrane are the characteristics that determine membrane propensity toward fouling. The hydrophobic membrane prefers to interact with foulants other than water (Gourley et al., 1994). The deposition of microorganisms on membranes can also be affected by the roughness of the membrane. Vrijenhoek et al (2001) found that the roughness of the membrane adversely affected the permeate flux of the membrane. This is because the valley structure of the rough surface forms suitable sites for microorganisms deposition. Additionally, the rough membrane surface gives more surface area as compared to the smooth surface, and this in turn can facilitate the microbial adhesion. Moreover, the roughness of the membrane surface can decrease the Lifshitz-van der Waals and electrostatic double layer interactions of the membrane (Walz, 1998; Brant and Childress, 2002).

The concentration of the nutrients in the bulk solution has a considerable impact on biofilm growth on the membrane surface. The sources of the nutrients in

the water can be esteemed from the existing contaminants in the water or the leached materials from the substratum (Flemming, 2011) which could be synthetic coating materials of the membrane or pipes in the water treatment plant.

The concentration of microorganisms in the solution is considered to be the most important factor in microbial adhesion to membrane surfaces. Koop et al. (1989) found that increasing the concentration of microorganisms in the solution can lead to increase the aggregation rate of microorganisms on surfaces. Fletcher (1977) reported that the increase in the concentration of *marine pseudomonad* onto a polystyrene surface led to increase the number of the cells attached to the polystyrene surface. The concentration of microorganisms in the solution is associated with the concentration of the secretion of microorganisms. Some of these secretions such as EPS, have the capability of fouling the membrane surface and negatively affecting membrane productivity (Herzberg et al., 2009).

2.4 Methods for controlling biofouling–an overview

Many efforts have been made to alleviate the formation of biofouling on RO or other membrane systems. Studies in the field of membrane systems, particularly in biofouling, have resulted in the emergence of number of techniques designed to decrease the deposition of microorganisms and biotic materials on the RO membrane. Generally, the reported techniques in the literature for controlling biofouling in the RO membrane system can be categorised into three main groups:

1) Techniques which are capable of reducing the formation of biofouling on the membrane surface through adjusting the characteristics of the membrane surface in a way that decreases membrane propensity toward biofouling (Kang and Choi, 2005; Hong et al., 2008).

2) Techniques that have the potential to degrade the structure of biofilm (Flemming, 2011; Richards and Cloete, 2010).
3) Techniques that can reduce the formation of biofouling on the RO membrane by removing or deactivating the planktonic microorganisms existing in the upstream of the membrane (Prihasto et al., 2009; Hori and Matsumoto, 2010).

2.4.1 Membrane surface modification techniques

The formation of biofouling on the RO membrane can be mitigated by modifying the properties of the RO membrane surface using several techniques including grafting, physical coating and modified membrane synthesis (Louie et al., 2006). Grafting of the membrane surface can be conducted through number of techniques including photochemical, free radical, radiation, plasma-induced and redox-induced technique (Bhattacharya and Misra, 2004; Louie et al., 2006). The surface modification techniques are applied to improve some of the membrane characteristics that are associated with membrane fouling such as roughness, surface charge and membrane hydrophilicity against fouling.

As explained in the section 2.3.4, the rougher the membrane surface, the higher the rate of microbial adhesion. The roughness of the membrane surface can be smoothed using physical coating with polymers (Louie et al., 2006) or surfactant (Chapman Wilbert et al., 1998). The charge of bacterial surfaces in the aqueous solution is negative (Hori and Matsumoto, 2010). Therefore, altering the charge of the membrane to negative increases the electrostatic repulsion between the membrane and microorganisms, and as a consequence, membrane fouling decreases (Bos et al., 1999). Increasing the hydrophilicity of the membrane can lead to

decrease the attachment of the microorganisms to the membrane, as the hydrophilic membrane favours interacting with water molecules rather than microorganisms (Chapman Wilbert et al., 1998).

Adjusting the characteristics of the RO membrane does not always give positive results. This could be attributed to the complexity of the microbial surface structure, and the microorganisms ability to develop adhesion means under different environmental conditions. Some microorganisms' species possess proteinous appendages such as *pili* and *flagella* that cause deviation in the adhesion behaviour of the microorganisms from the anticipated behaviour by the thermodynamic approach, DLVO or the extended-DLVO (Hori and Matsumoto, 2010). Furthermore, improving the characteristics of the RO membrane against biofouling can promote other types of fouling. Not all foulants present in the water are negatively charged (Louie et al., 2006) and altering the charge of the RO membrane to negative can stimulate the deposition of positively charged foulants onto the membrane. Similarly, enhancing the hydrophilicity of the RO membrane can induce the fouling caused by the hydrophilic components of natural organic matter (Kwon et al., 2004).

Reducing the concentration of microorganisms in the feed solution of the RO membrane can be regarded as the only technique that ensures a consequent decrease in the microbial adhesion to membranes under various conditions. Busscher and Weerkamp (1987) reported that the decrease of the microorganisms concentration in the solution by a factor of ten, led to desorb the adhering bacteria from solid surfaces. Chen and Stewart (2000) stated that biofouling problems can be controlled through either reducing the number of the existing microorganisms in the treated solution or by the removal of EPS from the biofilm layer.

2.4.2 Biochemical techniques

The structure and the architecture of biofilm can be changed in a way that mitigates the negative effects of biofouling on systems by using biochemical materials such as enzymes, bateriophages and signalling molecules (Flemming, 2011).

The enzymatic control of biofilm involves using wide range of enzymes for degrading EPS (Flemming, 2011). The EPS structure consists of exopolysaccharides, proteins, glycoproteins, released nucleic acid, phospholipids and other surfactants (Richards and Cloete, 2010). Kristensen et al. (2008) stated that the most two prominent components of biofilm structure are polysaccharides and proteins, and hence they have been the focus of enzymatic studies for controlling biofouling.

The enzymes that have the capability of destroying the structure of EPS can be categorised into two groups: polysaccharide lyases and hydrolases (Richards and Cloete, 2010). Polysaccharide lyases is defined as a specific class of enzymes (EC 4.2.2.) that have the ability to cleave certain glycosidic bonds in acidic polysaccharide (Linhardt et al., 1987). The polysaccharide lyases cleave the polymer chain of the polysaccharide through the β-elimination mechanisms (Sutherland, 1995). In contrast, the hydrolases can degrade polysaccharide through catalysing the hydrolysis of specific bonds in the polysaccharide chain. Further details on the mechanisms of lyases and hydrolases in degrading the structure of polysaccharide are available in (Richards and Cloete, 2010; Sutherland, 1995).

Proteins are important constitutes of biofilm and they can be decomposed using some degrading enzymes called Proteases. There are two groups of protease: exopeptidases and endopeptidases (Richards and Cloete, 2010). These two protease groups cleave bonds from the protein chain at different locations of the chain.

Exopeptidases cleave bonds close to the ends of the polypeptide chain, while endopeptidases cleave the bonds of the inner peptides from the polypeptide chain (Richards and Cloete, 2010).

The low toxicity and biodegradability of the enzymes make them an attractive solution for biofouling problems (Richards and Cloete, 2010). Nevertheless, the use of enzymes for controlling biofouling is associated with some shortcoming such the inability to reuse them, high cost associated with producing them (Richards and Cloete, 2010), insufficient activity or stability under certain conditions (Sutherland, 1995) and very specific depending on the structure of polysaccharide (Flemming, 2011; Richards and Cloete, 2010; Leroy et al., 2007). Furthermore, some biofilm degrading enzymes could give adverse results when applied for controlling biofouling. For example, Amano protease was found to be serving as a substrate to support bacteria growth (Leroy et al., 2007).

Another way of controlling biofouling using biochemical techniques is by employing bacteriophages or signalling molecules that regulate the development of biofilm (Flemming, 2011). Webb et al. (2003) presented the cell death and lysis of *Pseudomonas aeruginosa* in the micro-colonies of the biofilm using bacteriophages. The death of the cells inside the micro-colonies of the biofilm can lead to disperse the sub-populations of the surviving cells in the biofilm matrix. The cell lysis and dispersion together may result in void formation in the biofilm (Webb et al., 2003). Davies and Marques (2009) proposed the use of *cis*-2-decenoic acid to stimulate the dispersion of the biofilms for number of microorganisms such as *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *S. pyogenes*, *B. subtilis*, *S. aureus* and yeast *Candida albicans*. Davies and Marques (2009) attributed the capability of *cis*-2-decenoic acid for inducing the dispersion response of biofilms to the similarity in structure and

functionality of this acid to the signalling molecules such as diffusible signal factor. The diffusion signal factor is responsible to the cell-cell communication in populations of bacteria and fungi. The use of signalling molecules for controlling the formation of biofilm cannot act on bacteria in viable but non-culturable state, while large portion of the bacterial population in the biofilm is in this state (Flemming, 2011). Therefore, the use of signalling molecules in reducing the formation of biofilms seems to be an unpractical technique for controlling biofilm.

2.4.3 Membrane filtration techniques

The use of pressure driven membranes such as micro-filtration (MF), ultra-filtration (UF) and nano-filtration (NF) prior to the RO membrane installation can reduce the concentration of microorganisms in the RO feed water as reported by Sadr Ghayeni et al. (1998) and Hilal et al. (2004). Low concentrations of microorganisms present in the RO feed can reduce the chance of microbial adhesion to the membrane. The pores' size and the required differential pressure (the applied pressure to push the water through the membrane) for MF, UF and NF membranes are different from one membrane to another as illustrated in Figure 2.4.

The use of membrane filtration pre-treatments is available in different forms such as the use of MF, UF or NF alone (Sadr Ghayeni et al., 1998), the combination of any of the filtration membrane with granular media filter (Zheng et al., 2009) or the conjunction of MF, UF and NF (Yu et al., 2010). The mechanism of membrane filtration technology such as NF in detaining microorganisms is a combination of two effects: the effect of physio-chemical interactions between the membrane and microorganisms and the sieving effect (Kosutic and Kunst, 2002; Van Der Bruggen et al., 1999). The microorganisms that have size bigger than the pore size of the

membrane are retained, and in a similar way, the membrane that is negatively charged retain the microorganisms through the repelling force (elucidated in section 2.4.1). In addition to the above mentioned effects, membrane filtration technology has the potential to inhibit the formation of biofouling through decreasing the nutrients required for biofilm development (Wolf et al., 2005)

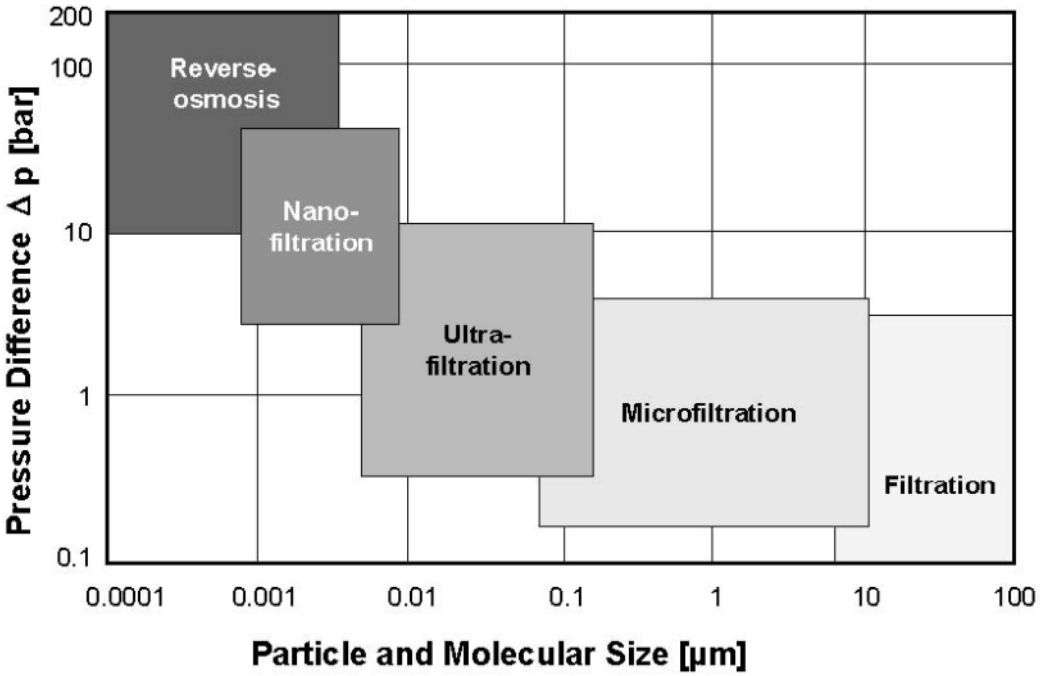


Figure 2.4: The separation capability of MF, UF, NF and RO with the corresponding differential pressures as reported by (Fritzmann et al., 2007)

The implementation of membrane filtrations as pre-treatment methods reduces the concentration of microorganisms in the upstream of the RO membrane (Sadr Ghayeni et al., 1998). However, the efficiency of filtration pre-treatment processes can deteriorate significantly due to the problems of fouling (Decarolis et al., 2001) and biofouling (Subramani and Hoek, 2008). Thus, alternative techniques for controlling biofouling are required.

2.5 Disinfection methods for controlling biofouling

The techniques of microorganisms deactivation in water vary from basic thermal treatments to chemical treatments, and more recently, physical treatments such as UV light, electrical, mechanical and ultrasound techniques. These techniques have different mechanisms through which they deactivate microorganisms. Some of these techniques have the potential to deactivate microorganisms through chemical effects, while others harness the physical effects or the combination of chemical and physical effects to deactivate the microbial cell. Disinfection techniques have their advantages and disadvantages, which will be reviewed in the following sub-sections.

2.5.1 Chemical techniques

The use of chemical methods for deactivating microorganisms was discovered when the German physician Robert Koch observed the bactericidal effect of chlorination in 1881 (Arrojo et al., 2008). Chemical methods for water disinfection involve adding various strong chemical oxidants to the water. These have the potential to pervade microorganisms and cause damage to the cells. The most popular disinfecting chemical agents include chlorine, chloramines, chlorine dioxide and ozone (Richardson et al., 2000; Oppenländer, 2002).

The chlorination process involves adding chlorine gas (Cl_2) or sodium hypochlorite (NaOCl) as a chlorine precursor to the contaminated water (Kim et al., 2009). When chlorine is added to the water, it immediately hydrolyses forming HOCl and Hydrochloric acids as shown in equation 2.1. The Hypochlorous acid (HOCl) is not stable; therefore it decomposes into OCl^- and H^+, equation 2.2 (Fritzmann et al., 2007).

$$Cl_2 + H_2O \longleftrightarrow HOCl + HCl \quad (2.1)$$

$$HOCl \longleftrightarrow H^+ + OCl^- \quad (2.2)$$

Both HOCl and OCl^- are strong oxidative agents, but the latter is considered weaker because it cannot penetrate the microorganisms that are negatively charged. In spite of the destructive effects of these oxidative agents on microorganisms, they have some disadvantages that can negatively affect the material of construction for the RO membrane and the quality of the treated water.

The use of free chlorine for water disinfection is associated with some advantages and disadvantages. Chlorine has the advantages of leaving residual effect that prevent the re-growth of microorganisms in the distribution system, and being effective in deactivating bacteria and viruses(Hand et al., 2010). However, the effectiveness of chlorine in deactivating protozoa and endospores ranges between poor to fair (Hand et al., 2010). The strong oxidative effect of chlorine may deteriorate the RO membrane (Kang et al., 2007). The use of chlorination is accompanied by the formation of carcinogenic Disinfection by-Products (DBPs) such as Trihalomethanes (THMs) and Halo acetic acid (HAAs) (Yang et al., 2007).

Richardson (2003) reported that the risk of DBPs on the public health lies in three main points:

1) More than a half of the formed DBPs in chlorinated water have not been identified yet, as the unknown organic halogen forms about 62.4% of the total DBPs of chlorine.
2) Chemical and toxicological studies are limited to identifying the effects of individual DBPs, while in reality humans are exposed to mixture of DBPs.

3) The ability of DBPs compounds to enter human body not just by ingestion (drinking water) but mostly by dermal absorption (showering and bathing).

More details on the DBPs are given in Appendix A.

In order to reduce the formation of DBPs in the treated water, chlorine may be replaced by chloramines. Chloramines are less reactive and thus it is more stable than free chlorine especially at high pH (i.e. pH more than 8.5) (Applegate et al., 1989). Chloramines are produced as a result of the reaction of aqueous ammonia with free chlorine (Kim et al., 2009):

$$HOCl + NH_3 \longrightarrow NH_2Cl + H_2O, \text{ pH} \geq 8.5 \quad (2.3)$$

$$HOCl + NH_3 \longrightarrow NHCl_2 + 2H2O, \text{ pH} \approx 4.5 \quad (2.4)$$

$$HOCl + NH_3 \longrightarrow NCl_3 + 3H_2O \quad (2.5)$$

Monochloramine (NH_2Cl) is more commonly used compared to di- and tri-chloramines because of its stability. However, this compound needs to be maintained at high pH otherwise it converts to di-chloramine and tri-chloramine.

The advantages of Mono-chlorine are represented by leaving residuals effects and high efficiency in deactivating bacteria (Hand et al., 2010). Despite the advantages of Monochloramine as disinfectant, it is application for water disinfection it restricted by some shortcomings such as low efficiency in deactivating viruses, protozoa and endospores (Hand et al., 2010), and causing damage to the RO membrane (Sorlini and Collivignarelli, 2005; Kang et al., 2007).

Another oxidative agent that has been used broadly for disinfection purposes is chlorine dioxide. This compound has captured the attention of industries and researchers because of its mild corrosive effects (Tanaka et al., 1994) and production

of fewer DBPs as compared to the aforementioned oxidative compounds. Chlorine dioxide is efficient in deactivating bacteria, viruses and protozoa, however its potency is less with endospores. Chlorine dioxide is produced from the reaction between sodium chlorite $NaClO_2$ and chlorine Cl_2 or hydrochloric acid HCl as given in equations 2.6 and 2.7 (Simonet and Gantzer, 2006):

$$2NaClO_2 + Cl_2 \longrightarrow 2ClO_2 + 2NaCl \quad (2.6)$$

$$5NaClO_2 + 4HCl \longrightarrow 4ClO_2 + 5NaCl + 2H_2O \quad (2.7)$$

Chlorine dioxide produces less DPBs as compared to chlorine (Petrucci and Rosellini, 2005), nonetheless, it produces Chlorite as a by-product which is mildly toxic to animals (Svecevičius et al., 2005). Furthermore, chlorine dioxide has lower limit of residuals compared to chlorine and mono-chlorine; about 0.8mg/L (Hand et al., 2010). Because of the possible adverse effects of chlorine compounds, other chemical techniques have emerged to substitute chlorination.

The use of ozone as an alternative disinfectant has received acceptance because of its powerful oxidation effects. Ozone is effective in deactivating bacteria, viruses, protozoa and endospores. Unlike chlorination, the use of ozone in water disinfection does not leave any residuals in finished water. Ozone decomposes spontaneously during water treatment forming hydroxyl free radicals OH^- (Hoigné and Bader, 1983). Hydroxyl free radicals are considered to be one of the most effective oxidizing agents in water and have the capacity to destroy microorganisms (Kim et al., 2003). Hydroxyl free radicals can recombine with each other producing hydrogen peroxide (H_2O_2) which is a strong oxidant itself (Al Bsoul et al., 2010). These two oxidants can attack and penetrate the cell wall of the microorganisms causing disorder in the cell physiology and thus resulting in cell death.

Ozonation has some disadvantages such as the instability of ozone which requires on-site production and the generation of mutagenic and carcinogenic agents such as bromide in the treated water (Richardson, 2003).

In spite of the advantages of chemical methods that are represented by the strong bactericidal effects, leaving residula and cost-effectiveness (Arrojo et al., 2008), chemical techniques have disadvantages that can reduce the value of these techniques. Such disadvantages include the production of carcinogenic agents and DBPs, transport and storage risks and membrane damage.

2.5.2 Thermal techniques

Traditional thermal treatments are used for deactivating microorganisms by applying two types of heating techniques: autoclave and ohmic heating. Autoclaving is a process of heating a suspension of microorganisms using a heat transfer medium (vapour) under specific pressure. Ohmic heating is defined as the process of passing electrical current through a suspension of microorganisms that can generate heat within the suspended cells in the medium due to the electrical resistance of the microorganisms (De Alwis and Fryer, 1992).

Traditional thermal treatments for water are regarded as an uneconomic choice owing to the large amount of energy that is required to heat the water up to the desirable temperatures. Zhang et al. (2007) reported that the lethal temperature for the vegetative bacteria ranges between 60 to 100°C. Thermal techniques used in water treatment should be repairable, low cost and inexpensive to construct (Davies et al., 2009). Thus, harnessing the abundant solar energy for water disinfection purposes has been suggested as an alternative thermal technique for water

disinfection, especially in the developing countries (Navntoft et al., 2008; Gill and Price, 2010; Kehoe et al., 2001).

The deactivation of microorganisms by solar disinfection techniques can be implemented by subjecting the water to the sunlight (Gill and Price, 2010). The first use of the sunlight for disinfection purposes was developed by Downes and Blunt in 1877. They discovered the bactericidal effect of sunlight on bacteria growing in unboiled Pasteur's solution (Downes and Blunt, 1877). Navntoft et al. (2008) investigated the effect of sunlight on deactivation of *E. coli* suspended in medium placed in PET bottles and borosilicate glass tubes. The suspension was exposed to cloudy and cloudless solar conditions. The concentration of *E. coli* in the PET bottles and the glass tubes was reduced by 10^6 CFU/mL when they exposed to sunny conditions (35 ± 5°C) for 180 and 120 hours respectively. However, the reduction in the concentration of *E. coli* in glass tubes was about 10^3 CFU/mL when exposed to cloudy conditions.

There are two main synergistic effects of solar treatment on microorganisms: the effect of ultraviolet radiation presented in the sunlight and thermal effect. Wegelin et al. (1994) reported that, increasing the temperature of the water to 50°C and above, can increase the deactivation rate of solar technique remarkably, while the increase of temperature from 20-50°C can cause a steady increase in the deactivation rate of viruses. This can be ascribed to the adverse effect of the heat rise on the microorganisms susceptibility to the treatment. Hurst (1984) reported that heating *E.coli* K12 to 48°C can result in a 20% loss of the *lipopolysaccharide* (LPS) layer. This layer constitutes the outer leaflet of the gram-negative cell membrane (Helander et al., 1997) and losing it may impair the resistance of microorganisms to

the synergistic treatment (Condón et al., 2005). The other bactericidal effect of solar disinfection is represented by the effect of UV presented in sunlight on microorganisms (Wegelin et al., 1994). This will be discussed in the UV section.

Solar disinfection offers a solution for the problem of drinking water scarcity in some developing countries (Gill and Price, 2010). However, this technique has some disadvantages that limit its application. The efficiency of solar disinfection depends on many parameters such as latitude, altitude, the intensity of radiation, and the proportion of UV to visible light (Davies et al., 2009). Moreover, this technique has no residual effects that prevent the re-growth of microorganisms in the treated water.

2.5.3 Ultraviolet light (UV) techniques

The use of UV light technique for water disinfection has gained considerable attention since the late 1990s when Bukhari et al. (1999) demonstrated the capability of medium pressure UV light for deactivating *Cryptosporidium*, as this specie of microorganisms is known for its resistance to the conventional chemical treatment (Clancy et al., 2000). The spectrum of UV light consists of four regions with different wavelength: vacuum UV (100~200nm) (VUV), UV-C (200~280nm), UV-B (280~315nm) and UV-A (315~400nm). VUV is not effective in deactivating microorganisms for two reasons:

1) The rapid dissipation of VUV in water (Choi and Choi, 2010).
2) The degradation of organic carbon in the presence of VUV (Thomson et al., 2004), which makes the organic carbons easy to consume by the microorganisms.

The bactericidal activity of UV resulted from the destructive effects of UV-B and UV-C on the microorganisms. The hydroxyl radicals OH^- are photolyzed from the

hydrogen peroxide H_2O_2 in the range of UV-C wavelength spectrum and these radicals are able to cause damage to microorganisms (Parsons, 2004). This process was also confirmed by Cho et al. (2004) who found that there was a linear correlation between the inactivation rate *E coli* and the concentration of the generated OH^- radical in the UV/TiO_2 disinfection process. Similarly, Paleologou et al. (2007) reported that OH^- can attack the polyunsaturated phospholipids components of the membranes' lipid causing disorder in the membrane integrity. The germicidal effects of UV light are not confined to the destructive effect of the UV-induced free radicals on microorganisms' membranes, there are other reported detrimental effects of UV on the Deoxyribonucleic acid (DNA) of the microorganisms. The effects of UV light on the DNA of microorganisms include photodimerization of pyrimidine bases, photohydratization of cytosine, and some inter- and intra-strand-specific cross links (Schwartz et al., 2003)

In addition to the ability of UV light in deactivating microorganisms, UV treatment can be used also for deactivating spores. Armstrong et al. (2006) used UV light to deactivate *B. Cereus* spores. They explained that the sporicidal effect of UV light is attributed to the detrimental effect of UV light on Thymine in the DNA strand. The effect of UV on Thymine can result in the production of spore photo-product which has a lethal effect on the spore. However, microorganisms which are situated in cluster form, can protect the cells in the inner side of the agglomeration from the germicidal effects of UV treatment. It was found that the endospores of *B. Subtilise* are 50 times more resistant to UV effects than the vegetative cell due to the presence of α/β-type small acid-soluble proteins that saturate the DNA of the spore (Setlow, 1992).

The sensitivity of microorganisms to UV treatment is influenced by many factors such as the growth medium, the stain of microorganisms and the culture stage (Harris et al., 1987). However, the performance of UV as a disinfectant is affected by many parameters including wavelength, UV dose, time of exposure and the way in which UV light is distributed in the medium. Increasing the time of exposure to UV and the UV doses, can increase the reduction of microorganisms (Armstrong et al., 2006; Paleologou et al., 2007). Adding to these factors, the efficacy of UV light in the contaminated water is susceptible to the presence of turbidity, ferric ions and humic acid where the germicidal activity of UV technique is inversely proportional to the increase in the concentration of the aforementioned contaminants in water (Lu et al., 2008).

Despite of the positive aspects of UV light such as low cost, easy to implement and the ability to deactivate *Cryptosporidium* (Clancy et al., 2000; Choi and Choi, 2010), UV techniques have some shortcomings that reduce its importance as an alternative disinfection method. Such shortcomings include causing mutagenic activity, low performance in light scattering water (Gómez et al., 2006), and breaking down the large molecules of natural organic matter into organic acids with low molecular weight. Converting natural organic matter into low molecular weight compounds can promote a further growth of microorganisms and subsequent formation of biofilm in water distribution systems (Choi and Choi, 2010). The other disadvantage of UV disinfection is the capacity of the treated microorganisms to self-repair (Sommer et al., 2000). The mechanisms of the DNA repair or the treated microorganisms are two types: dark repair which is induced by excision enzymes and photoreactivation which is induced by photolyase enzymes (Giese and Darby, 2000; Sommer et al., 2000). Furthermore, the potency of UV in deactivating protozoa is

excellent but it is less efficient in inactivating bacteria, viruses and endospores (Harris et al., 1987; Hand et al., 2010).

2.5.4 Electrical techniques

The common electrical methods used for water disinfection include electro-chemical techniques (Kerwick et al., 2005) and Pulsed Electric Field (PEF) (Guyot et al., 2007). These techniques are different in their mechanisms for deactivating microorganisms.

Electrochemical methods are defined as the methods that equip the synergistic effects of chemical and electrical techniques for water disinfection. These methods can be categorized into two groups: methods that use direct electrolysers and others that use mixed oxidant generators (Kerwick et al., 2005). The direct electrolysers interact directly with the microorganisms while with mixed oxidant generators produce oxidizing species that have the capability to damage microorganisms (Cho et al., 2001; Qin et al., 2002). The deactivation effectiveness of electrochemical methods is influenced by many factors such as the configuration of electrochemical cell, electrode material, the composition of the electrolyte, the species of microorganisms, current density and the flow rate of the water through the cell (Kerwick et al., 2005).

PEF is a disinfection technique that involves maintaining the suspension of microorganisms between electrodes and subjecting them to a high intensity short duration electric field (Guyot et al., 2007). PEF techniques can cause damage to the microorganisms directly by decomposing DNA or Ribonucleic acid (RNA) of the cell (Takayuki et al., 1999). The decomposition of DNA or RNA happens due to a disorganization in the intracellular (Aronsson et al., 2001), or deactivation of the

enzymes of the treated microorganisms (Van Loey et al., 2001), while the indirect effect of PEF on microorganisms is represented by the formation of bactericidal agents in the treated suspension (Reyns et al., 2004). There are two hypotheses that explain the deactivation mechanisms of PEF.

The first hypothesis explains the destructive effect of PEF by the dielectric rupture theory (Zimmerman, 1986) or the so-called electro-poration phenomenon. According to this phenomenon, the membrane cell acts as a capacitor and the exposure to an electric field can generate potential difference across the membrane (Guyot et al., 2007). The increase in the potential results in reduction of membrane thickness. When the potential of the membrane reaches the critical breakdown voltage, the breakdown of the cell membrane occurs.

The second hypothesis called thermo-poration hypothesis which ascribes the germicidal effect of PEF treatment partially to the thermal effects associated with the treatment. Guyot et al.(2007) demonstrated the synergistic role of PEF-induced heat and electro-poration of the membrane in damaging the microorganism as shown in Figure 2.5.

Figure 2.5 (1) represents an intact cell in the suspension before it exposes to PEF. After subjecting the suspension to PEF, the microorganism is polarized due to the electro-poration phenomenon leading to the leakage of the intracellular ions to the peripheral cellar sub-layer as illustrated in Figure 2.5 (2). The diffusion of the intracellular ions into the sub-layer in turn leads to an increase in the conductivity of this layer. With the effect of the electrical treatment, the temperature of this layer increases. Then, the cell experiences the thermo-poration phenomenon as well as the electro-poration phenomenon (Figure 2.5 (3)). Finally, the ions in the cellular sub-

layer diffuse to the medium (Figure 2.5 (4)) and this leads to mechanical damage in the cell membrane and eventually cell death.

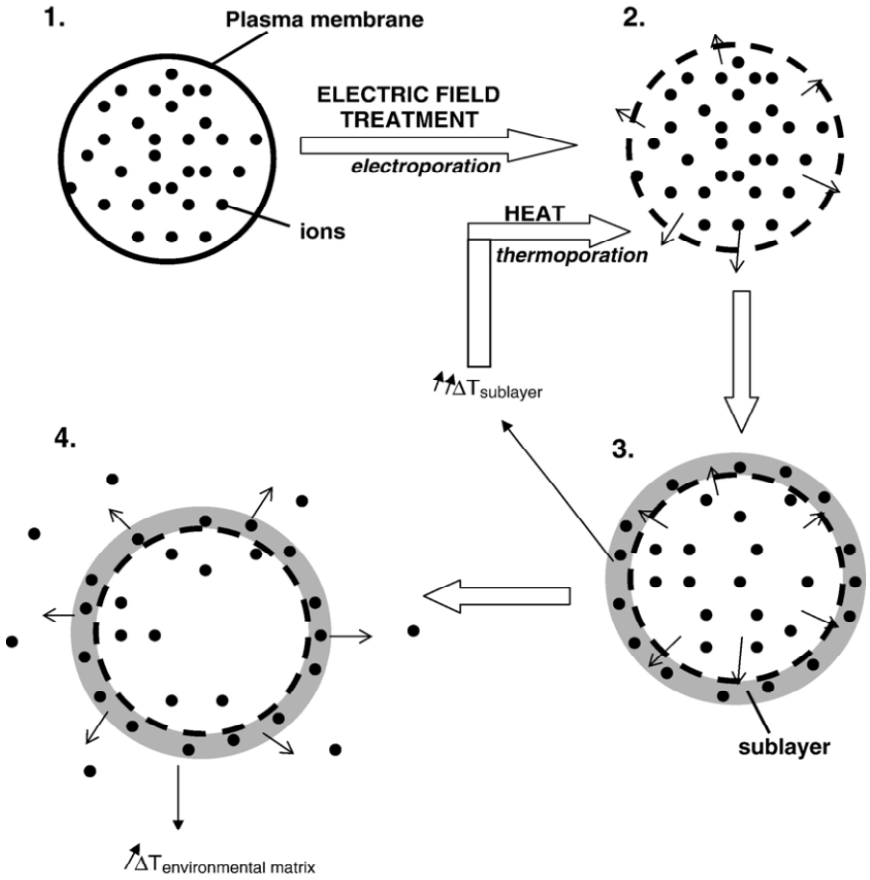


Figure 2.5: The physiological events of cell death under the effect PEF as proposed by (Guyot et al., 2007). The symbols:(●) represents ions, (ΔTx): temperature variations of the x medium, (⟹) induced event, and (→)

The advantages of the electrical disinfection methods include lower requirements to cooling process as compared to the conventional heat treatment (Dutreux et al., 2000) and the ability to deactivate microorganisms without producing a new generation of microorganisms that are tolerant to the treatment (Gusbeth et al., 2009). However, electrical treatments have some disadvantages that could limit their application, such as producing mutagenic components in the treated water (Reyns et

al., 2004), cathode fouling (Zhe et al., 2008) and the lack of this technique to residual effects.

2.5.5 Mechanical techniques

The techniques that rupture microorganisms through using pressure plus, shock wave and shear stress are classified as the mechanical methods for microorganisms deactivation. The common mechanical techniques include high pressure homogenization, hydrodynamic cavitation, bead mill and micro-fluidizer. These techniques are mostly used in food processing for extracting the intercellular products such as enzymes and proteins (Geciova et al., 2002). The mechanisms, the negative and the positives aspects of the mechanical techniques for microorganisms deactivation in water are summarized in Table 2.2. Further details on the mechanical techniques for microorganisms deactivation are provided in Appendix B.

Table 2.2: Mechanisms, the negative and the positive aspects of using the mechanical methods of microorganisms disruption for deactivation purposes

Mechanical techniques	Mechanisms	Positive aspects	Negative aspects
High Pressure Homogenization (HPH)	1) Effect of the generated eddies in the turbulent flow (Doulah et al., 1975). 2) Impingement of microorganisms onto the solid surfaces (Engler and Robinson, 1981). 3) Effect of cavitation (Save et al., 1994).	1) Free chemicals. 2) Possibility of scale-up (Geciova et al., 2002).	1) Low performance with filamentous microorganisms (Keshavarz et al., 1990). 2) Mechanical erosion in the equipments (Gogate and Kabadi, 2009). 3) Low efficiency (Gogate and Kabadi, 2009). 4) No residual effect.
Hydrodynamic cavitation (HC)	1) Chemical effect represented by the generation of $OH^{\cdot}$ (Arrojo et al., 2008). 2) Mechanical effects such as impingement, turbulences produced by high-velocity liquid jets, shear rates generated in the adjacent area to the jets and shock waves generated by bubbles explosion (Sawant et al., 2008).	1) Free chemicals 2) Possibility of scale-up (Sawant et al., 2008)	1) Difficulty in controlling the key parameters that influence the chemical and physical yield of cavitation in HC chambers (Arrojo and Benito, 2008). 2) Mechanical erosion in the equipments (Gogate and Kabadi, 2009). 3) No residual effect.
Bead mill	1) Shearing actions (Bunge et al., 1992; Melendres et al., 1992). 2) Collusion of microorganism with the beads (Macneill et al., 1985).	1) Free chemicals. 2) Possibility of scale-up (Geciova et al., 2002).	1) Effect of some of the process parameters on the efficiency of cell disruption is not clear such as bead size (Currie et al., 1972; Deters et al., 1976). 2) Low efficiency (Gogate and Kabadi, 2009). 3) No residual effect.
Micro-fluidizer	1) Impingements of microorganisms onto stationary surfaces (Geciova et al., 2002). 2) Shearing effects.	1) Free chemicals. 2) Possibility of scale-up (Geciova et al., 2002).	1) Low efficiency (Gogate and Kabadi, 2009). 2) No residual effect.

2.5.6 Ultrasound techniques

Ultrasound technology is suggested as an alternative method to replace or enhance the common methods for water disinfection such as chlorination and UV light (Hulsmans et al., 2009; Gibson et al., 2008). Integrating ultrasound technology to industrial processes can reduce the number of the synthetic steps in the processes or enable the processes to operate at milder conditions such as temperature and pressure (Thompson and Doraiswamy, 1999).

Ultrasound technology has been undergoing many developments since the date its spectacular effects were discovered. Table C.1 in Appendix C illustrates the important developments in the history of ultrasound technology. Recently, the research in the field of ultrasound technology has focused on extending the applications of ultrasound to include wide spectrum of industrial processes. A new application of ultrasound in water treatment process is proposed in this thesis. The application is represented by integrating ultrasound to the water treatment scheme in the aim of alleviating biofouling formation in the RO membrane system in an environmentally friendly way. Owing to the copious information available on ultrasound technology, the section of ultrasound treatment is divided into subsections.

2.5.6.1 Acoustic cavitation in water

The acoustic cavitation phenomenon occurs due to the fall of the ambient pressure under the saturated vapour pressure of the liquid as a result of ultrasound waves passage through the liquid. The formation of the voids takes place during the rarefaction period (i.e. the negative swing of the pressure), while the collapse of the

bubbles takes place during the compression period (i.e. the positive swing of the pressure) (Gogate and Pandit, 2000) as illustrated in Figure 2.6.

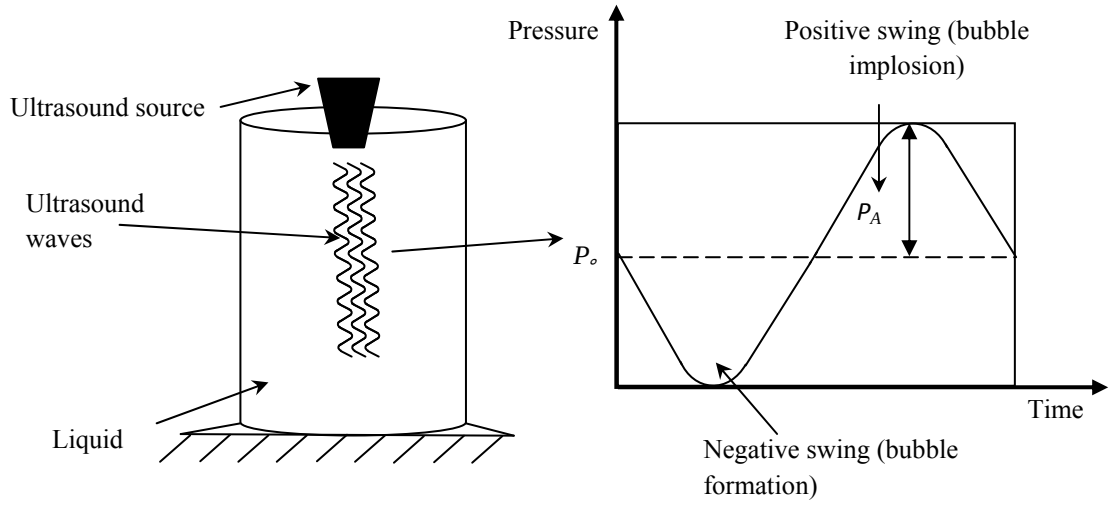


Figure 2.6: An illustration of the process of bubble formation in acoustic cavitation

The symbol P_A in Figure 2.6 denotes the amplitude pressure of ultrasound, while P_o represents the ambient pressure (the pressure of the medium before applying ultrasound pressure).

There are two types of cavitation that can be observed when irradiating a liquid depending on the path in which the bubble is created, homogenous and heterogeneous cavitations. Homogenous cavitation means the generation of the bubbles due to the interaction between liquid and vapour phases, whilst, heterogeneous cavitation is defined as the interaction between three phases, solid, liquid and vapour simultaneously. The locations at which the two types of cavitation occur are known as weaknesses or nucleation sites. The surface tension of the liquid at the nucleation sites is weak which allow the negative pressure of the sound wave to rapture the liquid and generate bubbles containing mixture of gas and vapour (Young, 1999). The term surface tension in pure liquid refers to the intermolecular

force that tends to hold the molecules of the liquid together and prevents the formation of voids. Brennen (1995) explained that the weaknesses in the liquid body can be in the form of micro-bubbles of dissolved air that are suspended in the liquid, crevices and irregularities on the surface of the vessel that contains the liquid or micro-sized particles. The formation of heterogeneous cavitation on surfaces with different characteristics is illustrated in Figure 2.7.

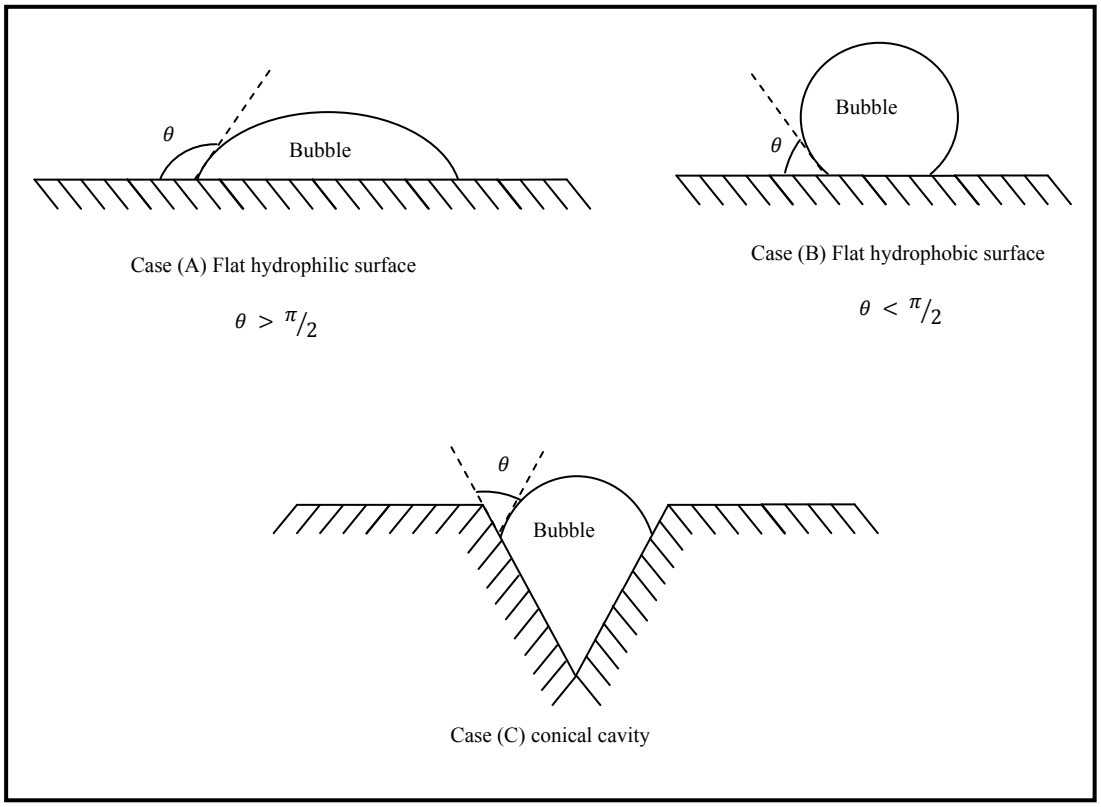


Figure 2.7: Effect of contact angle and surface hydrophobicity on the formation of heterogeneous bubbles in water (Young, 1999)

From Figure 2.7, it can be inferred that the formation of bubbles is affected by the contact angle between the bubble and the solid surface and the characteristic of the surface whether it is hydrophobic or hydrophilic. The water exhibits a high contact angle with the hydrophilic surface and thus gives less chance for bubble formation,

while when the contact angle between hydrophobic surface and the bubble is small, bubble formation is promoted (Gibson et al., 2008).

It was observed that there are two types of bubbles occurring in the liquid as a result of ultrasound irradiation: bubbles with gentle collapse (stable cavitation) and bubbles with violent collapse (transient cavitation) (Young, 1999). The latter exist normally for one cycle of the sound pressure in which bubbles expand to at least double their size and collapse severely, often disintegrating into small bubbles. It was suggested that the transient bubbles grow due to the gas expansion because of the pressure drop through the gas/liquid interface rather than mass transfer (Toegel and Lohse, 2003). This was attributed to the short time that does not allow the gas to transfer from or into the bubbles. In comparison, stable bubbles can oscillate for more than one cycle of sound pressure and grow due to mass diffusion through the bubble skin (Young, 1999).

2.5.6.2 Ultrasound processes

The synergistic combination of ultrasound with pressure (manosonication), temperature (thermosonication) or both (manothermosonication) was developed to enhance the effectiveness of ultrasound treatment in microbe deactivation and decrease the high energy consumption required to deactivate the most resistant microorganisms and spores (Raso et al., 1998). However, it should be noted that augmenting temperature or pressure with ultrasound can enhance the bactericidal effect of ultrasound to a certain level after which any increase in the pressure or temperature will result in no further change in ultrasonic deactivation. Raso et al. (1998) reported that increasing the temperature of MTS to high levels (higher than 58˚C) for *Yersinia enterocolitica* resulted in same reduction as thermal treatment at

the same temperature. Similarly, Neppiras and Hughes (1964) stated that the pressure increase could have a negative effect on the ultrasonic deactivation when it exceeds its optimum level. Therefore, with thermosonication, manosonication processes, the optimum level of temperature and pressure for certain operating conditions of the processes must be known.

2.5.6.3 Mechanisms of ultrasound in cell disruption

The mechanism of ultrasound in disrupting microorganisms lies in the combination of acoustic cavitation effects:

1. Mechanical effects results from the cavitation phenomena.
2. Chemical effects of cavitation phenomena which involve the generation of free radicals in the medium.
3. Heat effects represented by the generation of localized hot spots as a result of the rapid explosion of the bubbles (Hulsmans et al., 2009).

It was observed that the mechanical effects play the main role in destroying microorganisms while chemical and heat effects play a supporting role (Hulsmans et al., 2009).

The mechanical effects of cavitation phenomena encompass different form of mechanical energies that are produced as a consequence of oscillation and implosion of the stable and transient bubbles. The mechanical effects of acoustic cavitation include: high pressure, generation of turbulences as a result of liquid circulation and shear stresses (Gogate and Kabadi, 2009). Mason et al. (2003) reported that the micro-streaming resulting from bubble oscillation can generate stresses that have the potential to rupture microorganisms. Another scenario for the cell rupture under the effect of ultrasound was proposed by Doulah (1977) who assumed that, in ultrasound

treatment, the cell rapture occurs due to the exposure of the cell to viscous dissipative eddies that generate from the shock waves of bubble collapse. Yusaf (2011) has confirmed that the main cause of cell disruption in ultrasound treatment is the collapse pressure that results from bubble implosion.

2.5.6.4 Factors affecting the performance of ultrasound

The efficiency of ultrasonic deactivation is affected by several factors that determine the potential of this process, including:

1) *Type and characteristic of microorganisms*: It was reported that gram negative microorganisms and the rod-shaped bacteria are more sensitive to ultrasound treatment than gram positive microorganisms and bacteria with coccal forms (Davies, 1959). Additionally Ahmed and Russell (1975) reported that microorganisms with larger size are more susceptible to ultrasound treatment than the microorganisms with smaller sizes. The large cells have a larger surface area exposed to the pressure shock of ultrasound as compared to the small cells.
2) *Medium contents*: The physio-chemical properties of the medium can also impact on the efficiency of ultrasound in deactivating microorganisms. When using ultrasound with low viscosity medium, the penetration of ultrasound waves through the medium and the subsequent cavitational events are more effective than with the use of a high viscosity medium (Leadley and Williams, 2006).
3) *The nature of dissolved gasses or solid particles in the medium*: The dissolved gases or solid particles in the medium body can act as nucleation sites which will enhance bubble formation (Brennen, 1995). However, the

nature of these impurities can impact on the cavitational effect of ultrasound. For example, gases with high specific heat ratio could have more effect on the cavitational yield than the gases with low specific heat ratio (Thompson and Doraiswamy, 1999). In a similar way, the nature of the suspended solid particles can also impact on the cavitational events. Dadjour et al. (2005) found that the addition of TiO_2 pellets to a suspension of *E. coli* can improve ultrasonic inactivation for *E.coli*, and that was partially attributed to the catalytic effect of TiO_2.

4) *Type of ultrasonic reactor*: The type of ultrasonic reactor is another factor that can affect the potency of ultrasound in deactivating microorganisms. Some ultrasonic reactors such as the ultrasonic horn reactor have a limited area for cavitation activities as compared to multiple transducers reactor (Gogate, 2007). The larger the area of cavitation activities, the higher the deactivation of microorganisms that can be achieved.
5) *Volume of the treated water and initial concentration of microorganisms*: Hulsmans et al. (2009) showed that the reduction percentage of microorganisms is inversely proportional to the volume of the treated water and initial concentrations of microorganism.
6) *Process parameters of ultrasound treatments* which include ultrasonic power, frequency, treatment time, pressure and temperature of the medium.

2.5.6.5 Advantages and disadvantages of ultrasound

The advantages and disadvantages of using ultrasound for water disinfection are summarized in Table 2.3.

Table 2.3: Advantages and disadvantages of ultrasound as a disinfection technique

Advantages	Disadvantages
1. Free-chemical method. 2. The ability of ultrasound on breaking down clusters of microorganisms (Joyce et al., 2003). 3. Ultrasound can be combined with other techniques to enhance their performance (Hulsmans et al., 2009). 4. It can be used with high suspended solid solutions, and these solutions can even enhance the performance of ultrasound (Marschall et al., 2003; Borkent et al., 2007).	1. No residual effect. 2. High cost (Hulsmans et al., 2009).

2.6 Conclusions

An extensive bachground on RO technology, the biofouling problem and the possible techniques that can be used for alliviating biofouling problem in the RO membrane system was demonstrated in this chapter. There are many techniques that can be applied to reduce the formation of biofouling on the RO membrane. These techniques are: membrane surface modifications techniques, biochemical techniques, membrane filtration techniques and disifection techniques. Membrane surface modifications techniques reduce the depoesition of microorganisms on the membrane through modifying the charcteriscs of the RO membrane against biofouling. Biochemical techniches can be applied to remove a frormed biofilm on the RO membrane. Disifection and membrane filtration techniques have the potential to reduce the adhesion of microorganisms to the membrane through decreasing the concentration of existing microorganisms in the feed water of the RO system.

Disinfection techniques for controlling biofouling include a wide range of techniques such as filteration, chemical, thermal, electrical, mechanical techniques, UV and ultrasound. The use of free-chemical methods for water disinfection have recently gained considerable attention. This attention is a result of their environmentally benign effects. Among these free-chemical techniques, the use of ultrasound for water disinfection has captured the attention of many researchers (Mason et al., 2003). Owing to the promising results of the ultrasonic disinfection technique, and its advantages that outweigh the disadvantages, ultrasound has been proposed in this thesis as a new controlling technique for reducing the formation of biofouing on the RO mmebrane.

CHAPTER 3

Ultrasound treatment -theoretical considerations

3.1 The aim of the chapter

Ultrasound is a known effective technique for improving water quality (Furuta et al., 2004). Therefore it has been chosen in this work to reduce the concentration of microorganisms in RO feed water which, in turn, can reduce the formation of biofouling on the RO membrane. The performance of ultrasound in deactivating microorganisms is affected by a number of parameters as explained in Chapter 2 Section 2.5.6.4. Optimizing the parameters that influence ultrasound performance is of utmost importance and should be considered prior to the use of ultrasound in any application (Sivakumar and Pandit, 2001). Optimizing these parameters requires an understanding the theoretical bases of ultrasound.

The mechanical effect of ultrasound, represented by the release of high pressure from bubble implosion, was reported as the main cause of cell disruption (Yusaf, 2011). Thus, it is imperative to study the factors that influence the magnitude of the pressure released from bubble implosion ($P_{collapse}$). These factors include: ultrasonic intensity, frequency, pressure of the treated water, temperature of the treated water and the initial size of the bubble (Thompson and Doraiswamy, 1999; Adewuyi, 2005; Adewuyi, 2001; Gogate and Pandit, 2000; Lo"Rincz, 2004).

In this chapter, the effect of the ultrasonic intensity, frequency, temperature and pressure of the treated water on collapse pressure is theoretically investigated. The

effect of the initial bubble size on collapse pressure is also investigated (detailed information of this investigation is provided in Appendix D).

This chapter consists of the following sections:

1. Dynamics of the transient bubbles in homogenous acoustic cavitation.
2. Effect of ultrasonic intensity on collapse pressure.
3. Effect of ultrasonic frequency on collapse pressure.
4. Effect of pressure increase on collapse pressure (manosonication treatments).
5. Effect of temperature increase on collapse pressure (thermosonication treatments).
6. Conclusions.

3.2 Dynamics of the transient bubble in homogenous acoustic cavitation

To study the dynamics of a single transient bubble in water in the case of homogenous cavitation, the following assumptions are to be considered (Young, 1999; Brennen, 1995; Caupin and Herbert, 2006; Gogate and Pandit, 2000):

1. The shape of the bubble is spherical.
2. Bubble expansion is an isothermal process (Young, 1999)
3. The collapse of the bubble is isothermal process at the start and then becomes adiabatic (Young, 1999; Vichare et al., 2000), however the transition of the processes in the collapse phase from isothermal to adiabatic is governed by the Flynn's assumption (Gogate and Pandit, 2000). Flynn's assumption states that the collapse of the bubble becomes adiabatic when

the partial pressure of the gas inside the bubble becomes equal to the vapour pressure of the liquid medium (Gogate and Pandit, 2000).

4. Bubbles contain a mixture of water vapour and gas (air).
5. Gravity effect on the bubble is not considered.
6. Water is an incompressible material.

The effect of different parameters of ultrasound treatments on collapse pressure will be studied by adopting the Rayleigh-Plesset approach given in equation 3.1 (Young, 1999, Gogate and Pandit, 2000, Morch, 2009):

$$R\frac{d^2R}{dt^2}+\frac{3}{2}\left(\frac{dR}{dt}\right)^2=\frac{1}{\rho_L}\left(P_B-\frac{4\mu_L}{R}\frac{dR}{dt}-\frac{2\sigma}{R}-P_\infty\right), \quad (3.1)$$

where, dR/dt is bubble-wall velocity (m/s),

d^2R/dt^2 is the acceleration of bubble-wall (m/s^2),

ρ_L is the density of the liquid (kg/m^3),

μ_L is the dynamic viscosity (N.s/m^2),

P_B is the pressure inside the bubble at anytime (Pa),

P_∞ is the pressure in the surrounding liquid to the bubble (Pa),

R is the bubble radius at any time (m), and

σ is the surface tension of the water at a certain temperature (N/m).

The Rayleigh-Plesset equation will be solved numerically using the fourth order Runge-Kutta method. MATLAB software will be used to perform the numerical solution for the Rayleigh-Plesset equation. To solve the Rayleigh-Plesset equation, the value of the pressure inside the bubble at any time (P_B) and the time dependent pressure in the surrounding liquid (P_∞) should be known.

3.2.1 Bubble pressure

The lifespan of the transient bubble is divided into two thermodynamical phases; isothermal and adiabatic, as illustrated in Figure 3.1. In the isothermal phase, the bubble size changes while the temperature of the bubble as a system remains constant. In the adiabatic phase, the bubble size changes with no heat input to the bubble or heat release from the bubble. The growth of the bubble and the initial part of the collapse is assumed to be isothermal (the time period from 1 to 2 in Figure 3.1), while the process following the isothermal collapse is assumed to be adiabatic process (the time period from 2 to 3 in Figure 3.1) (Young, 1999; Gogate and Pandit, 2000; Vichare et al., 2000).

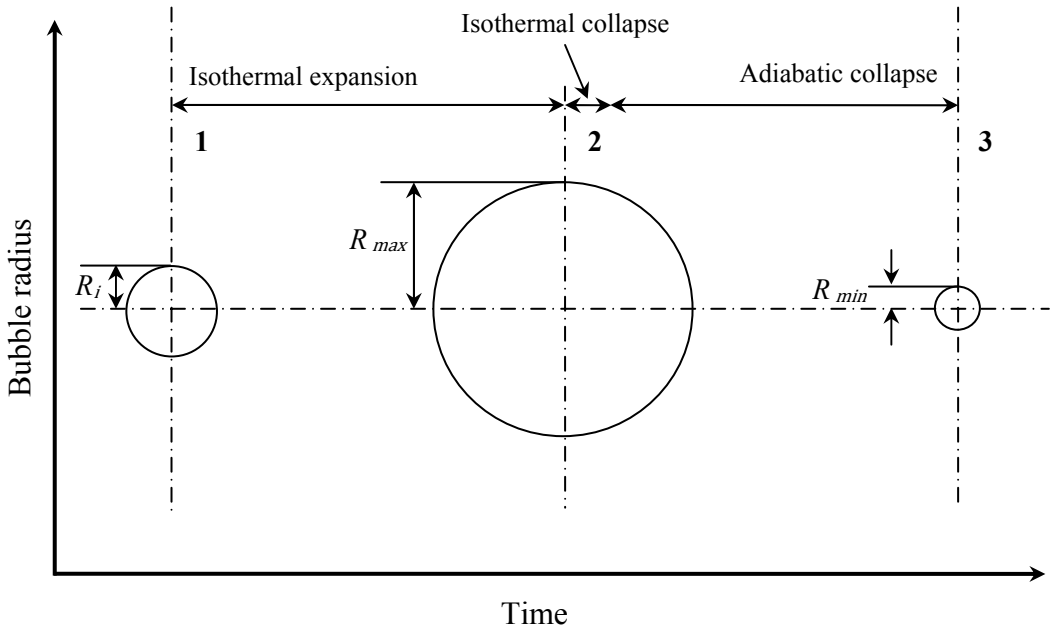


Figure 3.1: Lifespan of spherical transient bubble in acoustic cavitation

The magnitude of the pressure inside the bubble in each phase is different. During the isothermal phase the change in pressure of the gaseous mixture inside the bubble is given as:

$$P_1 V_1 = P_2 V_2, \quad (3.2)$$

where, P_1, P_2 are the bubble pressures at the start and the end of the isothermal process, and

V_1, V_2 are the bubble volumes at the start and the end of the isothermal process (m^3).

Since the geometrical shape of the bubble is assumed as a sphere, the volume of the bubble is replaced by a volume of sphere, and thus equation 3.2 can be re-formulated as:

$$P_B = P_{Bi}\left(\frac{R_i}{R}\right)^3, \tag{3.3}$$

where, R_i is the initial radius of the bubble (m) , and

P_{Bi} is the initial pressure of the bubble (Pa).

These two parameters can be set as the initial conditions for the isothermal phase (as given in equation 3.3).

The initial radius of the bubble in this work is assumed to be 0.01mm, which falls within the range of the initial bubble radii used for the simulation studies as reported by Gogate and Pandit (2000), whilst the initial pressure of the bubble can be found from the following equation (Young, 1999);

$$P_B - P_\infty = \frac{2\,\sigma}{R} \quad or \quad \Delta P = \frac{2\,\sigma}{R}. \tag{3.4}$$

where, ΔP is the pressure difference across the bubble film (Young, 1999).

By applying the initial conditions ($R = R_i$, and $P\infty = P_\circ$) in equation 3.4, the initial pressure of the bubble can be found as given:

$$P_{Bi} = P_\circ + \frac{2\sigma}{R_i}, \tag{3.5}$$

where, $P_\circ$ is the initial pressure of the treated solution by ultrasound and it is equal to the ambient pressure ≈ 101.3 kPa, when there is no external pressure applied on the treated solution.

The collapse transition from isothermal to adiabatic takes place when $P_g = P_v$, which gives $P_B = 2\ P_v$ (the Flynn assumption) (Flynn, 1964). The critical radius of the bubble, which is associated with the transition point, can be determined from equation 3.6 by adopting the Flynn assumption;

$$R_{crit} = R_i \left(\frac{P_{Bi}}{2P_v}\right)^{1/3}. \quad (3.6)$$

R_{crit} can be used as boundary conditions to test the transition of bubble collapse from isothermal to adiabatic in the numerical simulation as follows:

If $R \leq R_{crit}$, the collapse is isothermal and equation 3.3 is used to describe the pressure inside the bubble in the Rayleigh-Plesset approach, while in the case of $R > R_{crit}$, the pressure inside the bubble in the adiabatic process can be expressed by equation3.7:

$$P_B = 2P_v \left(\frac{R_{crit}}{R}\right)^{3\gamma}, \quad (3.7)$$

where, γ is the adiabatic index.

3.2.2 Pressure in the surrounding liquid of the bubble

According to Young (1999), when water is subjected to sound waves from an ultrasound device, these waves create negative pressure in the water. This negative pressure can drop the absolute pressure of the medium to less than the vapour

pressure of the same medium and this in turn causes bubble growth. Working under this assumption, the pressure in the adjacent areas to the bubble can be formulated as follows:

$$P_{\infty} = P_{\circ} - P_A \sin(2\pi f t), \quad (3.8)$$

where, f is the frequency (Hz),

t is time (s), and

P_A is the amplitude pressure of ultrasound (Pa) (see Chapter 2, Section 2.5.6.1), and it can be calculated from the following formula:

$$P_A = \sqrt{2I\rho C}, \quad (3.9)$$

where, C is the sound velocity in the water ≈ 1500 m/s (Gogate and Pandit, 2000), and

I is the ultrasonic intensity (W/cm^2).

Arrojo and Benito (2008) stated that the implosion of the bubble occurs in the adiabatic phase when the bubble radius reaches zero. The pressure developed in the bubble body, due to the change in the bubble size in the adiabatic phase, from the critical size to the minimum size will be released when the bubble implodes. This pressure can be found from the following equation:

$$P_{collapse} = 2P_v \left(\frac{R_{crit}}{R_{min}}\right)^{3\gamma} \quad (3.10)$$

where, $P_{collapse}$ is the pressure released from bubble implosion (Pa), and

R_{min} is the minimum radius of the bubble (m).

It can be observed from equation 3.10 that $P_{collapse}$ is inversely proportional with R_{min}. In addition to R_{min}, the maximum radius of the bubble (R_{max}) can also

affect collapse pressure, and bubbles that grow to larger sizes collapse more violently (Gogate and Pandit, 2000). Hence, the effect of the parameters of ultrasound treatments will be assessed depending on their effects on R_{max} and R_{min}. The values of R_{max} and R_{min} will be determined from the numerical solution of the Rayleigh-Plesset equation.

3.3 Effect of ultrasonic intensity on collapse pressure

The intensity of ultrasound can be calculated from equation 3.11 (Sivakumar and Pandit, 2001):

$$I = \frac{P_u}{A}, \tag{3.11}$$

where, P_u is ultrasound power (W), and

A is the area of the irradiating surface (cm^2).

The ultrasonic power that was applied in the ultrasound experiments was regulated through changing the amplitude of ultrasound device. Three levels of amplitude were applied in this study: 50% which gives ultrasonic power of 85 W, and 75% and 100% give 104 and 117 W respectively. These figures were taken from a previous study conducted by Yusaf and Buttsworth (2007) and Yusaf (2011) who used the same device in their study. The ultrasonic intensities that correspond to the applied amplitudes 50%, 75% and 100% were calculated using equation 3.11 and they are; 17.56, 21.49 and 24.17 W/cm^2 respectively.

The effect of the applied ultrasonic intensities of 17.56, 21.49 and 24.17 $W/cm^2$and fixed frequency of 55 kHz on the growth of a bubble at the atmospheric conditions is presented in Figure 3.2. Figure 3.2 and the other figures in this chapter

that show the evolution of bubble radius with time under different operating conditions were produced using MATLAB simulation for the Rayleigh-Plesset equation. The maximum and the minimum values of bubble radius in Figure 3.2 and the following figures in this chapter (Figure 3.3 through Figure 3.5) are the results of the numerical solution of the Rayleigh-Plesset approach.

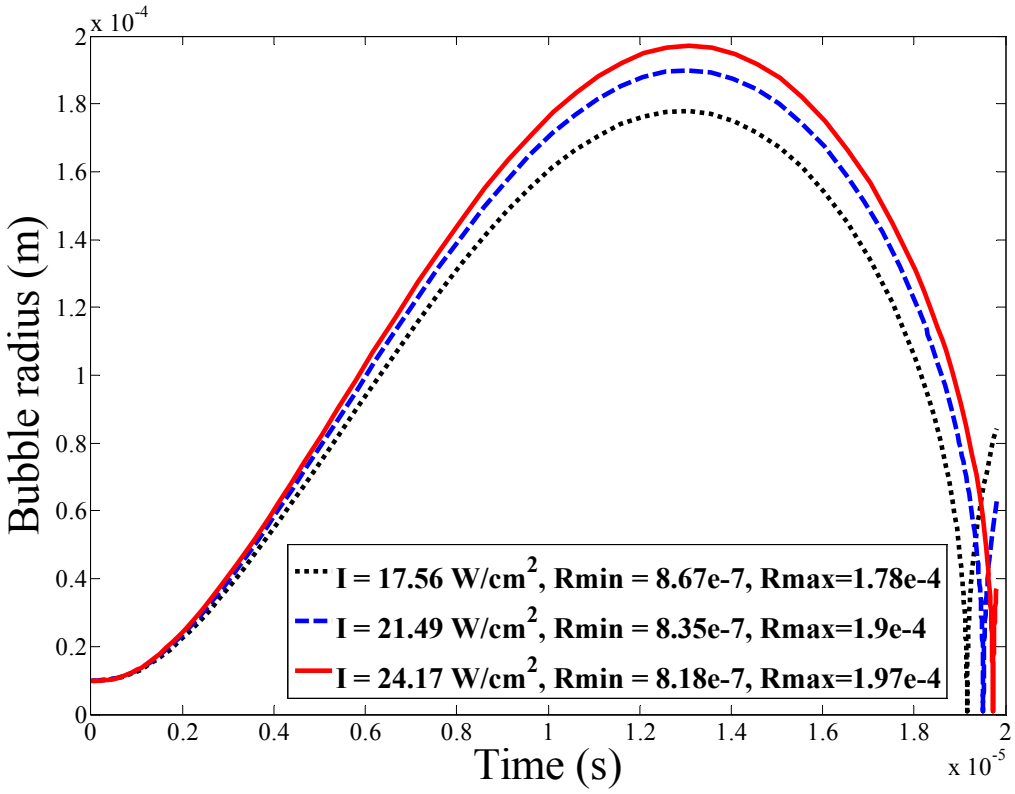


Figure 3.2: Evolution of bubble radius (R_i = 0.01 mm) with ultrasonic intensities of 17.56, 21.49 and 24.17 W/cm^2 and constant frequency of 55 KHz under atmospheric conditions ($P_\circ$ =101.3 kPa and T=25˚C)

From Figure 3.2, it can be said that the increase of the ultrasonic intensity leads to an increase in the maximum radius of the bubble as well as a decrease in the minimum radius of the bubble. Gogate and Pandit (2000) showed that increasing ultrasonic intensity results in an increase in the R_{max}. The increase in the R_{max} and the decrease in the R_{min} can lead to increase $P_{collapse}$. Thompson and Doraiswamy (1999) and Al Bsoul et al. (2010) reported that increasing the ultrasonic power delivered

into the liquid increases the cavitational yield of ultrasound. If the collapse pressure is the main cause of cell disruption in ultrasound treatments, then increasing the intensity is expected to result in an increase in the reduction of microorganisms. The effect of the intensity on ultrasonic reduction of microorganisms within the applied intensities in this study will be investigated experimentally in Chapter 4.

3.4 Effect of ultrasonic frequency on collapse pressure

Although the experiments of ultrasound treatments in this study were performed with a constant ultrasonic frequency, it is important to highlight the effect of ultrasonic frequency on $P_{collapse}$. Figure 3.3 illustrates the effect of three ultrasonic frequencies of 60, 70 and 80 kHz on the growth of a bubble at constant ultrasonic intensity of 17.56 W/cm^2 and at atmospheric conditions.

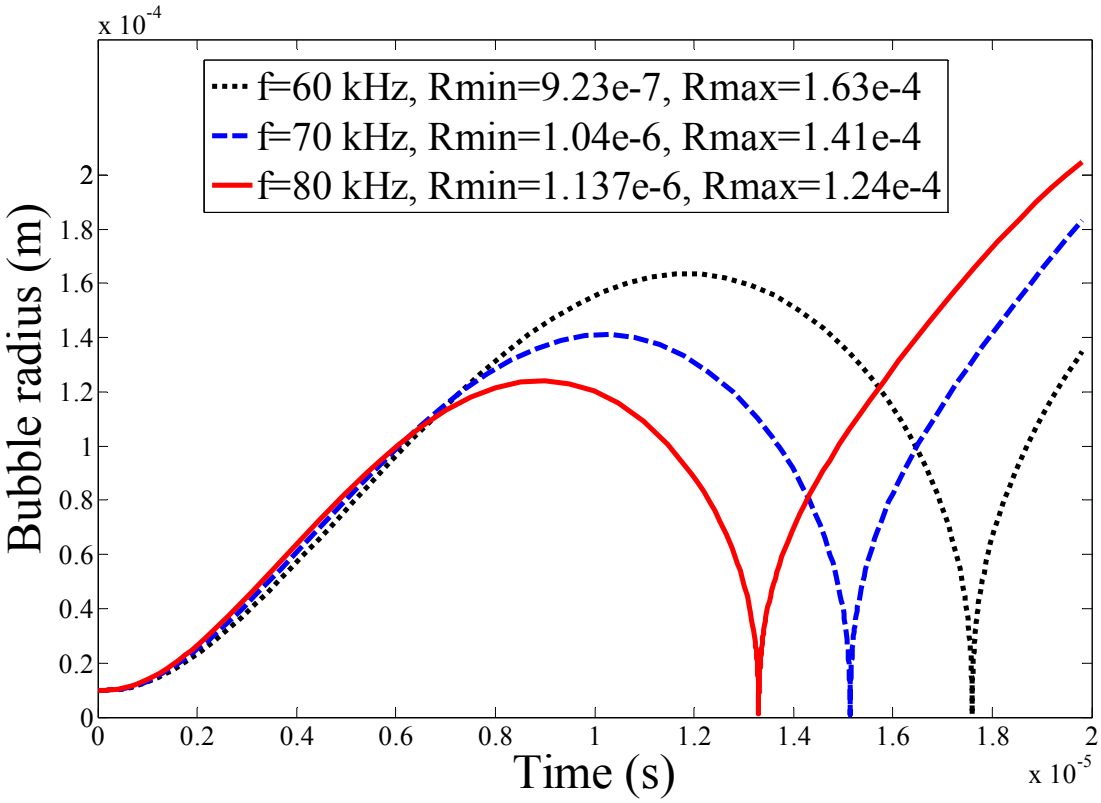


Figure3.3: Evolution of bubble radius (R_i =0.01 mm) with ultrasonic frequencies of 60, 70 and 80 kHz and constant intensity of 17.56 W/cm^2 under atmospheric conditions (P_o =101.3 kPa and T=25˚C)

Figure 3.3 shows that increasing ultrasonic frequency results in an increase in R_{min} and a decrease in R_{max} of the bubble which, in turn, can decrease $P_{collapse}$. It can be also noted that higher frequencies produce bubbles with a short lifespan, which means that higher frequencies can generate larger numbers of bubbles as compared to those produced with lower frequencies. Petrier et al. (1992) pointed out two effects of increasing the frequency. First, the energy release from bubble collapse is low and second, the bubbles form for a short period of time. Crum (1995) reported that the number of collapsing bubble with higher frequencies is higher than that of lower frequencies for the same acoustic intensities. However, the collapse of the bubbles of lower frequencies is more violent than that of higher frequencies.

Since the rupture of microorganisms in ultrasound treatment is attributed mainly to the collapse pressure, and low frequencies generate bubbles with high collapse pressure; low level ultrasonic frequency is recommended for microbial deactivation. Al Bsoul et al. (2010) showed that sonication treatment with low frequencies achieved higher reduction of *Mycobacterium* sp. 6PY1 as compared to sonication treatment with high frequencies.

3.5 Effect of temperature on collapse pressure

As a step toward developing a procedure for the thermosonication treatments of *E. coli* in water based-suspension, it is important to theoretically investigate the effect of increasing the treated water temperature from 45 to 50 and 55˚C on collapse pressure. It should be noted, that the temperature of 45, 50 and 55˚C is the temperature range that will be used in the thermosonication treatments of this study.

The increase in water temperature alters the physio-chemical and thermo-physical properties of the water, and as a consequence, affects the magnitude of

$P_{collpse}$. Table 3.1 shows the physio-chemical and thermo-physical properties of water for the temperatures 45, 50 and 55˚C. The properties of water presented in Table 3.1 were collected from the literature; Cengel and Boles (2008), Grosso and Mader (1972), Vargaftik et al. (1983), Lide (2005) and Lide (2010). From equation 3.5 and 3.6, changing the properties of the treated water affects $P_{B,i}$ and R_{crtit}. Therefore, the values of $P_{B,i}$ and R_{crtit} that correspond to these properties were calculated (using equations 3.5 and 3.6) and substituted in the numerical simulation.

Table3.1: Physio-chemical and thermo-physical properties of the treated water at temperatures of 45, 50 and 55˚C

T(˚C)	$P_v \times 10^3$ (Pa)	$\mu \times 10^{-4}$ (N.s/m^2)	ρ (Kg/m^3)	$\sigma \times 10^{-3}$ (N/m)	C (m/s)
45	9.5953	6.09	990.21	68.78	1536.409
50	12.352	5.468	988.04	67.94	1542.551
55	15.763	5.067	985.69	67.1	1547.382

The effect of increasing the temperature of the treated water, from 45 to 50 and 55˚C, on the growth of a bubble under constant ultrasonic intensity and frequency of 17.56 W/cm^2 and 55 kHz respectively was investigated in this study and the results are illustrated in Figure 3.4. The pressure of the treated water in the numerical simulation of Figure 3.4 was assumed to be atmospheric pressure.

It can be seen from Figure 3.4 that increasing the temperature of the treated water leads to two actions: increasing R_{max} and decreasing R_{min}. The values of R_{max} and R_{min} of Figure 3.4 are summarised in Table 3.2. The slight increase in the bubble radius (Figure 3.4) as a result of increasing the treated water temperature can be attributed to the change in the physio-chemical and thermo-physical properties of the treated water (Gogate and Pandit, 2000; Leadley and Williams, 2006; Lo˝Rincz, 2004).

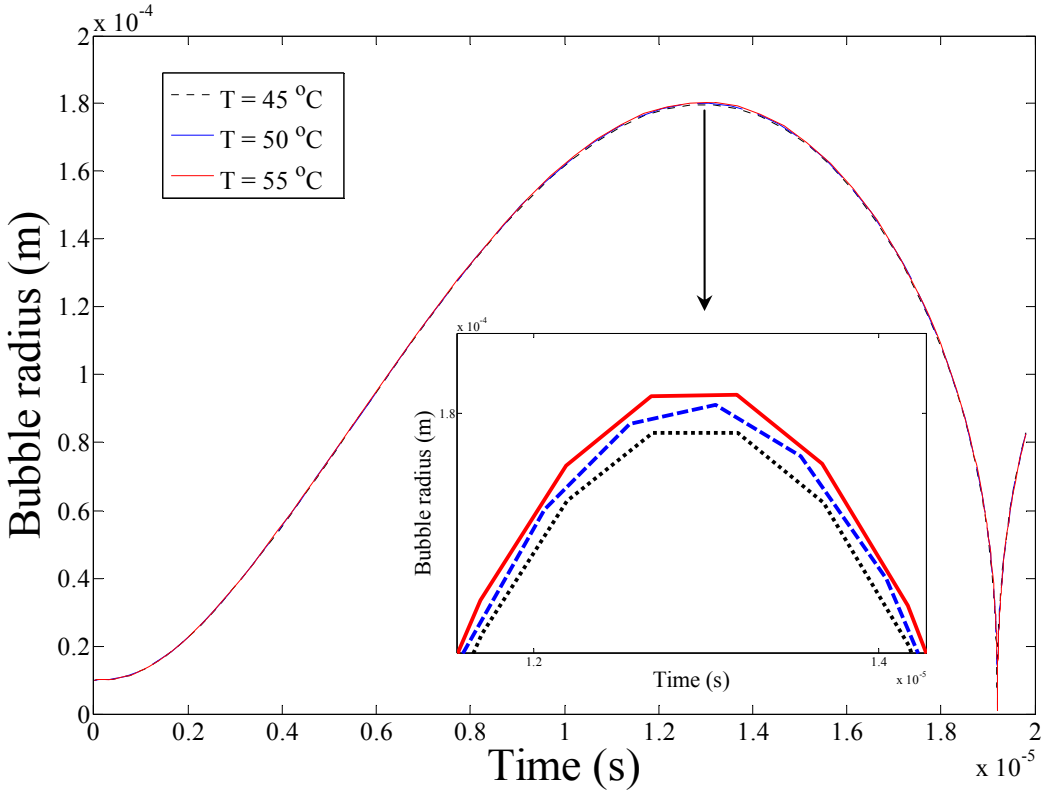


Figure 3.4: Evolution of bubble radius (R_i =0.01 mm) with ultrasonic intensity of 17.56 W/cm^2 and frequency of 55 kHz under atmospheric pressure ($P_\circ$ =101.3 KPa) and three different temperatures of 45, 50 and 55˚C

Table 3.2: R_{max} and R_{min} for the temperatures 45, 50 and 55˚C of thermosonication treatments

T (˚C)	$R_{max}\times10^{-4}$(m)	$R_{min}\times10^{-7}$(m)
45	1.797	8.6
50	1.801	8.590
55	1.804	8.581

Overall, increasing the temperature of the treated water in the range of 45 to 55˚C caused slight change (either increase or decrease) in the physio-chemical and thermo-physical properties of the treated water as presented in Table 3.1. The small change in the properties of the treated water led to a marginal change in the bubble growth (as illustrated in Figure 3.4), which signifies that temperature increase within the studied range has almost no effect on collapse pressure. The effect of increasing

the temperature of the treated water on the deactivation of *E. coli* in thermosonication treatments will be investigated experimentally in Chapter 4.

3.6 Effect of the applied pressure on collapse pressure

For meaningful analysis for the effect of increasing the applied pressure on the treated water in manosonication treatments on the deactivation efficiency of the treatments, it is imperative to study the effect of applied pressure increase on the collapse pressure in the first place. Thus, the influence of pressure increase of the treated water from 2 to 4 bars, on the collapse pressure, is theoretically studied in this section.

According to equations 3.5 and 3.6, changing the applied pressure on the treated water ($P_\circ$) can lead to changing the value of $P_{B,i}$ and as a consequence changes the value of R_{crtit} in the numerical simulation. The values of $P_{B,i}$ and R_{crtit} that correspond to each pressure were calculated (using equations 3.5 and 3.6) and substituted in the numerical simulation.

Figure 3.5 shows the effect of increasing the applied pressure on the treated water from 2 to 3 and 4 bars on the growth of a bubble under constant ultrasonic intensity of 17.56 W/cm^2 and frequency of 55 kHz. The effect of pressure change on bubble growth was theoretically investigated by assuming a constant ambient temperature.

Figure 3.5 shows that increasing the applied pressure on the treated water from 2 to 4 bars reduces the maximum radius of the bubble and subsequently increases the minimum radius of the bubble prior the implosion. The results in Figure 3.5 suggest that increasing the pressure of the treated water hinders the growth of the bubble and

forces the grown bubbles to collapse faster than normal under the atmospheric pressure. Such effects can lower the value of the produced pressure from bubble implosion. Lo″Rincz (2004) reported that increasing the pressure of the medium can increase the cavitation threshold which is defined as the limit of the medium pressure that needs to be exceeded by the amplitude pressure of ultrasound, in order for the acoustical cavitation to occur (Fry, 1978). Similarly, Raso et al. (1998), Thompson and Doraiswamy (1999) and Lee et al. (2009) showed in their studies that increasing the pressure of the medium beyond a certain limit can negatively affect the chemical and biological effect of ultrasound.

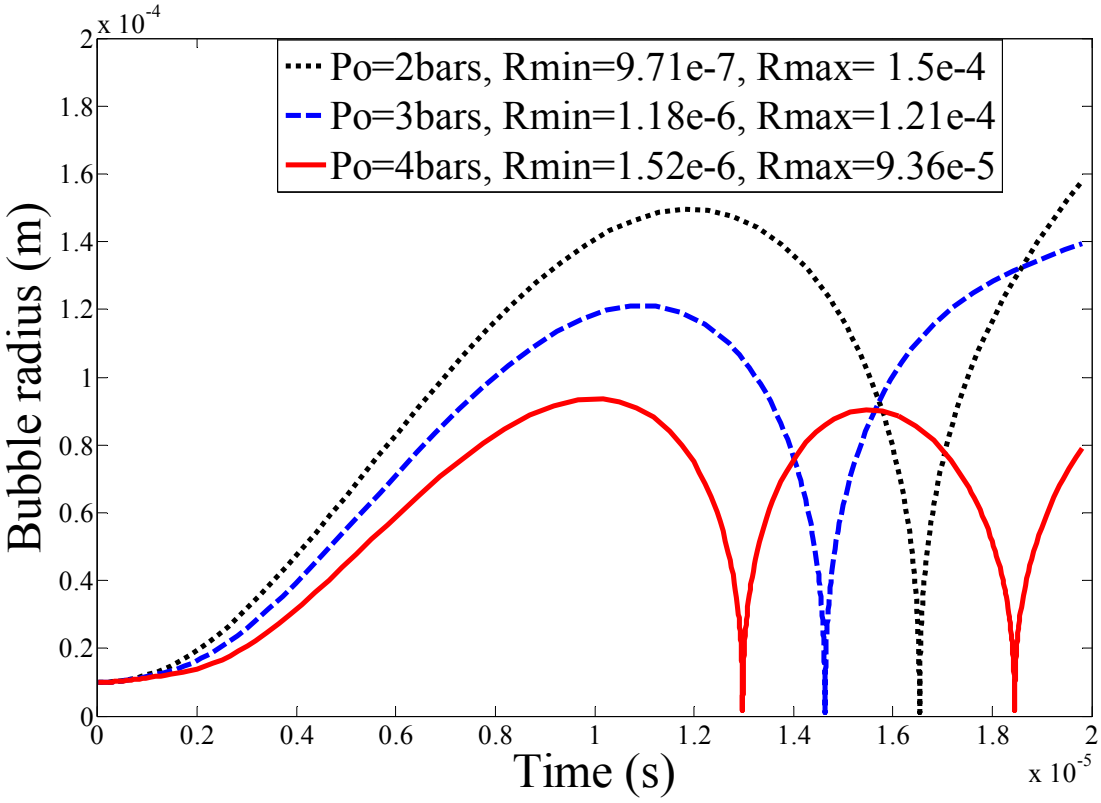


Figure 3.5: Evolution of bubble radius (R_i =0.01 mm) with ultrasonic intensity of 17.56 W/cm^2 and frequency of 55 kHz under ambient temperature (T=25˚C) and three different pressures of 2, 3 and 4 bars

In brief, the numerical solution for the Rayleigh-Plesset approach with three different levels of the applied pressure on the treated water showed that increasing

the applied pressure on the treated water negatively affect the growth of a bubble, and the collapse pressure. The effect of increasing the pressure of the treated water on the reduction of microorganisms will be thoroughly investigated in Chapter 4.

3.7 Conclusions

The theoretical study presented in this chapter involved exploring the effect of the process parameters of ultrasound treatments such as ultrasonic intensity, ultrasonic frequency, temperature and pressure of the treated water on the bubble growth as an indication of their effect on collapse pressure. The outcomes and the recommendations of the theoretical study are summarized as follows:

i) *Ultrasonic intensity:* Increasing ultrasonic intensity increases bubble growth and leads to decrease the minimum radius that the bubble can reach before the implosion. Such effects should increase the magnitude of the collapse pressure.

ii) *Ultrasonic frequency:* Bubbles generated in low ultrasonic frequencies grow to bigger size and eventually implode producing higher collapse pressure as compared to the bubbles generated in high ultrasonic frequencies.

iii) *Temperature increase:* Increasing the temperature of the treated water within the applied range has a marginal effect on bubble growth, therefore its effect on $P_{collapse}$ is expected to be slight.

iv) *Pressure increase:* Increasing the applied pressure on the medium adversely impacts on bubble growth, which in turn can have a negative effect on collapse pressure.

The effect of ultrasonic intensity, temperature and pressure of the treated water on the efficiency of ultrasonic deactivation of *E. coli* will be addressed in Chapter 4 of this thesis.

CHAPTER 4

Ultrasound treatment-experimental work

4.1 The aim of the chapter

The effect of the process parameters of ultrasound treatments namely intensity, frequency, pressure and temperature of the treated water on collapse pressure was investigated theoretically in Chapter 3. The theoretical study would carry less value without experimental work. Thus, a 55 kHz batch ultrasonic horn reactor with three levels of intensities; low (17.56 W/cm^2), intermediate (21.49 W/cm^2) and high (24.17 W/cm^2) was used to investigate the effect of ultrasonic intensity on the efficiency of ultrasonic deactivation of *E. coli* in water-based suspension. The effect of suspension temperature and pressure on *E. coli* deactivation was also examined through combining thermal treatment at the sub-lethal temperatures of *E. coli* of 45, 50, 55 and 60˚C and external pressures of 2, 3 and 4 bars with sonication treatments. In addition to ultrasonic intensity, temperature and pressure of the suspension, there are other intrinsic parameters of ultrasonic process that have significant effects on the ultrasonic deactivation of microorganisms. These parameters are treatment time and the arrangement of the ultrasound source in the suspension (Gogate and Kabadi, 2009; Hulsmans et al., 2009). Therefore, the effect of treatment time on the efficacy of *E. coli* deactivation using sonication, thermosonication and manosonication was considered within the range of 1 to 5 minutes. Prior conducting the experiments of sonication and thermosonication, the arrangement of ultrasonic horn probe was optimized, with regards to the radial direction, the vertical direction and the depth of the probe in *E. coli* suspension.

This chapter covers the following sections

1. Preparation of *E.coli* suspension in distilled water and viable cell count.
2. Description of ultrasound setups of sonication, thermosonication and manosonication treatments.
3. Optimization of the configuration of cavitation chamber (the beaker that carries *E. coli* suspension) for sonication and thermosonication set-ups.
4. Experimental procedures of sonication, thermosonication and manosonication treatments.
5. Investigation of the effect of the low osmolarity of the distilled water on the viability of *E. coli*.
6. Results and discussions of osmolarity test, sonication, thermosonication and manosonication treatments.
7. Comparison between sonication, thermosonication and manosonication in terms of energy consumption.
8. Conclusions

4.2 Preparation of water-based *E.coli* suspension and viable cell count

4.2.1 Preparation of E. coli water-based suspension

E. coli was selected to be the sample microorganism in this work owing to its widespread use as an indicator for microbial contamination in most water resources (Szewzyk et al., 2000), and its ability to adhere and subsequently develop biofilm on the RO membrane (Hori and Matsumoto, 2010; Danese et al., 2000).

A 75 µL of saline (autoclaved to 121˚C) was spread with 25 µL of *E. coli ATCC 25922* stock culture (cooked meat medium) on a Difco™ Nutrient Agar plate and was then incubated at 37˚C for 24 hours. Thereafter, the prepared plate was

washed with 2 mL of sterilised saline and then small amount of the suspending fluid was diluted with 10 mL of sterilised saline to prepare the inoculum of *E. coli*. The concentration of *E. coli* in the prepared inoculum was estimated by measuring the optical density of the inoculum at 625 nm (OD_{625}) using a Hach DR/2000 direct reading spectrophotometer. Saline was used as a blank in the spectrophotometer measurements. According to the Clinical and Laboratory Standards Institute (CLSI) standard (Clsi, 2006), when the optical density of the inoculum reaches between 0.08 and 0.13, this means that the concentration of *E. coli* in this inoculum is equivalent to approximately 10^8 CFU/mL. One millilitre of the prepared inoculum was added to 200 mL of distilled water to obtain suspension with concentration of *E. coli* about 10^6 CFU/mL.

4.2.2 Viable cell count

The viable cell count technique was adopted in the present work, to determine the concentration of *E. coli* before and after ultrasound treatments due, to its reliability in measuring the CFU per millilitre of the suspension after ultrasound treatment (Mason et al., 2003). The concentration of *E. coli* in the untreated suspension expressed in CFU/mL was initially evaluated using the optical density measurement within the range of 0.08–0.13 as explained in Section 4.2.1.

In the viable cell count procedure, the actual concentration of *E. coli* in the prepared suspension was measured by diluting 0.01 mL of untreated suspension with 10 mL of saline. Following this step, 0.1 mL of the diluted suspension was spread onto three plates of Difco™ Nutrient Agar. The three plates were then incubated at 37 ˚C for 18-24 hours. After the incubation time, the grown colonies on the plates were counted using a SUNTEX colony counter model 570 with electronic register as

shown in Figure 4.1. The number of colonies of the untreated suspension was obtained by taking the average of the colonies on the plates.

Figure 4.1: SUNTEX colony counter

The concentration of *E.coli* in the treated suspension was measured by applying appropriate serial 5-fold dilutions of the treated suspension in the viable cell count. The procedure of the viable cell count with 5-fold dilution series for the treated suspension is summarised by the following steps:

1) Five nutrient agar plates were prepared.
2) A 0.1 mL of the treated suspension was added to the first plate.
3) A 0.9 mL of the saline was added to 4 centrifugal tubes with volume of 1.5 mL, and the tubes were numbered to differentiate between the degree of concentration of each tube.
4) A 0.1 mL of the treated suspension was added to the first centrifugal tube.
5) After vortexing the mixture from step 4 vigorously, a 0.1 mL of the mixture was transferred to tube 2. The same procedure was repeated for the tubes 2, 3 and 4.
6) After getting all the tubes mixed well, a 0.1 mL of each tube was added to the agar plates, and then spread using a metal spreader.

7) The prepared plates were incubated at 37˚C for 18-24 hours and then counted.

To make sure there is no contamination occurred during ultrasound treatments, samples of the treated suspension were cultured in MacCONKEY agar. This agar displays the grown colonies of *E. coli* with a pink colour while other microorganisms are displayed in other colours (as illustrated in Figure 4.2). Additionally, triplication for the plates of the entire dilution series was applied to improve the accuracy of the viable cell count.

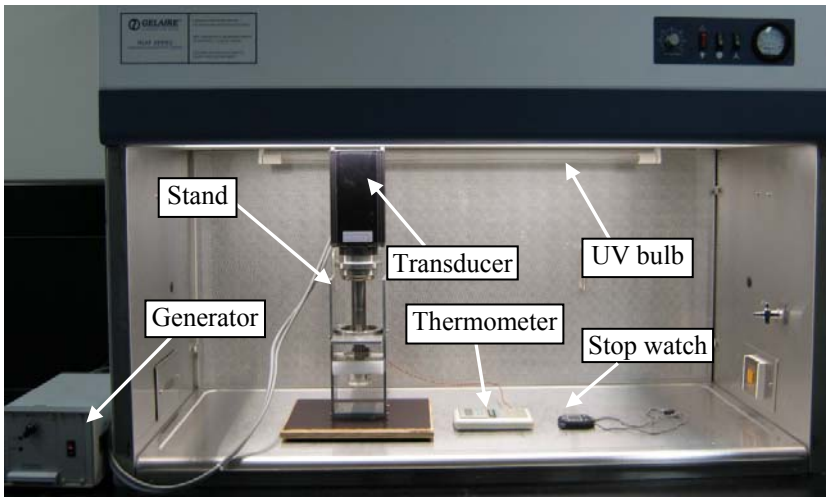
Figure 4.2: *E. coli ACCT 25922* colonies as displayed on CM0007 MacCONKEY agar

4.3 Description of sonication, thermosonication and manosonication set-ups

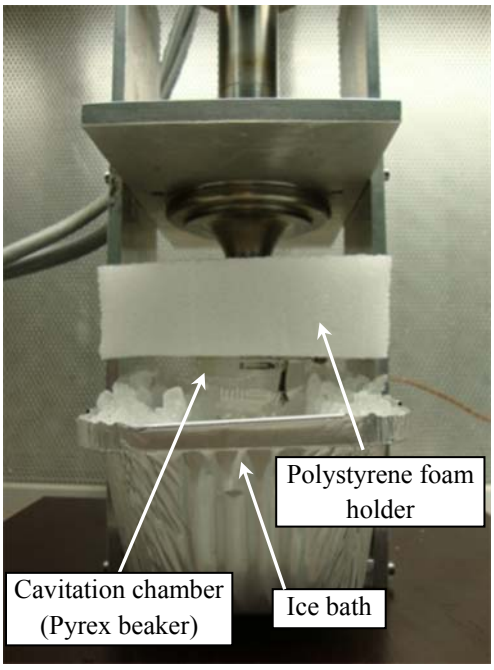
All the set-ups used to perform the experiments of sonication, thermosonication and manosonication are batch configuration set-ups. The batch configuration was selected in this study owing to the simplicity of controlling the process parameters of ultrasound treatments such as temperature, pressure and treatment time in this configuration.

4.3.1 Sonication set-up

The sonication set-up used to perform sonication experiments is presented in Figure 4.3. The ultrasound generator was placed outside the laminar flow, while the transducer rested on an aluminium stand inside the laminar flow.



(a)



(b)

Figure 4.3: Sonication setup (a) the entire setup inside the laminar flow (b) enlarged view of the beaker immersed in the ice bath

A Pyrex beaker was used as a cavitation chamber to carry the suspension of *E. coli* during the treatment. Before using the beaker for carrying *E. coli* suspension, the

beaker was autoclaved at 121˚C for 15 minutes. This beaker was fitted between two plates of the aluminium stand using a fitted holder made from foamed polystyrene (as illustrated Figure 4.3 b). The treatment time and the temperature of the treated suspension were measured using a stop watch and *DiGi-Sense* thermometer type K.

4.3.2 Thermosonication set-up

Thermosonication set-up is consisted of two elements: ultrasound set-up shown in Figure 4.3 and hot water bath used to heat *E. coli* suspension prior sonication treatment to the temperatures of 45, 50, 55 and 60˚C at the atmospheric pressure. The hot water bath and the ultrasound transducer were placed in the laminar flow, whereas the generator placed outside the laminar flow.

4.3.3 Manosonication set-up

In manosonication set-up, a steel chamber was used to carry the suspension instead of a beaker as illustrated in Figure 4.4.

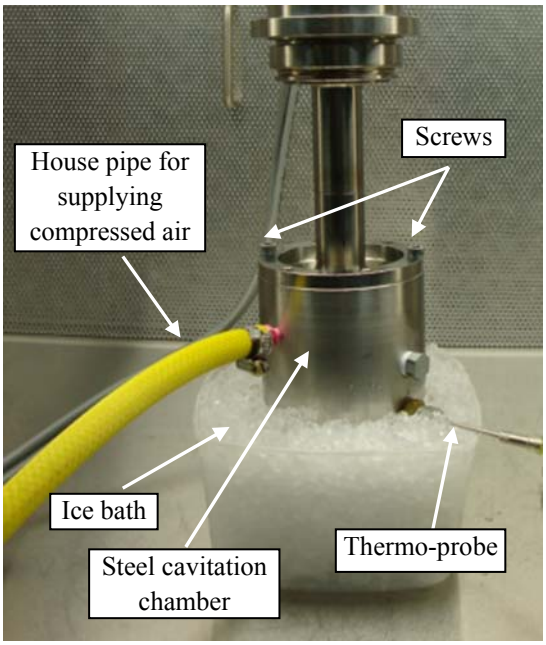


Figure 4.4: Enlarged view of cavitation chamber used in manosonication treatment

The chamber was pressurized with compressed air to three pressures: 2, 3 and 4 bars. The temperature of the treated suspension was monitored using a thermo-probe connected to *DiGi-Sense* thermometer type K. The whole setup was placed inside the laminar flow while the generator and the compressed air source were placed outside the laminar flow.

4.4 Optimization of the configuration of cavitation chamber for sonication and thermosonication set-ups

A commercial ultrasonic horn device (HielscherUIP500) with a 22 mm diameter horn tip (sonotrode, BS20d22 titanium) and a 55 kHz frequency was used to conduct the experimental work of sonication, thermosonication and manosonication. Prior using the ultrasound set-up for sonication and thermosonication experiments, the configuration of cavitation chamber was optimized. The features of the cavitation chamber that were optimized are:

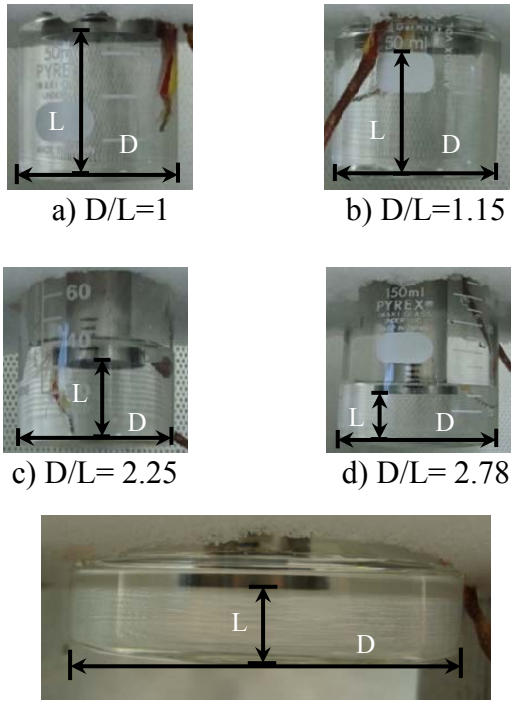
1. The ratio of beaker diameter to the vertical distance of the ultrasonic probe in the *E. coli* suspension (D/L).
2. The depth of ultrasonic probe in the *E. coli* suspension.

The optimization of cavitation chamber was performed using ultrasonic intensities of 17.56, 21.49 and 24.17 W/cm^2 for fixed treatment time of 4 minutes.

4.4.1 Optimizing the ratio of chamber diameter to vertical distance (D/L)

The process of selecting the optimum D/L ratio of the chamber used in sonication and thermosonication treatments was conducted using five Pyrex beakers with different diameters of 3.6, 3.8, 4.5, 5 and 6.5 cm. Changing the diameter of the chamber (D) that carried a constant volume of *E.coli* suspension (50mL) at horn

depth of 2 mm required changing the vertical distance between the irradiating surface of the probe and the bottom of the chamber (L) as presented in Figure 4.5.



a) D/L=1 b) D/L=1.15

c) D/L= 2.25 d) D/L= 2.78

e) D/L= 5.9

Figure 4.5: D/L ratios of five different beakers

The vertical distance of the ultrasonic probe in *E. coli* suspension was maintained within the effective axial range of ultrasonic horn reactor (between 0 to 4 cm) as reported by Gogate et al. (2002). The diameters with their corresponding vertical distance and D/L values are shown in Table 4.1.

Table 4.1: Beakers' radius with their corresponding vertical distance

Diameter (D) (cm)	vertical distance (L) (cm)	D/L
3.6	3.6	1
3.8	3.3	1.15
4.5	2	2.25
5	1.8	2.78
6.5	1.1	5.9

4.4.2 Effect of (D/L) on the viability of E. coli

The effect of increasing the D/L of the cavitation chamber on the reduction of *E.coli* at three ultrasonic intensities: 17.56, 21.49 and 24.17W/cm^2 for treatment time of 4 minutes was investigated and the results are presented in Figure 4.6. The solid line in Figure 4.6 depicts the reduction of *E. coli* for an intensity of 24.17 W/cm^2, whereas the dotted and the dashed lines in the same figure represent the intensities of 21.49 and 17.56 W/cm^2 respectively. The values of *E. coli* log reduction presented in Figure 4.6 and the other figures in this chapter are expressed by the mean value of the viable cell count of three plates and the error bars represent the standard error of the mean. It should be noted here that the variation in the error bars in Figure 4.6 and the other figures in chapter 4 and 5 that present log reduction or log survival of *E. coli* is attributed to the nature of the microbiological work.

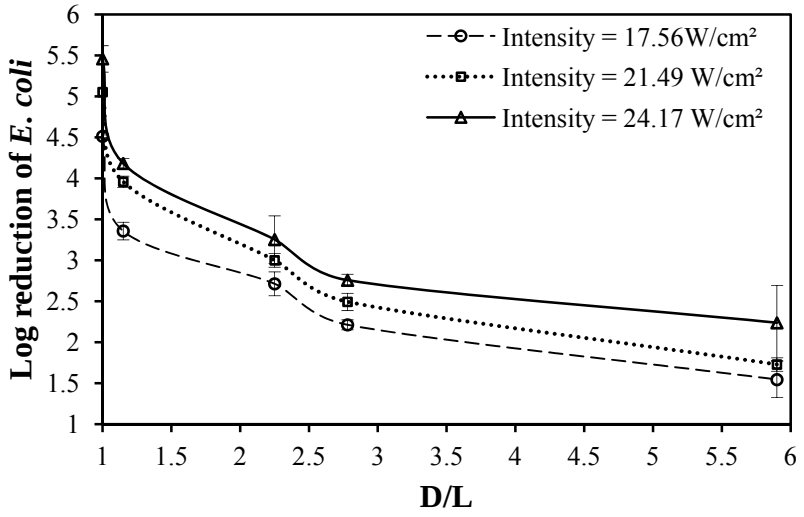


Figure 4.6: Log reduction of *E.coli* with different D/L ratio for three ultrasonic intensities (○) 17.56, (□) 21.49 and (△) 24.17 W/cm^2 and treatment time of 4 minutes

The general trend of Figure 4.6 suggests that increasing the D/L within the effective axial distance of the ultrasonic horn reactor led to a decrease in the log reduction of *E. coil*. The results in Figure 4.6 shows that the maximum reduction of

E.coli was achieved using cavitation chamber with D/L equal to 1, while the minimum reduction of *E. coli* was attained using cavitation chamber with D/L equal to 5.9. The reduction of *E. coli* for the other configurations of the cavitation chamber falls between the reductions of D/L=1 and D/L=5.9. The decrease in the log reduction of *E. coli* with increasing the D/L of the cavitation chamber can be attributed to the fact, that the cavitational effects of ultrasonic horn reactor are limited to a small area underneath the horn. These results are in agreement with the results obtained by Gogate et al. (2002), as the latter confirmed that the mechanical and the chemical effects of ultrasound for ultrasonic horn reactor occur effectively under the centre of the horn tip and started to decrease away from the horn centre up to a distance of 3 cm. Beyond this limit, the effect of ultrasound is insignificant.

The process of optimizing the D/L of the cavitation chamber shows that the smaller the D/L of the chamber, the higher reduction of *E. coli* obtained. However, the rate of temperature rise of the suspension due to ultrasound treatment in the cavitation chamber with small D/L was significantly quicker than that of large D/L, which can be a problem. It is worth mentioning that temperature rise in the cavitation chamber with small D/L can cause some problems such as the requirement for an extensive cooling process and giving misleading results of ultrasound treatments. The increase in the temperature of the treated suspension can affect the viability of microorganisms which makes it hard to identify and pinpoint the actual reduction of microorganisms caused by ultrasound. Condón et al. (2005) suggested that the change in the outer membrane of the gram-negative microorganisms due to the thermal effect of ultrasound can be a possible reason for the synergistic deactivation of the microorganisms.

Since, the use of cavitation chambers with small D/L for deactivating *E. coli* is associated with some technical problems, and the use of cavitation chambers with large D/L for the same purpose result in low reduction of *E. coli*, a cavitation chamber with an intermediate value of D/L (D/L=2.25) was chosen to be used in subsequent ultrasound treatments.

4.4.3 Depth of ultrasonic horn probe

The depth of the ultrasonic horn probe in the suspension of *E. coli* is another parameter that is associated with the configuration of the cavitation chamber of sonication and thermosonication set-ups. The effect of this parameter on the reduction of *E.coli* was investigated within a depth range of 2 to 10 mm. The ultrasonic horn was set on an aluminium stand at fixed level, while the depth of the horn probe in the suspension was changed by moving the beaker up with a step of 2 mm. The optimization of the probe depth in the *E. coli* suspension was conducted using the chosen cavitation chamber from Section 4.3.2 and a constant suspension volume of 50 mL.

4.4.4 Effect of probe depth on the viability of E. coli

The influence of increasing the depth of the horn probe from 2 to 10 mm on the viability of *E.coli* at three ultrasonic intensities of 17.56, 21.49 and 24.17W/cm^2 for treatment time of 4 minutes was examined and the results are shown in Figure 4.7. The log reductions of *E. coli* presented in Figure 4.7 for the intensities of 24.17, 21.49 and 17.56 W/cm^2 are plotted as solid, dotted and dashed lines respectively. The results presented in Figure 4.7 manifests that increasing the depth of the probe from 2 to 10 mm resulted in a marginal effect on the log reduction of *E.coli* of approximately 0.5 $\log_{10}$for all the applied ultrasonic intensities.

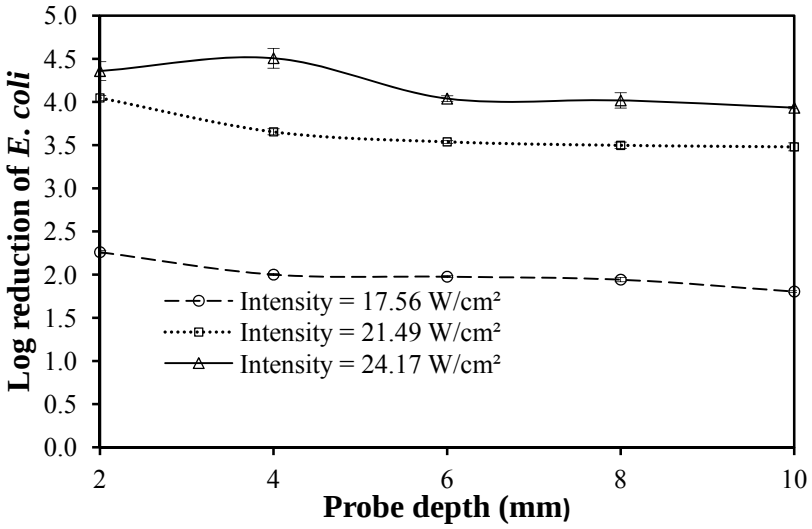


Figure 4.7: The change in log reduction of *E.coli* versus probe depth for three ultrasonic intensities; (○) 17.56, (□) 21.49 and (△) 24.17 W/cm2 and treatment time of 4 minutes

From Figure 4.7, it can be seen that the log reduction of *E.coli* of 17.56 and 21.49 W/cm^2 decreased with increasing the depth of the probe in the suspension from 2 to 10 mm. This could be attributed to the increase in the lost volume of the suspension that is not subjected to ultrasound as the probe depth increases (as illustrated in Figure 4.8).

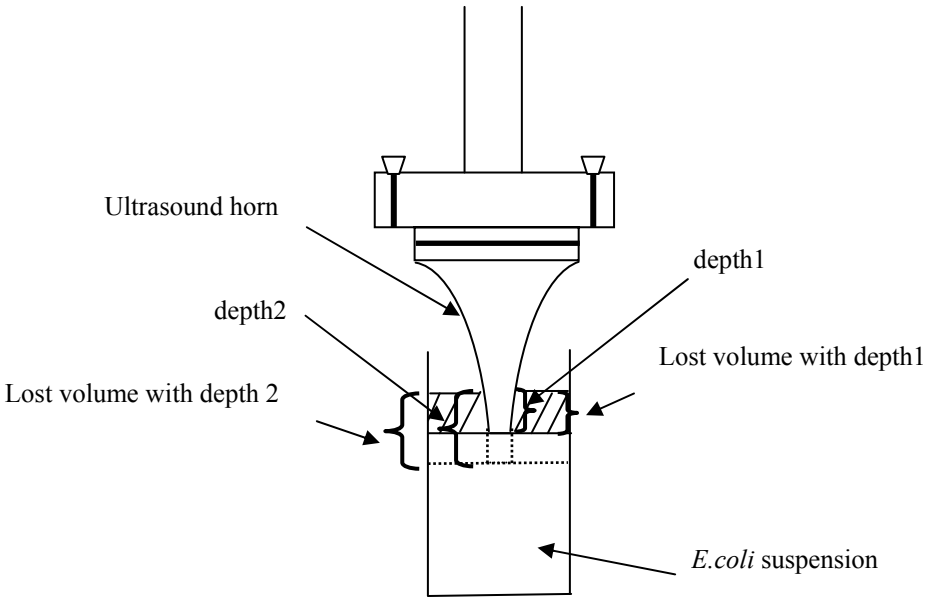


Figure 4.8: Demonstration for the effect of probe depth on the lost volume of the suspension

The cells of *E. coli* that are floating in the lost volume of the suspension are exposed to a lesser effect of acoustic cavitation as compared to the ones beneath the radiating surface. Many studies conducted on mapping ultrasonic horn reactors, such as Chivate and Pandit (1995), Romdhane et al. (1995a), Romdhane et al. (1997) and Gogate et al. (2002), confirmed that the ultrasonic activities of the horn reactor occur in the area below the irradiating surface of the horn.

Log reduction of *E. coli* for the intensity of 24.17 W/cm^2 increased when increasing the depth of the probe in the suspension from 2 to 4 mm and then decreased with increasing the depth from 4 to 10 mm. This can be explained as subjecting the suspension, with a small volume of 50 mL, to ultrasonic intensity of 24.17 W/cm^2 was found to cause high level of turbulences in the suspension, especially at the surface. When the depth of the ultrasonic probe is as small as 2 mm, the turbulences at the suspension surface would allow the air from the atmosphere to penetrate into the suspension and cause the formation of large air bubbles near the irradiating surface (as presented in Figure 4.9). The formation of large bubbles near the ultrasound horn presented in Figure 4.9 was captured using a SONY hybrid Handycam. The presence of the large bubbles in the affinity of the irradiating surface could hinder the transition of ultrasonic energy into the suspension. The increase in the log reduction of the intensity 24.17 W/cm^2 at the depth of 4 mm suggests that, at this depth, ultrasonic energy can easily transmit into the suspension body without obstacles (formation of large bubbles). This discussion is valid when the ultrasonic horn reactor used to treat a solution is open to the atmosphere.

The outcome of investigating the effect of ultrasonic probe depth on the reduction of *E. coli* showed that the smaller the depth of the probe, the higher the reduction of *E. coli* obtained for low and intermediate ultrasonic intensities.

However, in the case of high ultrasonic intensity, the probe has to be immersed to a depth that is sufficient to prevent any air penetration from the atmosphere.

Figure 4.9: Generation of large air bubbles in the suspension with ultrasonic intensity of 24.17 W/cm^2

4.5 Experimental procedures of sonication, thermosonication and manosonication

4.5.1 Osmolarity tests

Prior the experiments of sonication, manosonication and thermosonication, testing for the effect of distilled water osmolarity on the viability of *E. coli* were conducted. *E. coli* was suspended in distilled water for 5 minutes during which samples of 2 mL of the suspension were taken at 1, 2, 3, 4 and 5 minute to be cultured using nutrient agar plates. The plates were incubated at 37˚C for 24 hours and later the colonies were counted as explained in Section 4.2.2.

4.5.2 Sonication experiments

Prior to performing any experiments, the following precautions were adopted to avoid possible contamination:

1. UV light in the laminar flow was switched on for one hour before starting the experiments to sterilise the air inside the laminar flow.

2. Ethanol 75% (volumetric percentage) was used to sterilise the steel bottom of the laminar flow.
3. The Ultrasound horn probe and the probe of thermometer were sterilised with ethanol.

The Pyrex beaker selected through the optimization process was used to hold the suspension. The beaker was filled with 50 mL of *E. coli* suspension and placed in between the plates of the aluminium stand (Figure 4.7 b). After that, the horn probe of the ultrasound device was immersed in two different depths in the suspension depending on the supplied ultrasonic intensity. The depth of the ultrasonic horn probe was 2 mm in the case of low and intermediate ultrasonic intensities (17.56 and 21.49) W/cm^2, and 4 mm in the case of high ultrasonic intensity (24.17 W/cm^2).

The pressure of the suspension in sonication treatment was constant at the atmospheric pressure as the suspension was exposed to the atmosphere, while the temperature of the suspension was varied due to ultrasound treatment. The temperature was controlled using an ice bath set below the suspension beaker to cool down the suspension and maintain the temperature to less than 40˚C.

The generator of the ultrasonic device was operated at the targeted intensities for 5 minutes during which a 2 mL sample of the treated suspensions was withdrawn at 1, 2, 3, 4 and 5 minutes for analyses. It is noteworthy to mention here that pre-tests on using sonication treatments for a longer time (6 to 10 minutes) were conducted to set a timeframe for the experimental work. It was found that 6 minutes of treatment time, even with the lowest ultrasonic intensity of 17.56 W/cm^2, was sufficient to obtain a complete reduction of *E. coli*. Therefore, treatment time for sonication, thermosonication and manosonication in this study was limited to the time intervals of 1, 2, 3, 4 and 5 minutes.

4.5.3 Thermosonication experiments

The procedure of sonication experiments was repeated here with an additional step which involved pre-heating the suspension of *E. coli* to four different temperatures, 45, 50, 55 and 60˚C. The lethal effects of thermosonication on the microorganisms can be attributed to two effects; thermal effect and the effect of ultrasound. To differentiate between these two effects, thermal tests at the temperatures of 45, 50, 55 and 60˚C for one minute were conducted. Samples of the treated suspension by thermal treatments were analysed using viable cell count.

4.5.4 Manosonication experiments

A steel chamber was filled with 50 mL of *E. coli* suspension and then assembled with the ultrasound horn as illustrated in Figure 4.10.

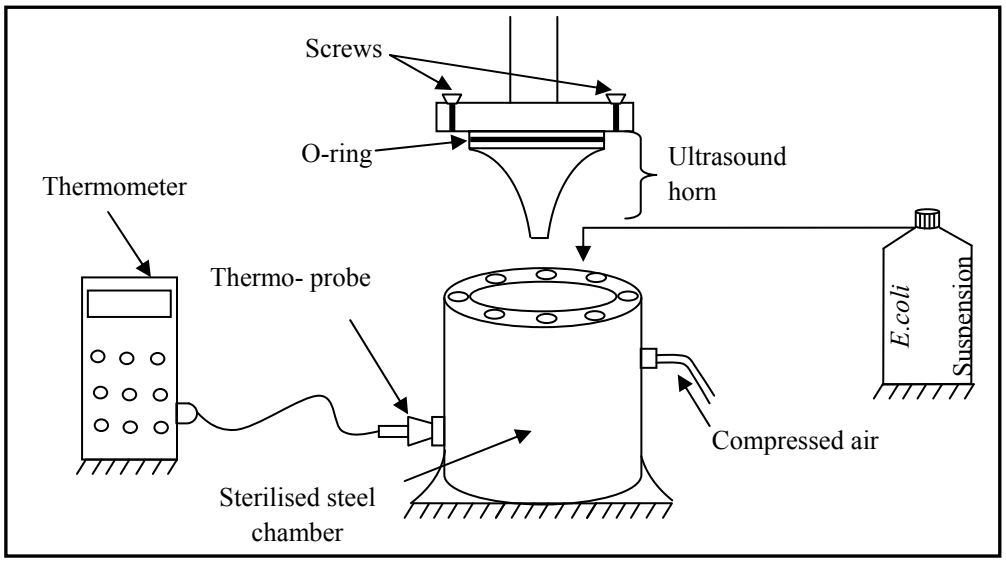


Figure 4.10: Schematic demonstrates the procedure of manosonication experiments

The chamber was sterilised by ethanol 75% before it was used in manosonication experiments. The periphery of the ultrasound horn is surrounded by a rubber O-ring to prevent the leakage of the supplied air when pressurizing the

chamber. The assembly of the ultrasound horn and the steel chamber was tightened with screws and the compressed air was supplied through a hosepipe with different pressures of 2, 3 and 4 bars. For monitoring and controlling the temperature rise, the same procedure as that of sonication and thermosonication was followed.

4.6 Results and discussions

4.6.1 Effect of distilled water osmolarity on the viability of E. coli

The medium that was used in this work to dissolve the colonies of *E. coli* is distilled water. Since distilled water has low osmolarity about 0 osmol/kg (Perchec et al., 1996), it is expected that this low level of osmolarity can affect on the viability of *E. coli*. Bayer (1967) found that suspending *E. coli* in a low osmolarity medium resulted in generating mechanical stress on the cell wall of *E. coli*. The stress generated on the cell wall is attributed to the inflow of water in the low osmolarity medium into the protoplasm, which in turn can cause swelling of the plasma membrane (Bayer, 1967). The expansion in the size of the protoplasm applies a pressure on the cell wall causing either osmotic injury (Scheie, 1973) or osmotic death (Bayer, 1967) of *E. coli* cell. Therefore, it is advisable to specify the lethal effect of the medium osmolarity on *E. coli* cell before commencing ultrasound treatments. This will help avoid confusion between the lethal effects of ultrasound with that of medium osmolarity.

The results of suspending *E. coli* in distilled water for treatment time intervals of 1, 2, 3, 4 and 5 minutes with no ultrasound treatments are presented in Table 4.2. The values of *E. coli* concentration in Table 4.2 are expressed as a mean value with the standard of error for plate triplication. Table 4.2 illustrates that increasing the treatment time within the time frame of the experimental work (1 to 5 minutes) can

reduce the viability of *E. coli* due to the osmolarity of the distilled water. The maximum reduction of *E. coli* that was caused because of the osmotic death was about 0.05 $\log_{10}$ for a treatment time of 5 minutes, which is considered to be very small compared to the initial concentration of *E. coli*. It can be said that the contribution of distilled water osmolarity to the death of *E. coli* within the range of treatment time of 5 minutes is marginal.

Table 4.2: Change in the viability of *E. coli* in distilled water with no ultrasound treatments

Time (min.)	Log reduction of *E. coli* (CFU/mL)
0	6.34 ± 0.059
1	6.34± 0.027
2	6.325± 0.015
3	6.31± 0.023
4	6.3± 0.022
5	6.29± 0.04

4.6.2 Sonication treatments

The log reductions of *E. coli* at three different ultrasonic intensities of 17.56, 21.49 and 24.17 W/cm^2 for treatment time intervals of 1, 2, 3, 4 and 5 minutes are presented in Figure 4.11. In general, increasing ultrasonic intensity increases log reduction of *E. coli* and these results confirm the theoretical expectations presented in Chapter 3, Figure 3.2 and consistent with the reported results in the literature. Furuta et al. (2004) reported that the inactivation rate of *E. coli* is directly proportional with ultrasonic amplitude. Hulsmans et al. (2009) demonstrated that increasing ultrasonic power increases log reduction of *E. coli*. Another study conducted by Al Bsoul et al. (2010) showed that increasing ultrasonic density (the supplied ultrasonic power divided by the volume of the treated suspension) led to an increase of the removal percentage of *Mycobacterium* sp.6PY1.

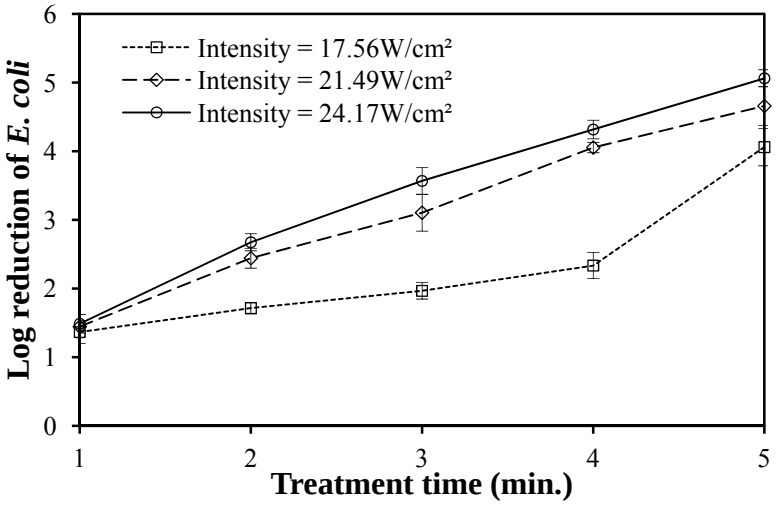


Figure 4.11: Log reduction of *E. coli ATCC 25922* versus treatment time for sonication *E. coli* suspension with different intensities of 17.56, 21.49 and 24.17 W/cm^2 under temperature range of 21 to 40˚C and atmospheric pressure (complete elimination of *E. coli* was achieved at all of the used intensities with treatment time of 6 minutes)

The results in Figure 4.11, the *E. coli* log reduction obtained with 17.56 W/cm^2 was lower than that of 21.49 and 24.17 W/cm^2 by a noticeable difference. In comparison, the *E. coli* log reduction of 21.49 W/cm^2 was slightly less than the log reduction of 24.17 W/cm^2 during different treatment time intervals. It can hence be said that, the ultrasonic intensity of 21.49 W/cm^2 represents the optimum intensity for the ultrasonic device used in this study, and increasing the intensity to a level higher than 21.49 W/cm^2 has insignificant effect on the log reduction of *E. coli*. These results agree with the results obtained by Sivakumar and Pandit (2001) and Contamine et al. (1995) who found the ultrasonic intensity has an optimum limit after which any further increase in the intensity will have an insignificant effect on the produced ultrasonic activities. This could be attributed to the fact that increasing the ultrasonic intensity more than its optimum level will generate a cloud of bubbles near the irradiating surface that impairs the transition of ultrasonic energy into the

body of the suspension (Thompson and Doraiswamy, 1999; Gogate and Pandit, 2000).

When comparing the results obtained in sonication experiments with the theoretical results presented in Chapter 3 (Figure 3.2), it can be noted that the values of R_{max} and R_{min} for 21.49 and 24.17 W/cm^2 are close, and thus, the magnitude of $P_{collapse}$ for these two intensities should be close. Since the shock pressure of ultrasound has the dominant role in ultrasound deactivation (Yusaf, 2011), the intensities that have close values of $P_{collapse}$ should have the same opportunities of rupturing the cell of *E. coli* and this explains the results in Figure 4.11.

The effect of treatment time on the log reduction of *E. coli* in sonication experiments showed that increasing the treatment time for ultrasonic intensity of 17.56 W/cm^2 has a slight effect on the log reduction of *E. coli* <1 $\log_{10}$ over a period of 1 to 4 minutes. However beyond this time range, increasing the treatment time resulted in a pronounced increase in the log reduction of *E. coli* by almost 1.8 $\log_{10}$. The significant increase in the reduction of *E.coli* for the low ultrasonic intensity after 4 minutes could be attributed to an increased degree of cell damage after this time. Allison et al. (1996) demonstrated using photos of electron microscopy that, 50% of the *E. coli* cells treated with low ultrasonic power for 4 minutes, experienced a severe damage in their cell wall. In contrast, increasing the treatment time for the ultrasonic intensities of 21.49 and 24.17 W/cm^2 causes a moderate increase in the order of 1 $\log_{10}$ for treatment time intervals of 2 to 5 minutes.

Overall, the results obtained from sonication experiments suggest that ultrasonic intensity has an optimum level after which any increase in the intensity does not result in a significant increase in the reduction of microorganisms. Increasing the treatment time for high and intermediate ultrasonic intensities led to a

moderate and gradual increase in the log reductions of *E. coli*. Increasing the treatment time to more than 4 minutes for low ultrasonic intensities resulted in a significant increase in the log reduction of *E. coli*.

4.6.3 Thermosonication treatments

Combining thermal treatment with sonication treatment can improve microbial deactivation of sonication treatment through the effect of temperature rise on bubble collapse and the lethal and sub-lethal effects of thermal treatment on *E. coli*. The effect of increasing the temperature from 45 to 55˚C on the collapse pressure of the bubbles was studied in Chapter 3 Section 3.5 (Figure 3.4). The results demonstrated that the increase in the temperature within the studied range (45 to 55˚C) has a marginal effect on the intensity of bubble collapse. To identify the lethal effect of thermal treatment on *E. coli* in thermosonication experiments, diagnostic thermal tests for *E. coli* suspension at 45, 50, 55 and 60˚C for one minute were conducted.

The results of thermal tests showed that heating the suspension of *E. coli* to 45˚C did not result in any detected reduction of *E. coli*. In comparison, treating the suspension of *E. coli* with thermal treatment at 50, 55 and 60˚C led to1.1±0.031, 1.576±0.047 and 5.385±0.021 log reductions respectively. The values of *E. coli* log reductions of the thermal tests are expressed in the mean value of plate triplication with the standard of error of the mean. The lethal and sub-lethal effects of thermal treatment on *E. coli* are attributed to the detrimental effect of temperature rise on the lipid element of the *E. coli* outer membrane (Gusbeth et al., 2009). Such effects may cause thinning of the membrane which in turn can lead to pore formation on the membrane surface (Shillcock and Seifert, 1998). When the pores formed on the

membrane surface, *E. coli* may lose some of the intracellular contents that could lead to the injury or death of *E. coli*.

Treating the suspension of *E. coli* with thermosonication of 60˚C achieved complete elimination of *E. coli* (reduction of 6 $\log_{10}$) even with the least treatment time of 1 minute using different ultrasonic intensities of 17.56, 21.49 and 24.17 W/cm^2. From the results of thermosonication and thermal treatments at 60˚C for 1 minute (5.385±0.021 log reduction of *E. coli*), it can be noticed that the log reduction of thermal treatment is very close to that of thermosonication. This means that the effect of thermal treatment at 60˚C on *E. coli* is almost equal to that of thermosonication treatment at the same temperature.

Thermosonication treatment at 55˚C and different ultrasonic intensities for 2 minutes resulted in complete elimination of *E. coli*. Whilst 1 minute of thermosonication treatment at 55˚C resulted in 1.9±0.036, 2.5±0.1 and 2.97±0.097 log reductions of *E. coli* for the intensities of 17.56, 21.49 and 24.17 W/cm^2 respectively. Log reduction of *E. coli* for the thermal treatment at 55˚C for 1 minute is less than that of thermosonication treatment at 17.56 W/cm^2 for the same temperature by a slight difference of approximately ≤ 0.4 $\log_{10}$. In contrast, the log reductions of thermosonication treatments at 55˚C and ultrasonic intensities of 21.49 and 24.17 W/cm^2 were higher than that of the thermal treatment by a significant difference of approximately ≤ 1.5 $\log_{10}$.

Thermosonication treatments at low sub-lethal temperatures of *E. coli*, 45 and 50˚C respectively needed more treatment time to achieve complete elimination of *E. coli*. The results of using thermosonication treatments at 45 and 50˚C are shown in Figure 4.12.

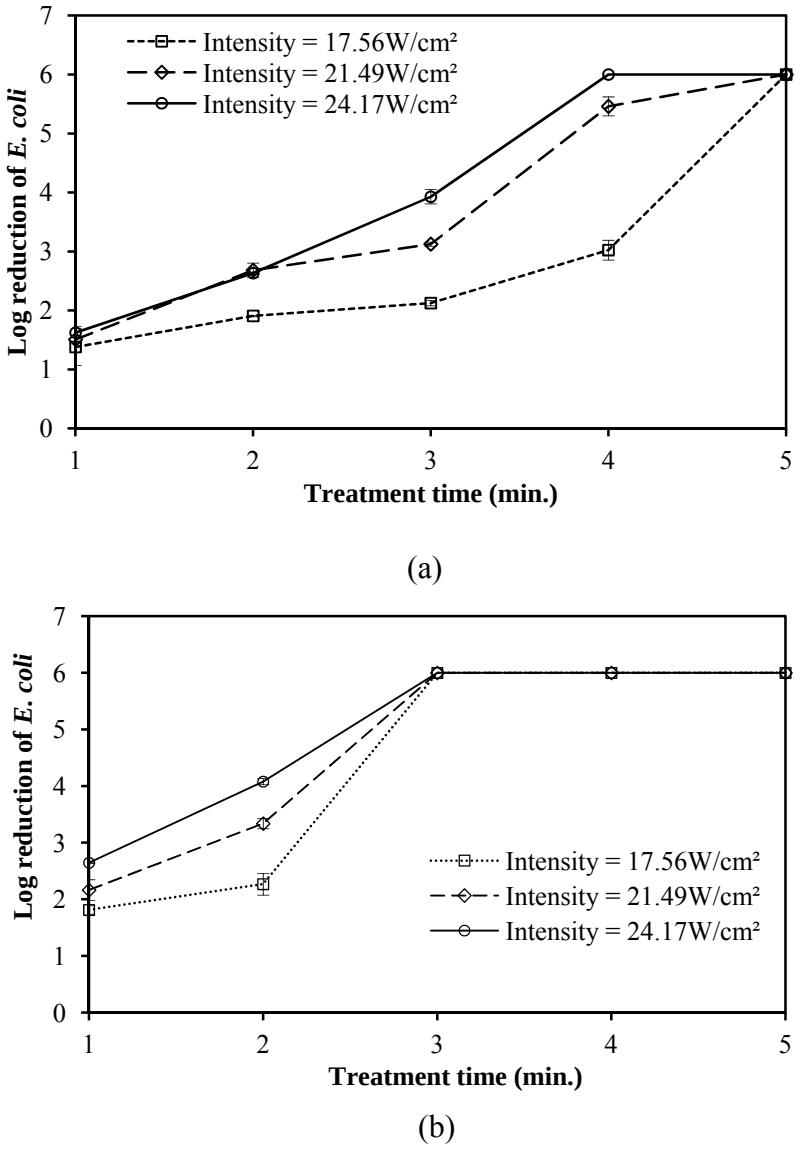


Figure 4.12: Log reduction of *E. coli ATCC 25922* versus treatment time for sonication *E. coli* suspension with different intensities of 17.56, 21.49 and 24.17 W/cm^2 at (a) 45 and (b) 50˚C with an atmospheric pressure

It can be seen from Figure 4.12 that increasing the temperature of the suspension reduces the required treatment time for complete reduction of *E. coli*. In the thermosonication treatment at intensities of 17.56 and 21.49 W/cm^2 and 50˚C, 3 minutes of treatment time was found to be sufficient to attain complete killing of *E. coli*. In comparison, the complete elimination of *E. coli* in thermosonication at 17.56 and 21.49 W/cm^2 and 45˚C was obtained at 5 minutes. These results are consistent

with Lee et. al.'s (2009) results. They reported that thermosonication treatment can shorten the required time to achieve a certain log reduction of *E. coli*.

The results of thermosonication treatments suggest that the increase in the log reduction of *E. coli* for thermosonication treatments as compared to sonication, is attributed mainly to the destructive effect of the heat on the structure of *E. coli*. The results also show that the lethal effect of thermal treatment on the viability of *E. coli* become very obvious at high sub-lethal temperatures of 55˚C and above. Raso et al. (1998) reported that the time required to deactivate 90% of *Yersinia enterocolitica* using manothermosonication (sonication combined with pressure and temperature) at 58˚C is equal to that of thermal treatment. This study recommends that the temperature level in thermosonication treatment for *E. coli* should not exceed 55˚C. Beyond this temperature, the lethal effect of thermosonication was ascribed mainly to the thermal effects. Combining thermal treatment with sonication treatment can improve the efficiency of ultrasonic deactivation of *E. coli* through two effects:

1) Decreasing the required time to obtain a certain level of *E. coli* reduction.
2) The lethal and the sub-lethal effects of thermal treatment that have the potential to disintegrate the cell of *E. coli* or impair its resistance against sonication treatment.

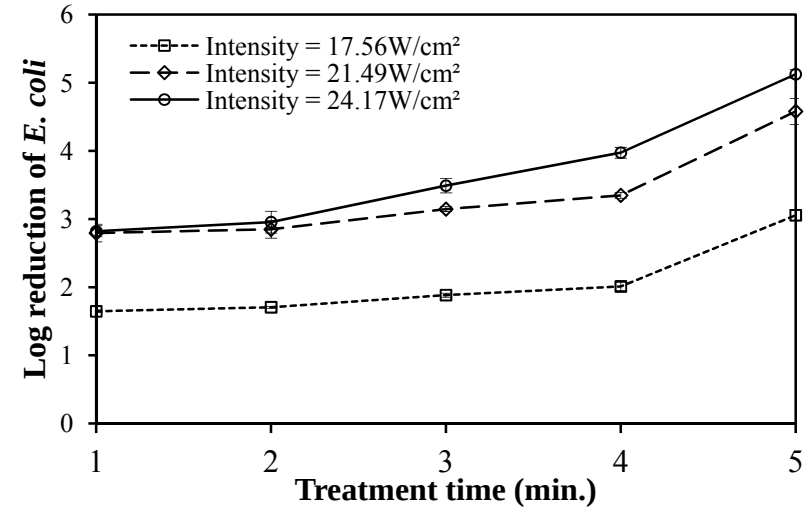
4.6.4 Manosonication

Log reductions of *E. coli* for manosonication treatments with different ultrasonic intensities of 17.56, 21.49 and 24.17 W/cm^2 at pressures of 2, 3 and 4 bars are shown in Figure 4.13 a, b and c respectively. From Figure 4.13, it can be noticed that the difference between the log reductions of manosonication treatments for the intensities 21.49 and 24.17 W/cm^2 at different pressures is small, in the order of < 1

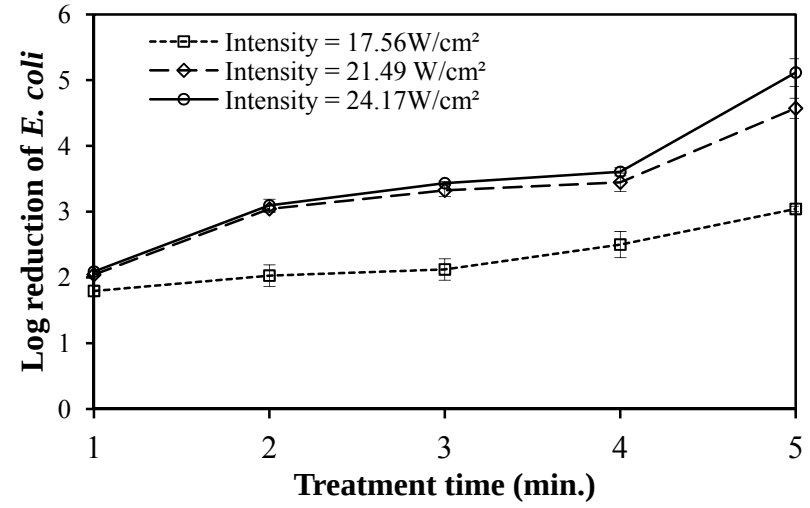
log_{10}. This agrees with sonication and thermosonication results, which showed that increasing the intensity beyond 21.49 W/cm^2 would have a slight effect on the *E. coli* reduction. Log reductions of *E. coli* for manosonication treatments with ultrasonic intensity of 17.56 W/cm^2 at 2 and 3 bars were close, while the log reduction of the same ultrasonic intensity at 4 bars was slightly higher.

Combining three external pressures of 2, 3 and 4 bars with sonication treatments (manosonication treatments) at different ultrasonic intensities resulted in an insignificant increase in the log reduction of *E.coli* as compared to sonication treatments alone (Figure 4.11 versus Figure 4.13). This could be explained as increasing the pressure of the suspension have a negative effect on the growth of the bubbles and thus produce less violent collapse as explained in Chapter 3 Section 3.6.

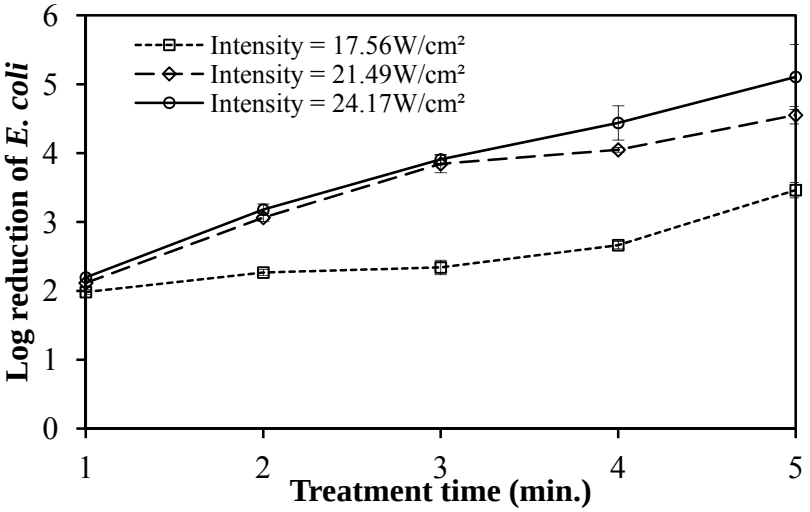
When comparing the reduction of *E. coli* for the intensities of 17.56, 21.49 and 24.17 W/cm^2 at different pressures (i.e. 2, 3 and 4bars), it can be seen that the differences in the log reductions of *E. coli* at 2 bars and the other pressures are marginal. For example, the log reduction of *E. coli* for manosonication treatment at 24.17 W/cm^2 and 2 bras was 5.12 log_{10}, while the log reductions of *E. coli* for the same ultrasonic intensity and pressures of 3 and 4 bras were 5.11 log_{10} and 5.10 log_{10} respectively. Therefore, it can be said that two bars is the upper limit of the pressure in manosonication treatments of *E. coli ATCC25922* in this study. After this limit, any increase in the applied pressure on the suspension can impair bubble growth and as a consequence negatively affect *E. coli* deactivation. Lee et al. (2009) found that the upper limit of pressure in manosonication treatment for *E. coli K12* was 3 bars. Raso et al. (1998) reported that the upper pressure limit in manosonication treatment for *Y. enterocolitica* is 4 bars and increasing the pressure to 6 bars gave a barely measurable increase in the deactivation rate.



(a)



(b)



(c)

Figure 4.13: Log reduction of *E. coli ATCC 25922* versus treatment time for sonication the test water with different intensities (17.56, 21.49 and 24.17 W/cm^2) under temperature range of 14 to 30˚C and pressures of (a) 2, (b) 3 and (c) 4 bars.

From the results obtained in this study and the results presented in the literature, it can be suggested that considerable attention needs to be given when increasing the applied pressure in manosonication treatment. Increasing the applied pressure in manosonication treatment may not result in a significant increase in the reduction of microorganisms, and it would be a waste of additional energy.

4.7 Comparison between sonication, thermosonication and manosonication in terms of energy consumption – case study

Three tests were conducted using sonication, manosonication at 2 bars and thermosonication at 45˚C to achieve 5 $\log_{10}$ reduction of *E. coli* in 50 g water-based suspension in the aim of assessing the deactivation efficiency of ultrasound treatments with regards to energy requirements. Since the application of ultrasound in this study is to pre-treat the feed solution of the RO membrane , and the efficiency of the pre-treatment technique is gauged by its capacity for deactivating microorganisms, high reduction of *E. coli* (5 $\log_{10}$) was selected to be the limit upon which the deactivation efficiency of ultrasound treatments was assessed.

The deactivation efficiency of each treatment was calculated by dividing the specific energy by the log reduction of *E. coli* for each treatment. The specific energy was chosen as an indicator for the energy requirements in ultrasound treatments because it includes the consumed ultrasonic power, treatment time and the mass of the treated suspension collectively (Hulsmans et al., 2009). The specific energy required to obtain 5 $\log_{10}$ reduction of *E. coli* using sonication, manosonication and thermosonication was calculated in this study using equations 4.1, 4.2 and 4.3 respectively:

$$1.\, e_{sonication} = \frac{P_u}{m} \times t_{treatment}, \qquad (4.1)$$

$$2.\, e_{manosonication} = \left(\frac{P_u}{m} \times t_{treatment}\right) + {}^{V\Delta P}/_{m}, \quad (4.2)$$

$$3.\, e_{thermosonication} = \left(\frac{P_u}{m} \times t_{treatment}\right) + C_p \Delta T, \quad (4.3)$$

where, e is the specific energy (kJ/kg)

P_u is the ultrasonic power (W),

$t_{treatment}$ is the treatment time,

V is the volume of the suspension (m^3),

ΔP is the applied pressure in manosonication treatments (Pa),

m is the mass of the suspension,

C_p is the specific heat of water (kJ/kg.K), and

ΔT is the difference between the applied temperatures in thermosonication treatments and room temperature (K).

Table 4.3 illustrates specific energy required to achieve 5 $\log_{10}$ reduction of *E. coli* and the deactivation efficiency represented by (kJ/kg)/ (log reduction) of sonication, manosonication (2 bars) and thermosonication (45˚C). The results presented in Table 4.3 shows that the specific energy required to obtain 5 $\log_{10}$ reduction of *E. coli* in sonication, manosonication and thermosonication are 702, 690 and 565.5 kJ/kg respectively. From the results presented in Table 4.3, it can be seen that thermosonication treatment possesses the highest deactivation efficiency among the other two ultrasound treatments. The specific energy required to achieve 1 $\log_{10}$ reduction of *E. coli* in thermosonication treatment is113.1 kJ/kg, which is lower than that of manosonication (138 kJ/kg) and sonication (140.4 kJ/kg). Thus

thermosonication treatment at 45˚C is regarded as the optimum ultrasound treatment in this study.

Table 4.3: Comparison between the deactivation efficiency of sonication, manosonication (2 bars) and thermosonication (45˚C)

Treatment	Log reduction of *E. coli* (CFU/mL)	Specific Energy (kJ/kg)	Deactivation efficiency (kJ/kg)/(log_{10} reduction)
Sonication	5	702	140.4
Manosonication	5	690	138
Thermosonication	5	565.5	113.1

4.8 Techniques to reduce energy requirements in ultrasonic deactivation

The energy demand in ultrasonic deactivation process can be reduced by intensifying the cavitation phenomenon through number of approaches such as seeding solid particles (Dadjour et al., 2005) or injecting steam bubbles (Mahulkar et al., 2008) into the treated suspension. In this study, another technique was introduced to boost the cavitation phenomenon in the treated suspension by immersing a corrugated surface in the suspension to stimulate the formation of heterogeneous bubbles. This technique was found to be a good approach to increase the reduction of microorganisms in sonication treatment especially at low ultrasonic intensity. This in turn, can reduce the energy requirement associate with the use of sonication to achieve certain level of microorganisms deactivation. The increase in the log reduction of *E. coli* for sonication treatment at 17.56, 21.49 and 24.17 W/cm^2 as a result of immersing corrugated surface in the treated suspension was approximately 0.5, 0.3 and 0.2 log_{10} respectively. The effect of a corrugated surface as a mean of reinforcing the cavitational events in ultrasonic deactivation of *E. coli* is illustrated in Figure E.2, Appendix E.

It should be noted here that, using a corrugated surface to improve ultrasonic deactivation, was not applied when using ultrasound for controlling biofouling formation in the RO membrane (Chapter 5). The eroded particles from the corrugated surface can clog membrane pores, which make it hard to differentiate between the effects of biofilm on membrane productivity and the effect of the eroded particles.

4.9 Conclusions

The use of sonication, thermosonication and manosonication treatments for *E.coli* deactivation was investigated in the chapter. Two types of the cavitation chamber were used in the treatments: Pyrex beaker in sonication and thermosonication treatments and steel chamber in manosonication treatment. The configuration of the cavitation chamber of sonication and thermosonication set-ups was optimized with regards to the D/L ratio and the depth of the ultrasonic probe in the suspension prior to the sonication and thermosonication experiments. The effect of the low osmolarity of distilled water on the viability of *E. coli* was investigated before proceeding with sonication, thermosonication and manosonication experiments. The outcomes of the experimental studies are summarized in the following points:

1. Cavitation chamber with intermediate D/L value was found to be the optimum chamber to be used in sonication and thermosonication treatments.
2. The probe of ultrasonic horn for high ultrasonic intensity needs to be large enough to prevent air penetration into the suspension from the atmosphere.
3. The effect of osmolarity on the viability of *E. coli* within the studied time range of this study was found to be marginal.
4. Ultrasonic intensity has an optimum limit after which any increase in the intensity will have an insignificant effect on the reduction of

microorganisms. To reduce the energy requirements in ultrasound treatments, the optimum limit of ultrasonic intensity has to be applied in ultrasound treatments.

5. The increase in the log reduction of *E.coli* for sonication when combined with the thermal treatment (thermosonication), was mainly due to the detrimental effect of heat on the cell of *E. coli*.
6. Combining thermal treatment with sonication treatment can reduce the required energy and treatment time to obtain a certain level of *E. coli* reduction.
7. The reduction of *E. coli* in manosonication treatment was found to be slightly better than that of sonication treatment. This was explained through theoretical study, as increasing the applied pressure of the treated water negatively affected bubble growth and consequently collapse pressure.
8. The optimum limit of the applied pressure in manosonication treatments for *E. coli* was 2 bars. The log reduction of *E. coli* for manosonication at 3 and 4 bars was slightly different than that of manosonication at 2 bars.
9. Increasing treatment time leads to an increase in the reduction of *E. coli* in all ultrasound treatments. However, four minutes of treatment time seemed to be a critical limit for *E. coli* in all ultrasound treatments. Increasing the treatment time for ultrasound treatments from 4 to 5 minutes resulted in a dramatic increase in the log reduction of *E. coli*.
10. Thermosonication treatment at low sub-lethal temperature of *E. coli* (45˚C) was found to be the optimum ultrasound treatment to be applied for *E. coli* deactivation. Therefore, these conditions of ultrasound treatment will be applied to pre-treat the feed water of reverse osmosis system in Chapter 5.

CHAPTER 5

Thermosonication as a selected pre-treatment method for RO

5.1 The aim of the chapter

The adherence and subsequent growth of microorganisms on the RO membrane in wastewater reclamation and seawater desalination systems has been an ongoing problem that impairs the performance of the membrane. In spite of the large number of techniques that have been proposed in the literature to reduce the formation of biofouling on RO membrane, biofouling formation on the membrane remains an unresolved problem in water treatment plants employing membrane technology. Therefore, the use of ultrasound technology as a pre-treatment method for decreasing the formation of biofouling in a batch RO membrane system is suggested and investigated in this chapter.

The efficacy of ultrasound treatments for deactivating microorganisms (*E. coli* in this study) in a batch configuration system was investigated in Chapter 4. The outcomes showed that thermosonication treatment at 45˚C is the optimum ultrasound treatment for deactivating *E. coli* in water-based suspension of 50 mL. Therefore, thermosonication at 21.49 W/cm2 and 45˚C for 4 minutes has been chosen as the ultrasonic treatment to treat the feed water of the RO system before the water deliver to the RO unit. The RO system used in this study is a batch configuration system. Although the use of thermosonication for deactivating microorganisms was investigated in few applications such as food processing (Zenker et al., 2003) and water decontamination (Lee et al., 2009), apparently there is no reported study on the

use of thermosonication as a pre-treatment for membrane systems, which is the focus of this study.

The performance of thermosonication was assessed based on two measurements: i) the permeate flux of the RO unit and ii) the development of the biofilm on the RO membrane. Staining and epiflourescence microscopy techniques were used for biofilm analysis. The permeate flux and the developed biofilm on the RO membrane for the treated *E.coli* suspension was compared to that of the untreated suspension, which acted as a control.

This chapter will cover the following:

1. Preparation of broth-based suspension of *E. coli*.
2. Viable cell count and cell injury estimation.
3. Description of the RO set-up.
4. Illustration of biofouling experiments.
5. Biofilm analyses.
6. Thermosonication as a pre-treatment for the RO system (results and discussions).
7. Conclusions.

5.2 Methods and analyses

5.2.1 Preparation of broth-based suspension of E. coli ATCC25922

The method of preparing *E. coli* suspension is similar to the method explained in Chapter 3 except MacCONKEY broth was used as a medium instead of distilled water.

MacCONKEY broth was used in this study as a medium to provide *E. coli* with sufficient nutrients to grow and develop biofilm during the operation of the RO unit. The MacCONKEY broth consists of four chemical constitutes; peptone as a source of carbon and nitrogen for the growing microorganisms in the form of amino acids, lactose as another source of carbon, Ox-gall as an inhibitor salt for the growth of gram positive microorganisms and brom cresol purple as a pH indicator (Difco-Manual, 1998).

The broth was prepared by adding 7g of the MacCONKEY broth powder into 200 mL of distilled water. After vortexing the mixture until the powder dissolved completely, the broth solution was autoclaved at 121˚C for 15 minutes. Then the broth was cooled until it reached room temperature. It should be noted here, that the pH of MacCONKEY broth is approximately 7.3±0.1 at 25˚C (Difco-Manual, 1998), which is within the operating pH range of the RO membrane used in this study. The MacCONKEY broth gives an indication for the growth of microorganisms by changing the colour of the broth. With the growth of microorganisms, the colour of the MacCONKEY broth turns gradually from its original purple colour to a brownish colour as illustrated in Figure 5.1.

(a) After 24 hours (b) After 48 hours

Figure 5.1: Display of the broth colour change during (a) 24 and (b) 48 hours of the operating time of RO unit

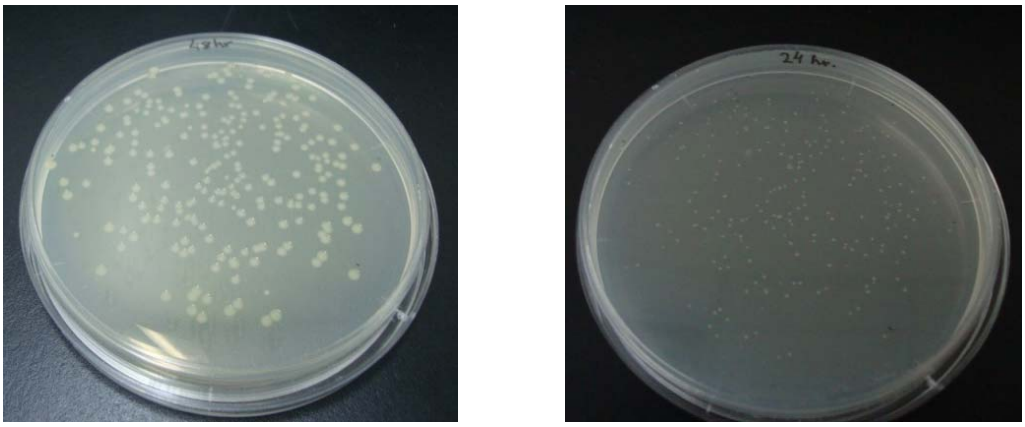
5.2.2 Viable cell count and estimation of cell injury

Viable cell count was used to measure the concentration of *E. coli* at different stages of both treatments; thermosonication and RO. The procedure of viable cell count was discussed in Chapter 4 Section 4.2.2. However, detecting and measuring cell injury of *E. coli* will be covered in this chapter. The concentration of the injured cells was measured in this study owing to its reported effect on the formation of biofouling (Broekman et al., 2010).

To measure the number of the injured cells of *E. coli* in thermosonication treatment, samples of the treated suspensions for 1, 2, 3 and 4 minutes were plated in parallel onto two mediums: Tryptone Soya Agar (TSA) as a non-selective medium and CM0007 MacCONKEY Agar as a selective medium. A non-selective medium provides the microorganisms with a nutritious environment, so the injured cells can grow and multiply, whereas the selective medium is considered a harsh environment for the injured microorganisms to grow and produce colonies (Besse, 2002; Gnanou Besse et al., 2000). It should be noted that an appropriate serial 5-fold dilution of the treated suspension was applied in the viable cell count. After plating the treated microorganisms onto the two mediums, the plates were incubated for 24 and 48 hours for selective and non-selective mediums respectively.

The treated suspension of *E. coli* was plated on the non-selective medium for a longer incubation time to avoid inaccuracy in counting the grown colonies. Some of the colonies on the non-selective medium were very small or almost undetectable at an incubation period of 24 hours. These colonies were believed to be formed by the injured cells. Therefore the incubation period for the non-selective medium was extended to 48 hours, as increasing the incubation period resulted in increasing the

colony size so it became countable as shown in Figure 5.2. The number of the injured cells was obtained by subtracting the number of the colonies of the non-selective medium from those of the selective medium.



(a) (b)

Figure 5.2: Colonies of the treated *E. coli* on TSA for a period of (a) 24 and (b) 48 hours

5.2.3 Reverse Osmosis membrane

The RO membrane was obtained from the Sterlitech Corporation USA. The trade name of this membrane is the Toray flat sheet membrane UTC-70UB. The membrane is a thin-film composite polyamide, composed of polyamide layered on the top of a polysulfone backing. The operating pH range for the membrane is between 4 and 11 at 25˚C.

The membrane was supplied as a roll of flat sheet with dimensions of 45.7× 45.7 cm. The sheet was cut into circular sheet-filters with diameter of 9 cm, so it can be used in the stirred cell. Prior to installing the filter in the RO unit, the filter was soaked with nanopure water for one hour (as recommended by the manufacturer). The circular filter was installed onto the bottom of the cell between the supporting

screen of the membrane and a silicon O-ring. Once the membrane was set, the whole cell was assembled and ready to use.

5.2.4 Reverse Osmosis unit

Reverse Osmosis treatment for four different feeds; nanopure water, MacCONKEY broth, treated suspension and untreated suspension (control) was conducted using an RO stirred cell. Nanopure water is purified water with a Barnstead/Thermolyne Nanopure lab system. RO technology is used to purify the water in this system. The RO stirred cell was placed on an HB502 magnetic stirrer that helps in homogenizing the suspension during the treatment. The stirred cell was provided by the same supplier as the RO membrane, and its specifications are presented in Table 5.1.

Table 5.1: The specifications of the RO stirred cell UHP 90

Specifications	**Limits**
Maximum Pressure	450 kPa
Capacity	600 ml
Weight	2.2 kg
Height	23 cm
Diameter	12.2 cm
Filter diameter	90 mm

The parts of the stirred cell are demonstrated in a schematic shown in Figure 5.3. The cell comprises of a barrel made from acrylic with two open sides (label 20), the lower side set on the bottom of the cell and the upper side is covered by a polyacetal cap (label 15). The bottom of the cell consists of three parts: a cap made of polyacetal that has a grooved inner surface to carry permeate (label 22), polypropylene screen to support the membrane (label 21) and a silicon O-ring (label 19). The circular cut-out of the RO membrane sheet is installed on the bottom of the cell between the polypropylene screen and the O-ring. The upper cap of the cell is

connected to polyacetal stirring rod from the inner side (labels16-18). The outer side of the upper cap has a pressure relief valve (labels 7 and 8) and two apertures, one for the pressure inlet line (labels 1-6) and the other one is an access for the cell feed (labels 9-14). The pressure relief valve in the stirred cell is designed to control the excessive pressure. All parts of the RO cell are held together by three 10 mm screws (label 23).

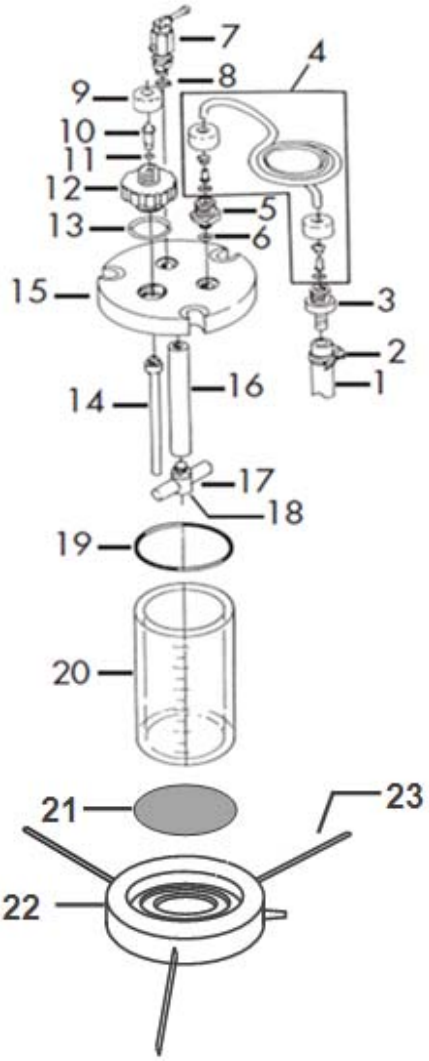


Figure 5.3: A schematic of RO stirred cell (Sterlitech Corporation USA).

5.2.5 Biofouling experiments

Biofouling experiments were conducted following control experiment procedure, with four different runs: untreated suspension (Run 1), treated suspension (Run 2), nanopure water (Run 3) and MacCONKEY broth (Run 4) as demonstrated in Figure 5.4. The purpose of using nanopure water and MacCONKEY broth as reference feeds is to:

i) Identify the membrane productivity with pure water and compare it with the other feeds as standard productivity of the membrane (Run3).

ii) Differentiate between the fouling of the broth contents and that of the microorganisms' adhesion (Run4).

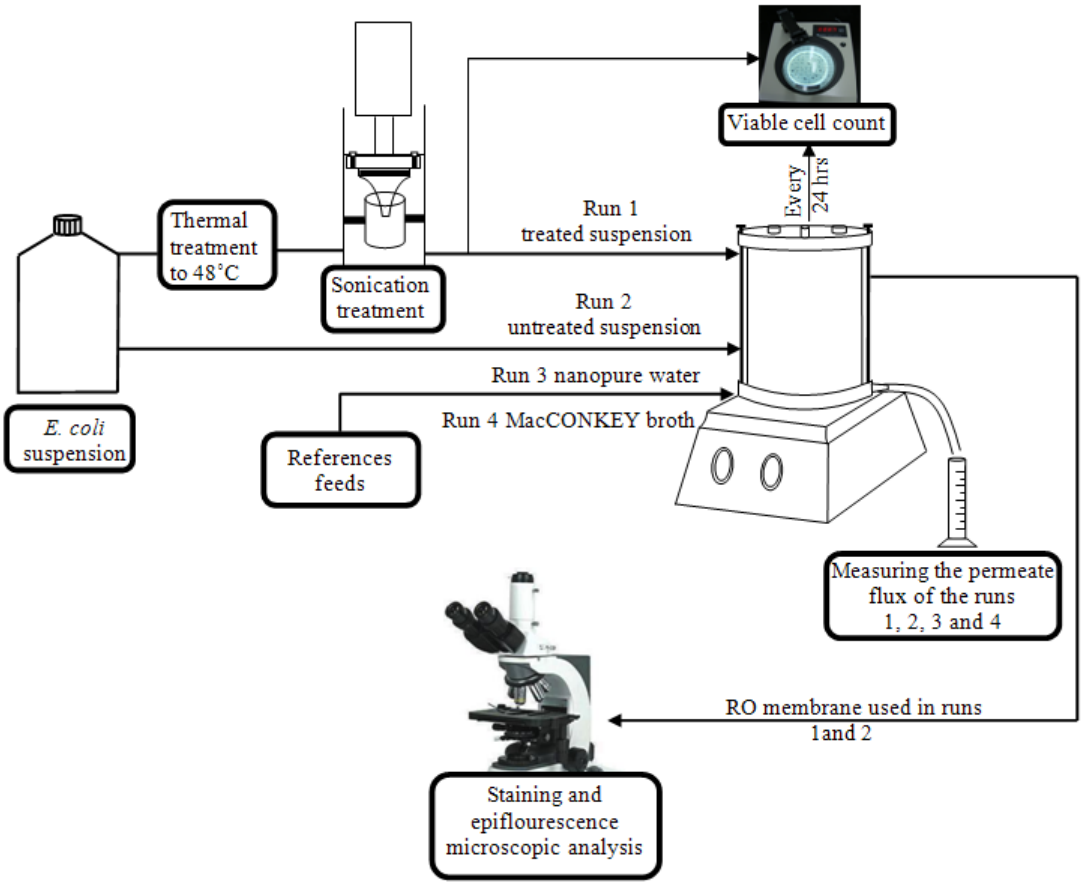


Figure 5.4: Schematic diagram of the procedure of control experiments

The stirred cell was loaded with 200 ml of feed solution and pressurized with compressed air of 4.5 bars. The stirred cell was operated for 60 hours with each Run during which the permeate flow rate was measured using a graduated cylinder every 6 hours. The measured permeate was returned to the cell after each reading to keep the cell loaded with the same amount of solution. In the case of Runs 1 and 2, a one millilitre sample of the feed solution was taken every 24 hours to measure the concentration of *E. coli* in the bulk solution during the operating time of the RO cell. The membranes used in Runs 1 and 2 underwent staining and epiflourescence analyses. After each Run with a different feed, the cell was dismantled and sterilised with commercial bleach diluted with distilled water by a volumetric percentage of 5% (as recommended by the manufacturer).

5.2.6 Biofilm analyses

The analyses of the developed biofilm on the RO membrane involved using staining and epiflourescence microscopy techniques as shown in Figure 5.4. Evaluating the formation of biofouling on the RO membrane through numerating the attached live and dead cells of *E. coli* on the membrane using staining and epiflourescence microscopy techniques was applied in this study owing to the following reasons:

1) Both live and dead cells are considered to be important constitutes of biofilm and enumerating them through staining techniques can give an idea about the density of the formed biofilm on the RO membrane. Danese et al. (2000) reported that the main two features of biofilm are micro-colonies and water filled channels and that the micro-colonies in turn consist of cells surrounded by extracellular polymeric substance.

2) Live cells of *E. coli* are the source of EPS. The number of *E. coli* cells adhered to the RO membrane can determine the amount of the produced EPS that can foul the membrane (the other constitute of biofilm). Recent study conducted by Patrick et al. (2011) showed that reducing the concentration of the active microorganisms in the feed solution can reduce the deposition of biomass and biopolymer onto the membrane.
3) Numerating the attached active cells of *E. coli* on the RO membrane is considered to be a crucial factor in biofouling formation. Chua et al. (2003) stated that the accumulation of active microorganisms on an RO membrane may accelerate the degradation of the membrane.

After using the RO membrane for 60 hours with untreated and treated suspensions, the membrane was cut into small swatches and subjected to two types of stains; Acridine Orange and *LIVE/DEAD BacLight*^{TM} Bacterial Viability Kit for microscopy and quantitative assays (Invitrogen Molecular Probes, Victoria-Australia).

Acridine Orange stain: The swatches of the used RO membrane were stained with Acridine Orange by covering the swatches of the membrane with fair amounts of the stain and leaving them in the dark for 5 minutes. Following this step, the swatches were washed with nanopure water to remove the excess stain and then incubated in the dark at room temperature for 1 hour to dry.

LIVE/DEAD BacLight^{TM} *Bacterial Viability Kit for microscopy and quantitative assays:* this stain consists of three main components; SYTO 9 dye (3.34mM), Propidium iodide dye (20mM) and *BacLight* mounting oil. SYTO 9 stains the intact cells of microorganism with green fluorescent, while Propidium iodide stains the damaged membrane cells of microorganism with red fluorescent. The pack

of the stain components was stored at −60˚C as recommended by the manufacturer. The staining procedure with *LIVE/DEAD BacLight*^{TM} stain was obtained from the literature (Khan et al., 2010). A 1.5µl of each dye; SYTO 9 and Propidium iodide was added to 2 ml of Milli-Q water and, after a well vortexing, the mixture was spread on the top of the membrane swatches. The excess dye on the membrane was washed with Milli-Q water. After that, the stained membrane swatches were incubated in the dark for 1 hour.

The cells stained with both types of stain were examined with an epiflourescence microscope (Nikon, Eclipse, E600) applying a magnification power of a 40× objective for Acridine Orange stain and 20× objective for *LIVE/DEAD BacLight*^{TM} stain. Two types of microscopic filters were used: a triple DAPI, FITC and Texas Red (D-F-T) filter and a green excitation filter (G-2A). Images of the biofilm were captured with the aid of MicroPublisher 5.0 RTV camera which is equipped with a fluorescence microscope and computer. The captured microphotographs of the biofilm were optimized using *Analysis FIVE* software.

5.3 Results and discussions

5.3.1 The concentration of E. coli injured cells in the treated suspension

Log survival of *E. coli* in the treated suspension with thermosonication trialled by parallel plating on CM0007 MacCONKEY Agar and TSA is shown in Figure 5.5. The values of log survival of *E. coli* in Figure 5.5 and the other figures in this chapter are expressed in the mean value and the error bars represent the standard error of the mean. The solid line in this figure symbolizes log survival of *E. coli* in the treated suspension plated on CM0007 MacCONKEY Agar, while the dashed line depicts log survival of *E. coli* in the treated suspension plated on TSA. The vertical distance

between the dashed and the solid lines represents the number of the injured cells expressed by log CFU/mL.

Figure 5.5 shows that the number of the injured cells of *E. coli* decreased at the treatment time interval of 2 minutes as compared to the interval of 1 minute and then increased at 3 and 4 minutes as compared to 1 and 2 minutes. However, the general trend of Figure 5.5 can be described as a direct relationship between the number of the injured cells of *E. coli* and treatment time (where the number of the injured cells was obtained by subtracting A value from B value as illustrated in Figure 5.5). The increase in the number of the injured cells of *E. coli* is not pronounced over the period of 3 minutes of treatment time ($\leq 0.2 \log_{10}$) but it doubled when the treatment time was increased to 4 minutes ($\geq 0.4 \log_{10}$).

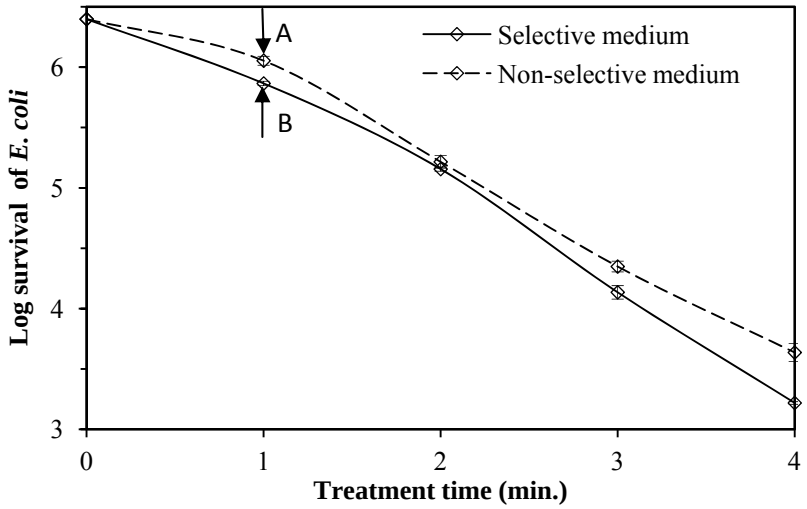


Figure 5.5: Survival curves for the treated *E. coli* plated on a selective medium (CM0007 MacCONKEY agar) and non-selective medium (TSA)

The results obtained from this study are consistent with the results reported in the literature (Perni et al., 2007; Allison et al., 1996). Perni et al. (2007) found that the concentration of the injured cells of *E. coli* treated with pulsed electric fields rises with increase in the exposure time and became noticeable with treatment time of 4

and 5 minutes. Similarly, Allison et al. (1996) showed that the amount of the released protein from *E. coli* treated with sonication increased by increasing the treatment time. They considered this increase in the released protein as an indicator of the cell damage due to sonication treatment.

In short, Figure 5.5 shows that increasing the treatment time of thermosonication can increase the number of the injured cells in the treated suspension. The most important point in this figure is the last point (treatment time = 4 minutes), as this treatment interval will be applied when treating the RO feed.

5.3.2 Effect of medium contents on the variability of E. coli treated with thermosonication

To assess the effect of using MacCONKEY broth as a medium on the viability of *E. coli* treated with thermosonication, pre-tests using distilled water as a medium for *E. coli* were conducted and the results were compared to that of the broth as presented in Figure 5.6. This figure shows that the concentration of the viable *E.coli* cells in the treated broth is more than that of the treated distilled water by more than 1 $\log_{10}$ for all the treatment intervals. A similar observation was reported by Lee et al. (1989), who found that treating *Salmonella eastbourne* suspended in two different mediums, peptone water and milk chocolate, with sonication for 10 minutes resulted in 2.5 and 0.13 $\log_{10}$ reductions respectively.

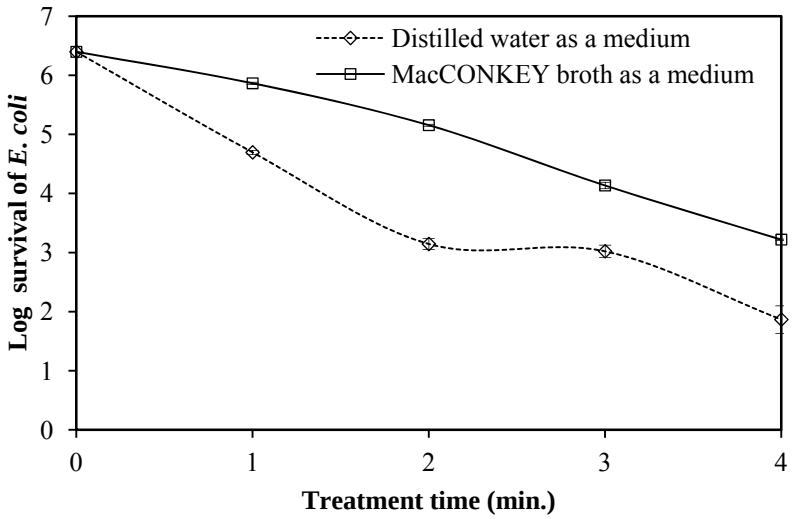


Figure 5.6: Survival curves for the treated *E. coli* in two mediums: MacCONKEY broth (solid line) and distilled water (dotted line)

The difference in the survival population of *E. coli* in the treated mediums; distilled water and MacCONKEY broth can be attributed to one the following reasons:

1) The reaction of the free radicals generated due to ultrasound irradiation with some of the broth components, such as amino acid liberating gases, (Johnson et al., 1989; Heusinger, 1990) that could contribute to an excessive generation of bubbles with large size in the broth body. The presence of the large number of bubbles with big size in the suspension can hinder the transmission of ultrasonic intensity into the suspension, especially when the bubbles cluster close to the irradiating surface (Thompson and Doraiswamy, 1999).
2) Sonoprotective effects of lactose (one of MacCONKEY broth components) could strengthen the resistance of *E.coli* to ultrasound treatment. Gera and Doores (2011) observed that the addition of lactose to a simulated milk ultrafiltrate that contains *E. coli* inoculated in it, can increase the resistance

of *E. coli* to sonication treatment. The increase in the resistance of *E. coli* was ascribed to a decrease in the water activity (Alvarez et al., 2003) and the stabilization of the biomolecules of the microorganism membrane such as phospholipids due to their interaction with the sugar molecules (Molina-Hoppner et al., 2004).

3) The difference in the osmolarity of the two mediums, distilled water and MacCONKEY broth can be another reason that affected the lethality of thermosonication treatment for *E. coli*. The mechanical stress that can be generated in the *E. coli* cell wall when suspended in distilled water can cause injury or death to *E. coli* as explained in Chapter 4 Section 4.6.1. In comparison, such stress could be absent or less in the case of the MacCONKEY broth. The effect of the distilled water osmolarity on the viability of *E. coli* for treatment time of 1, 2, 3, 4 and 5 minutes was investigated in chapter 3 section 3.7.1. It was found that the effect of distilled water osmolarity on the viability of *E. coli* was marginal in the range of approximately 0.05 $\log_{10}$. This means that the effect of the osmolarity of distilled water on *E. coli* within the time range of 1 to 5 minutes is more likely to be confined to the cell injury that rendered *E. coli* susceptible to thermosonication treatment.

The results presented in Figure 5.6 suggest that the characteristics of the medium could have a significant impact on ultrasonic deactivation for microorganisms and, therefore, it should be considered before applying an ultrasound treatment.

5.3.3 The reproduction of E. coli in untreated and treated suspension during the operating time of RO

Figure 5.7 shows the reproduction of *E. coli* in untreated and treated suspension for the interval of 54 hours of RO running time. The proliferation of *E. coli* in the untreated and treated suspensions has an important role in the development of biofouling and, therefore, it was monitored every 18 hours using viable cell count technique.

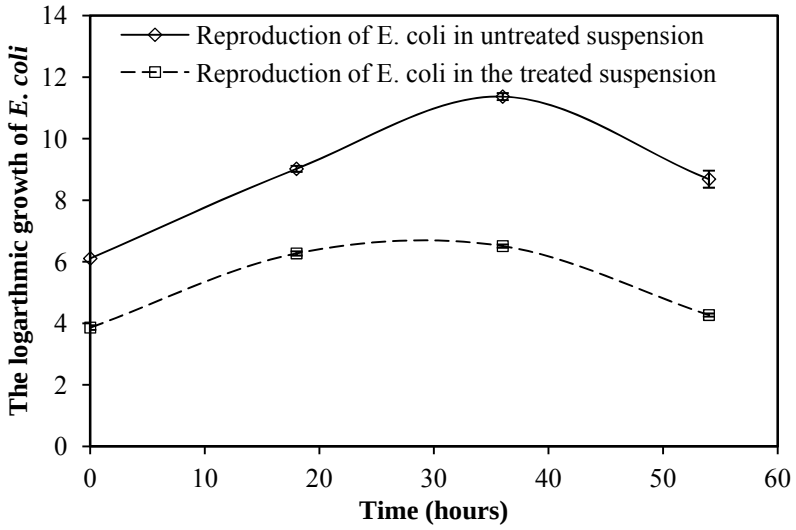


Figure 5.7: The proliferation curves of *E. coli* during the operating time of RO for untreated suspension (solid line) and treated suspension (dashed line)

Overall, the increase in the concentration of *E. coli* in both suspensions reached its peak in 36 hours, and after this time the concentration started to decrease. However, the reproduction of *E. coli* in the treated suspension was found to be less than that of the untreated suspension. The concentration of *E. coli* in the untreated suspension increased from its initial value of 6.1 $\log_{10}$ to its maximum value of 11.368 $\log_{10}$ (86.36% increment), while this increase of *E. coli* concentration in the treated suspension was lower from 3.86 $\log_{10}$ to 6.5 $\log_{10}$ (68.4% increment). This difference between the proliferation of *E. coli* in the untreated and treated suspensions could be attributed to two causes: the effect of thermosonication

treatment on the reproduction of *E. coli*, and the high consumption of nutrients by the injured cells that in turn can cause starvation in the whole population of *E.coli* in the broth (Broekman et al., 2010). Lo"Rincz (2004) stated that ultrasonic treatment can impair the capability of treated cells for cellular division. Similarly, the predominance of starvation condition in the medium impacts adversely on the ability of *E. coli* to proliferate (Desnues et al., 2003).

Figures 5.5 and 5.7 demonstrated that thermosonication has the potential to reduce the concentration of intact cells in the treated suspension and hamper a further reproducibility of the survived cells in the suspension. This in turn can deteriorate the ability of the microorganisms to develop a biofilm on the RO membrane.

5.3.4 Observation of the developed biofilm on RO membrane using Acridine Orange and LIVE/DEAD BacLight stains

Figure 5.8 presents top view microphotographs of the accumulated biofilm on the centre of the RO membranes for untreated (left side) and treated suspensions (right side) stained with *LIVE/DEAD BacLight*^{TM} (1-4) and Acridine Orange (5-8). The density of the biofilm seemed to vary from one membrane location to another. This suggests that there are uneven areas of the membrane that facilitate the deposition of the microorganisms onto the membrane. Subramani and Hoek (2008) explained through their observation, that the initial deposition of microorganisms occurred in discrete locations on the RO membrane, as the initially attached microorganisms to the membrane serve as seeds for further depositions. The uneven characteristic of the RO membrane is believed to be due to scratches, particle marks or creases associated with the processes of storing, cutting or setting the membrane.

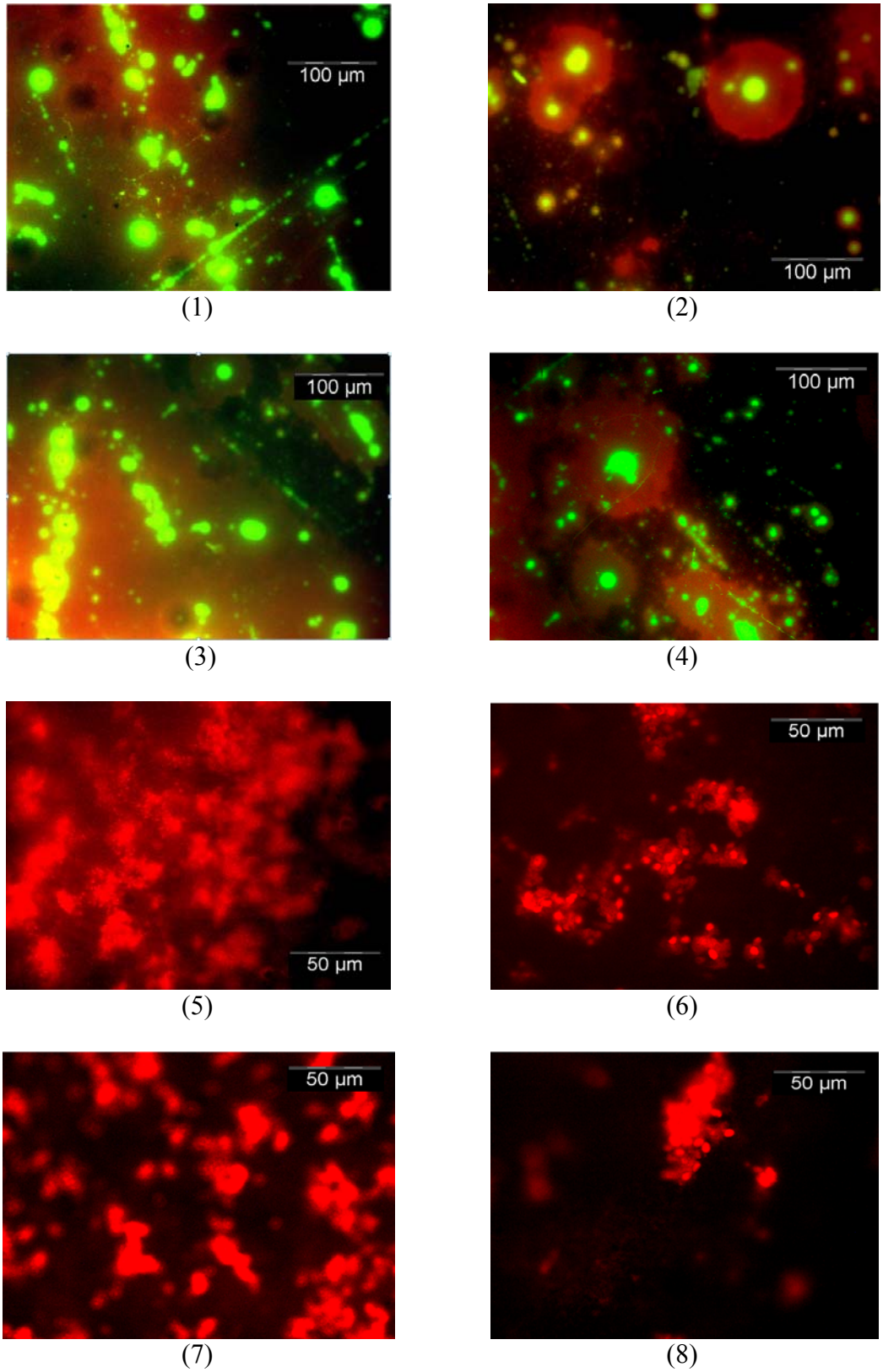


Figure 5.8: Distribution of biofilm on the centre of RO membranes using *LIVE/DEAD BacLight* (1-4) and Acridine Orange (5-8), the left side represents the used membrane with untreated suspension and the right side represents the used membrane with treated suspension

It can be noticed from the images presented in Figure 5.8 that the colonies of the untreated cells are bigger than their counterparts of the treated cells. This could be ascribed to two effects: first, the adverse effect of nutrient limitations in the treated suspension on the reproducibility (Figure 5.7) and the size of the treated cells (Özkanca and Flint, 1997; Desnues et al., 2003), and second the effect of the ultrasound treatment on the poration of the microorganism's cell wall (Ward et al., 1999) and cell division (Lo"Rincz, 2004). A similar finding was reported by Broekman et al. (2010) who demonstrated, with photos, that cells treated with ultrasound formed disaggregated and smaller colonies as opposed to that of the untreated cells.

The microphotographs of biofilm in Figure 5.8 show that the treated cells of *E. coli* with thermosonication could not build a biofilm with a coverage area of the membrane as wide as that of the untreated cells. This observation is likely to be attributed to the following:

1. When comparing the adhesion of *E. coli* in both untreated and treated suspensions, the injured cells of the surviving population lack some of the adhesion such as *Pilis* and EPS. These mediating tools are considered to be essential for *E. coli* adhesion to the RO membrane (Hori and Matsumoto, 2010; Danese et al., 2000), and thus losing these tools adversely affect the adhesion.
2. The concentration of the intact microorganisms in the treated suspension is lower than that of the untreated suspension. Liew et al. (1995) found that the increase in the concentration of the intact cells' density in the bulk solution can lead to an increase in the MF fouling associated with particle loading such as cell debris and cell contents. In addition, Goosen et al. (2005) pointed out that the efficacy of bacterial adhesion to the membrane is associated with the existing number of the

intact cells in the solution. This can be explained as the adhesion of microorganisms to the membrane takes place through two stages: initial adhesion facilitated by the physiochemical interactions between the cell and the RO membrane, and the adhesion mediating tools of the microorganisms. Following this phenomenon, a secondary adhesion is mediated by the attraction between the adhered microorganisms and the suspended microorganisms in the solution starts (Brant and Childress, 2002). Hence, the initial and the secondary adhesion of microorganisms to the membrane depend on the available number of the intact cells in the solution.

3. Possible detachment of the developed biofilm in the early time of the experiments related to starvation conditions (Hunt et al., 2004). It was reported that the starvation of the cells in the feed solution could lead to the development of a biofilm with incomplete coverage over the area of the membrane (Goosen et al., 2005), as it is the case in the present study which is illustrated in Figure 5.8. Furthermore, Busscher and Weerkamp (1987) explained in their study on the bacterial adhesion to solid substrata, that the decrease in the concentration of microorganisms in the suspension can lead to desorbing of the adhering bacteria from the solid substratum.

5.3.5 Effect of thermosonication treatment on the permeate flux

Figure 5.9 shows the permeate fluxes of RO stirred cell for untreated and treated suspensions compared to the reference feeds, panel (a) and untreated suspension compared to the treated suspension, panel (b).

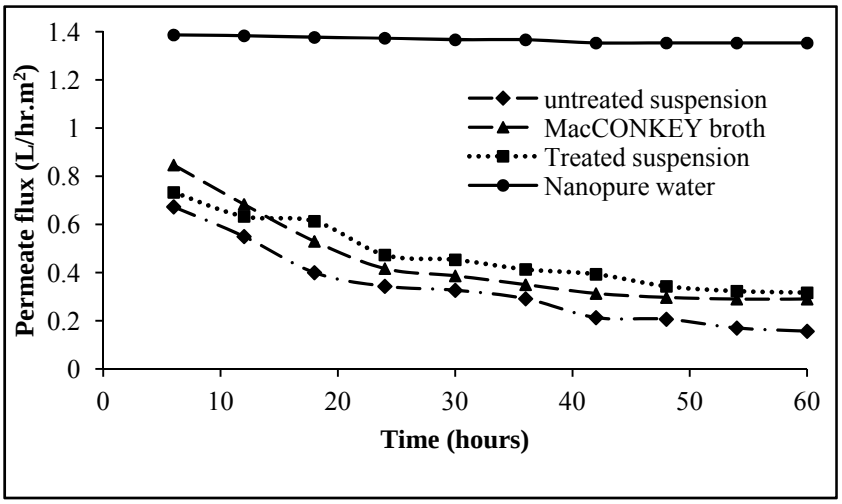
The permeate flux of the RO cell was calculated from the following formula (Mousavirad et al., 2009):

$$J_p = \frac{Q_p}{A}, \tag{5.1}$$

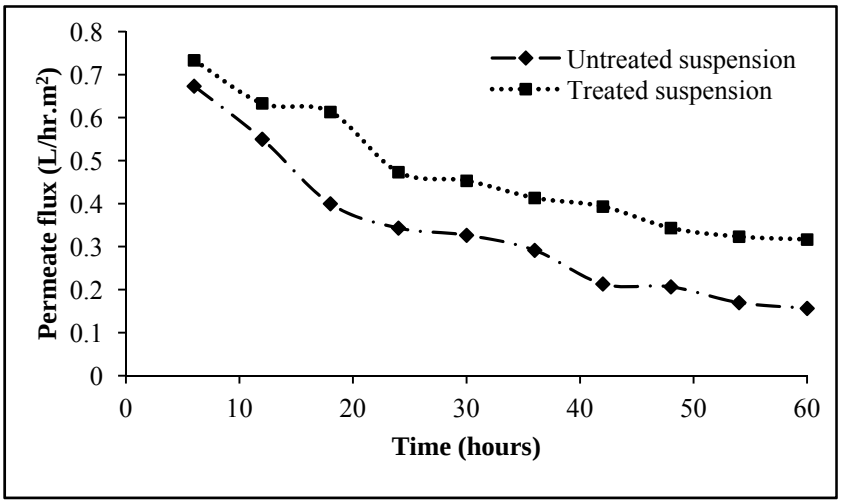
where, J_p is the permeate flux (L/m^2.hr),

Q_p is the permeate flow rate (L/hr), and

A is the filter area 0.005m^2.



(a)



(b)

Figure 5.9: Permeate fluxes of (a) untreated (centre line) and treated (dotted line) suspensions compared to the reference feeds, (b) treated suspension compared to the untreated suspension

The permeate flux of the nanopure water was almost constant at 1.367 L/hr.m^2 during the operating time of RO. In a graphical concept, the vertical distance between the curves of the permeate fluxes of nanopure water and any of the other feeds represents the decline in the permeate flux due to the fouling caused by the comparative feed. It can be seen from Figure 5.9 a, that the permeate flux of the untreated suspension was continually lower than that of the MacCONKEY broth during the whole operating time of the RO stirred cell. This trend was expected, as the particle loading in the untreated suspension including cell debris, cell contents and medium components is higher than that of the broth which includes only the medium components.

In contrast, the graphical comparison between the permeate fluxes of the treated suspension and the MacCONKEY broth followed a different pattern from that of the untreated suspension. In the first 12 h of the RO running time, the permeate flux of the broth was higher than that of the treated suspension. However, in the following period the case was overturned, as the permeate flux of the treated suspension became higher than that of the broth. The change in the curve trajectory of the permeate flux for the treated suspension in Figure 5.9 a after the first 12 hours is believed to be due the propensity of some of the broth components, such as peptone and lactose to foul the RO membrane (Chai et al., 1999; Madaeni and Mansourpanah, 2004). The recovery of the injured cells could have happened simultaneously throughout this interval which can negatively affect the adhesion of *E.coli* and the stability of the biofilm developed throughout the first 12 hours.

The measured permeate fluxes of the treated and untreated suspensions in Figure 5.9 b are consistent with the results in Figure 5.7. The decrease in the *E. coli* population after 36 hours in Figure 5.7 coincides with the decrease in the effect of

the fouling on the permeate flux decline in Figure 5.9 b. This could be due to the high consumption of nutrients available in the broth during the first 36 hours which could lead to the death of *E. coli* in the subsequent period of the RO operating time because nutrients are limited. The combination of *E.coli* death and the consumption of the nutrients could result in a decrease in the permeate flux decline.

In summary, the comparison between the fluxes of untreated and treated suspensions presented here, shows that pre-treating the suspension of *E.coli* with thermosonication recovered the permeate flux of the suspension by more than 0.1 L/hr.m^2 during most of the operating time of RO.

5.4 Conclusions

To reduce the formation of biofouling on the RO membrane (stirred cell), thermosonication treatment at power intensity of 21.5 W/cm^2 and temperature of 45˚C was applied for 4 minutes using batch configuration.

The results presented in this chapter demonstrated that thermosonication pre-treatment has the capacity to reduce the formation of biofouling in the RO membrane and, as a result, the permeate flux of *E. coli* suspension was improved by more than 0.1 L/hr.m^2. The results obtained in this chapter suggest that the potential of thermosonication in reducing the adhesion of *E.coli* to the RO membrane lies in the following effects:

1. The ability of thermosonication to decrease the concentration of intact *E. coli* cells in the feed solution which, in turn, decreases the number of the adhering cells to the membrane. The concentration of the intact *E. coli* cells decreased from 6.4 $\log_{10}$ to 3.6 $\log_{10}$ due to thermosonication treatment.

2. Part of the surviving cells was injured (approximately 10% of log survival of *E. coli*) which could affect, not only the reproducibility of the microorganisms in the treated suspension, but also the stability of the formed biofilm.
3. The negative effect of thermosonication on the capability of *E. coli* for reproduction. The increase in the population of the treated *E. coli* (from 3.86 $\log_{10}$ to 6.5 $\log_{10}$) was less effective than that of the untreated *E. coli* (from 6.1 $\log_{10}$ to 11.368 $\log_{10}$).

CHAPTER 6

Conclusions and future work

Reverse Osmosis technology offers an effective solution for the problem of water scarcity through its potential to reclaim the wastewater and desalinate sea/brackish water. However, the RO system has suffered from the problem of microbial adhesion to the membrane surface causing biofouling which, in turn, deteriorates membrane performance and increases operational costs. Many techniques for alleviating the problem of biofouling have been suggested by a large number of researchers. Most of the suggested techniques have some shortcomings that restrict their implementation as demonstrated in Chapter 2.

Ultrasound technology has been proposed in this thesis, as a new pre-treatment technique to reduce the formation of biofouling on the RO membrane. The main research questions of this thesis were:

1. Can ultrasound technology reduce the formation of biofouling on the RO membrane?
2. Which ultrasound treatment of sonication, thermosonication and manosonication is the most suitable method for microbial deactivation? And what are the optimum operating conditions for the most suitable method?

The factors that affecting the intensity of bubble collapse such as ultrasonic intensity, ultrasonic frequency, temperature of the treated water and pressure of the treated water were theoretically studied in Chapter 3. The effect of these factors on the intensity of bubble collapse was evaluated through their effect on the R_{max} and

R_{min} of the bubble. The theoretical prediction for the effect of the ultrasonic process parameters on collapse pressure demonstrated that:

1. Increasing the intensity of ultrasound has a positive effect of the bubble collapse. When the intensity increased from 17.56 to 24.17 W/cm^2, the R_{max} increased from 1.78×10^{-4} to 1.97×10^{-4} m, while the R_{min} decreased from 8.67×10^{-7} to 8.18×10^{-7} m.
2. The increase in the ultrasonic frequency adversely affects the growth of the bubble. The increase of ultrasonic frequency from 60 to 80 kHz led to decrease the R_{max} form 1.63×10^{-4} to 1.24×10^{-4} m and increase R_{min} from 0.923×10^{-6} to 1.137×10^{-6} m.
3. Increasing the temperature of the treated water from 45 to 55˚C resulted in marginal effects on the evolution of bubble radius. When the treated water temperature increased from 45 to 55˚C, the R_{max} increased from 1.797×10^{-4} to 1.804×10^{-4} m and the R_{min} decreased from 8.6×10^{-7} to 8.581×10^{-7} m.
4. Increasing the pressure applied on the treated water was found to have negative effect on bubble growth. The increase of the applied pressure on the treated water from 2 to 4 bars resulted in decreasing the R_{max} from 1.5×10^{-4} to 0.936×10^{-4} m and increasing the R_{min} from 0.971×10^{-6} to 1.52×10^{-6} m.

The theoretical study was accompanied with an experimental study that involved investigating the effect of ultrasonic intensity, treated water temperature, treated water pressure and treatment time on the viability of *E. coli*.

The experimental results presented in Chapter 4 were found to be consistent with the results of the theoretical study. The experimental results confirmed that the increase in the ultrasonic intensity for sonication, thermosonication and

manosonication can increase the log reduction of *E. coli*. However, the increase in the log reduction of *E. coli* due to ultrasonic intensity increase has an optimum limit (21.49 W/ cm^2) after which any increase in the intensity will have insignificant effect on the reduction of *E. coli*.

In relation to the effect of treated water temperature, this study showed that increasing the temperature of the treated water from 45 to 50 and 55°C caused an increase in the log reduction of *E. coli* as compared to sonication. In addition to that, the increase in the treated water temperature shortened the treatment time required to achieve complete reduction of *E. coli*. For instance, the treatment time required to achieve complete reduction of *E. coli* in thermosonication treatment at 45˚C and 17.56 W/cm^2 was 5 minutes, whereas, the complete reduction time in thermosonication treatment at 50˚C and 17.56 W/cm^2 was 3 minutes.

The experimental results of manosonication treatments for *E. coli* suspension demonstrated that increasing the applied pressure on the treated water resulted in an insignificant increase in the reduction of *E. coli* as compared to sonication treatments. The log reduction of *E. coli* obtained in manosonication treatment at 2, 3 and 4 bars and intensity of 24.17 W/cm^2 for 5 minutes was approximately 5.12, 5.11 and 5.10 $\log_{10}$ respectively. In comparison, sonication treatment at 24.17 W/cm^2 for 5 minutes achieved around 5.06 $\log_{10}$ reduction of *E. coli*.

With regards to treatment time, the increase in the treatment time of sonication, thermosonication and manosonication led to increase the log reduction of *E. coli* for all ultrasonic intensities. It was observed that increasing the treatment time of ultrasound treatments at 17.56 W/cm^2 to more than 4 minutes causes noticeable increase in the log reduction of *E. coli*.

It was found that thermosonication treatment at low sub-lethal temperature of *E. coli* (45˚C) is the optimum ultrasound treatment, as the energy required for 1 $\log_{10}$ reduction of *E. coli* in thermosonication treatment was the least, 113.1 kJ/kg, as compared to sonication, 140.4 kJ/kg and manosonication at 2 bars, 138 kJ/kg.

Although some literature point to the high energy demand associated with ultrasound use for deactivating microorganisms, the study presented in this thesis (Chapter 4) illustrated that the cost associated with ultrasound use can be reduced through three mechanisms:

1. Identifying the optimum level of ultrasonic power for the used ultrasound device. For example, the reduction of *E. coli* using ultrasonic intensity of 21.49 W/cm^2 was very close to that of 24.17 W/cm^2 for all ultrasound treatments. Therefore, from the energy consumption viewpoint, the intensity of the ultrasonic device used in this study should not exceed 21.49 W/cm^2 when the device used for microbial deactivation purposes.
2. Augmenting ultrasound with other techniques such as thermal techniques that have the potential to increase the reduction of microorganisms and decrease the treatment time.
3. Promoting the cavitational events of ultrasound through invigorating the formation of heterogeneous bubbles. When corrugated surface was immersed in *E. coli* suspension as heterogeneous cavitation nuclei, the log reduction of sonication treatments at 17.56, 21.49 and 24.17 W/cm^2 increased by 0.5, 0.3 and 0.2 $\log_{10}$ respectively.

Once thermosonication treatment was selected as the optimum ultrasound treatment, thermosonication treatment at mild temperature and intermediate intensity

for 4 minutes was applied to reduce the formation of biofouling on the RO membrane in fouling experiments that lasted for 60 hours. This, in turn, reflected on the permeate flux of the treated suspension which was higher than that of the untreated suspension by approximately 0.1 $L/m^2.hr$. The capacity of thermosonication in reducing the formation of biofouling on the RO membrane lies in three mechanisms:

1. *Decrease the concentration of microorganisms*: Thermosonication treatment at 21.49 W/cm^2 and 45˚C for 4 minutes reduced the concentration of *E. coli* from 6.4 $\log_{10}$ to 3.6 $\log_{10}$ in broth-based suspension.
2. *Cause cell injury*: The concentration of the injured *E. coli* cells in the treated suspension was approximately 0.4 Log_{10}.
3. *Negatively affect the reproducibility of the microorganisms*: The measurements of the logarithmic growth of the untreated and treated *E. coli* showed that, the increment of untreated *E. coli* population during 56 hours was 86.36%, while the increment of treated *E. coli* population during the same period of time was 68.4%.

These effects led to the formation of biofouling with smaller colonies and less coverage area than that of the untreated suspension.

Overall, the results presented in this study concluded that ultrasound can be used as an effective pre-treatment technique to alleviate the problem of biofouling in the RO membrane system. However, the following points are recommended to be considered for future work:

1. The effect of the operation conditions of ultrasound and the properties of the liquid on the number of collapsing bubbles in the liquid.

2. The effect of thermosonication treatment on the physiochemical interactions between the RO membrane and the cells of microorganisms.
3. The effect of thermosonication treatment on the tendency of EPS to foul the RO membrane and the capability of the treated *E. coli* to secrete EPS.
4. The efficiency of thermosonication in reducing biofouling formation in a large scale membrane system.

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Appendix A

The negative impacts of DBPs on public health

The awareness against Disinfection By-Products (DBPs) has launched in the 1970s. Chloroform and the other Trihalomethanes (THMs) were identified as first DBPs in chlorinated water in 1974 (Rook, 1974). Since then health organisations all over the world started warning from the serious health problems that these compounds could pose to the public. In 1976 the results of the study that was conducted by US national cancer institute showed the relationship between chloroform and cancer case in laboratory animals (Richardson, 2003). After the discovery of first DBPs in the chlorinated water, this method was replaced by using ozone, chlorine dioxide and chloramines to reduce the formation of DBPs. However these alternative methods such as ozonation has witnessed the formation of bromate and this also showed carcinogenicity effects in the laboratory animals (Kurokawa et al., 1986). Since the early detection of DBPs until 2002, the number of DBPs reached more than 500 compounds (Richardson, 2003). Although most of these compounds are public health concerns, brominated and iodinated compounds such as iodoacids, brominated forms of MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) and nitrosodimethylamine (NDMA) are considered to be most serious DBPs.

The formation of chlorinated DBPs was attributed to the reaction between chlorine compounds with organic contaminants in water. In contrast , iodinated and brominated DBPs are formed from the reaction of the disinfectant with the natural bromide or iodide that present in the natural water (Plewa et al., 2003). It was pointed out that the natural bromide and iodides are existed abundantly in the coastal

areas and its neighbourhood. Therefore, this illuminates a point that chemical treatments have to be avoided in the water plants that are installed in these areas. Additionally the formation of NDMA in the chlorinated can result from the reaction of sodium hypochlorite with dimethylamine (Richardson, 2003).

It is essential here to demonstrate some results of the toxicological and epidemiological studies on the possible effects of DBPs on the human health. Iodinated DBPs were deemed to be more toxic than its chlorinated counterparts as well they were found to be more biological reactive than brominated DBPs (Plewa et al., 2003). Komulainen et al.(1997) reported the possibility of MX compounds being carcinogenic in laboratory animals. Additionally, the high cytotoxicity and genotoxicity of brominated nitromethanes was observed in laboratory study on mammalian cells (Richardson, 2003). Furthermore, preliminary study of iodoactic acid has manifested the toxicity of this compound and its negative effect on mouse embryo (Plewa et al., 2003). More importantly, some of the DBPs such as NDMA have been suspected to be as a possible human carcinogen (Najm and Trussell, 2001). The epidemiological studies also illustrated the possible developmental effects of DBPs such as low birth weight, intrauterine growth retardation and spontaneous abortion (Richardson et al., 2002).

Appendix B

Mechanical methods for deactivating microorganisms

B.1 High Pressure Homogenization (HPH)

The homogenization technique emerged in 1900, when Auguste Gaulin invented the first homogenizer and presented it at the World Fair in Paris (Diels and Michiels, 2006). However, the earliest attempt to use HPH for microorganism cell disruption dates back to 1932 (Middelberg, 1995). After this time, many studies investigated the use of the HPH technique for extracting enzymes and intracellular products from various species of microorganisms such as *yeast* (Follows et al., 1971), *E. coli* (Higgins et al., 1978), *Bacillus* (Augenstein et al., 1974) and filamentous fungi *Aspergillus niger* (Zetelaki, 1969).

A standard homogenizer consists of positive displacement pump, valve seat, homogenizer valve and impact ring as shown in Figure B.1. (Middelberg, 1995, Diels and Michiels, 2006).

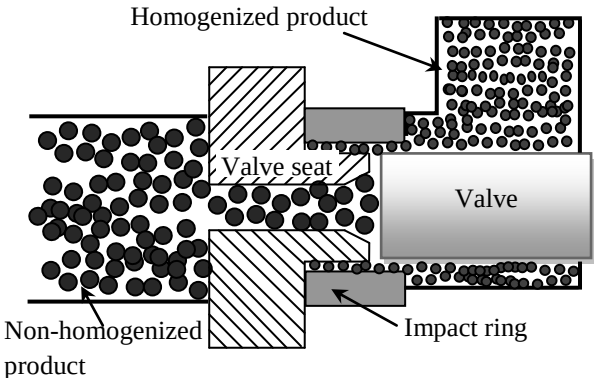


Figure B.1: Schematic diagram for standard homogenizer (Diels and Michiels, 2006)

The pump is used to force the fluid through a small orifice between the valve and the valve seat. When the fluid leaves the aperture between the seat and the valve, it strikes the impact ring and after that the homogenized fluid arrives at the discharge side. The pressure of the homogenizer can be regulated by changing the force applied to the valve (Middelberg, 1995).

The main mechanisms through which the HPH disrupts the cell of microorganism has been a controversial issue (Diels and Michiels, 2006). However, the most acceptable reported scenarios in the literature are presented herein:

1) *Rupture of microorganism's cell under the effect of the generated eddies in the turbulent flow (Doulah et al., 1975)*

Doulah et al.(1975) postulated that most of the compression energy applied to the homogenizer valve converts to kinetic energy dissipated in the liquid. The dissipated kinetic energy in the fluid contributes to the generation of turbulences in the liquid body. The turbulences, in turn lead to the formation of eddies with different scales and intensities. The larger eddies can only cause cell movement, while the smaller ones cause cell rupture. Small eddies have the potential to impart the dissipated kinetic energy in the fluid on the cell of microorganisms and thus cause the rapture. The condition for cell disruption can be met when the kinetic energy of the small eddies exceeds the strength of the cell wall.

2) *Rupture of microorganism's cell under the effect of impingement (Engler and Robinson, 1981)*

It was postulated that the microbial disruption in HPH happens when the high velocity jets of the suspensions strike the stationary surfaces in the homogenizer. The impact area of the stationary surfaces can have influence on the efficacy of the microbial disruption and this influence varies from one species of microorganism to

another. Middelberg (1995) reported that increasing the impact distance in HPH can decrease the disruption of *S. cerevisiae* and *E. coli*, however the decrease in the microbial disruption was more pronounced with *E. coli* than *S. cerevisiae*.

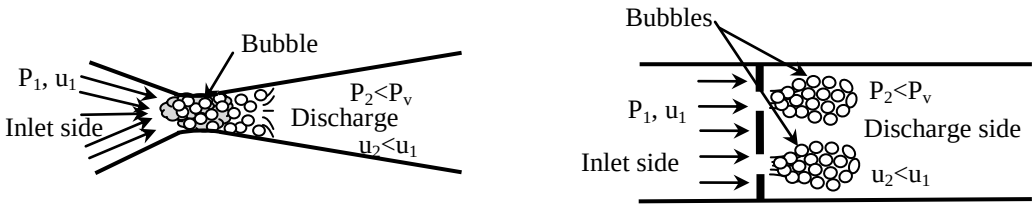
3) Rupture of microorganism's cell under the effect of cavitation (Save et al., 1994)

The microbial cell disruption in this scenario is attributed to the effect of hydrodynamic cavitation on the cell of microorganism. Hydrodynamic cavitation phenomenon can occur in particular parts of the homogenizer where the pressure of the fluid drops below the saturated vapor pressure, such as the orifice and the gap between the valve and the valve seat. The formation of hydrodynamic cavitation in throttling flow geometries, such as orifice and venturi, and its mechanisms in rupturing the cell of microorganism is explained in details in the next section.

B.2 Hydrodynamic cavitation (HC)

Hydrodynamic cavitation is defined as the process of generating, growing and subsequent collapsing of bubbles resulting from the pressure fall of the liquid under the saturated vapor pressure at a certain temperature. As the velocity of the liquid increases in the discharge side of cavitation chamber, the pressure decreases. The common design of a HC chamber includes venturi and orifice configurations as illustrated in Figure B.2 (a) and (b).

The most important parameters in HC deactivation include pressure parameters and time scale parameters. The pressure parameters include inlet pressure, minimum pressure and the recovered pressure. The time scale parameters of HC include three time scales, time for pressure decrease, rarefaction period, and time for pressure recovery (Arrojo and Benito, 2008).



(a) Venturi configuration (b) Orifice configuration

Figure B.2: Standard configurations for the hydrodynamic cavitation chamber

The disruption of microorganisms with the HC technique can happen through chemical and mechanical effects. Chemical effects are represented by the liberation of OH^-, which is an unstable radical. This radical can react with its counterparts producing H_2O_2. OH^- and H_2O_2 are strong oxidant agents that have the potential to rupture the cells of microorganisms. The reported mechanical effects of the hydrodynamic cavitation include the impingement of microorganisms onto the solid surfaces, turbulences produced by high-velocity liquid jets, shear rates generated in the adjacent area to the jets and shock waves generated by the bubbles' explosion (Sawant et al., 2008). The latter can be considered the dominant effect among the other mechanical effects of HC.

Sawant et al. (2008) tested the feasibility of hydrodynamic cavitation as a disinfection technique to deactivate zooplankton in seawater. The configuration of the cavitation chamber that was used for this purpose was an orifice design. They suggested that the opened area of the orifice could impact on the viability of microorganisms, as the achieved killing percentage of the zooplankton with an opened area of the orifice of 25%, 50% and 75% was around 79% , 78%, and 82% respectively. Another study of using HC for microbial disruption was carried out by Arrojo et al (2008) to deactivate *E coli* in the wastewater. Arrojo et al (2008) used two types of cavitation chamber designs; orifice with various numbers of holes and

venturi different cross-sectional areas. The outcomes of Arrojo et al.(2008) study showed that the venturi configuration is more effective than the orifice configuration. Among the different venturi configurations; the venturi with the smallest cross sectional area was the best in terms of *E. coli* deactivation.

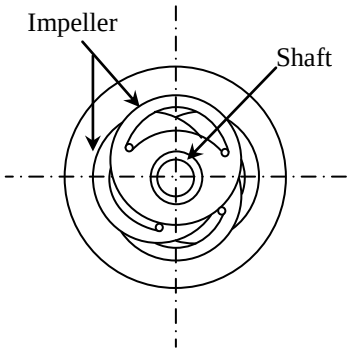
B.3 Bead mill

This technique was used first for wet-grinding of pigments in the paint industry (Kula and Schütte, 1987) and the grinding of ceramic and limestone (Bunge et al., 1992). The standard design for the bead mill device consists of a jacketed chamber that has a rotating shaft located in the centre of the chamber. The shaft has agitators that are attached to it in different configurations and designs, as shown in Figure B.3 (Middelberg, 1995).

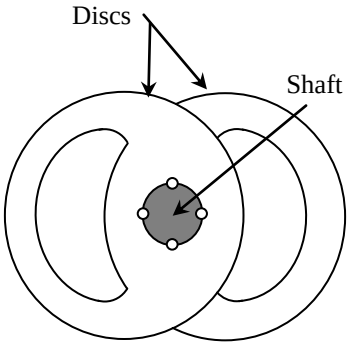
The function of the agitators is to transfer the energy that results from the rotation of the shaft to the solution body in order to induce the collision of beads in the solution. Part of the energy that is generated due to the rotation of the shaft converts to thermal energy, therefore the chamber of the bead mill is usually surrounded by a cooling jacket. The beads remain in the chamber using a sieve with size smaller sized holes than that of the beads (Macneill et al., 1985).

Middelberg (1995) reported that the mechanism of cell disruption in the bead mill could be due to shearing or compaction actions (Bunge et al., 1992, Melendres et al., 1992) and the effect of the transferred energy from beads to the cells of microorganisms (Macneill et al., 1985). The effectiveness of the cell disruption in the bead mill is influenced by many factors including; bead size, agitator peripheral

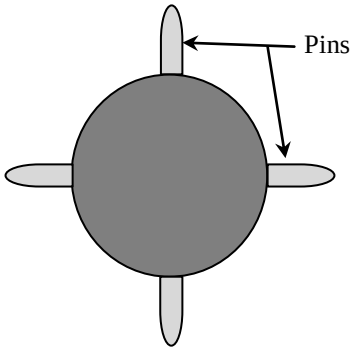
velocity, flow rate, concentration of microorganisms, volume fraction of beads in the chamber, and temperature.



Agitator with eccentrically impellers attached to the shaft.



(a) Agitator with double disc stirrer.



(b) Agitator with pins

Figure B.3: The common agitator designs of bead mill (Middelberg, 1995)

The effect of bead size on the efficacy of cell disruption was investigated by many studies and the outcomes of these studies were all different. Currie et al. (1972) showed that cell disruption with small beads is better than that with larger beads especially with low concentration of microorganisms, however small beads tend to float in a suspension with high concentration of microorganisms. Garrido et al.(1994) pointed to another advantage of using small beads, as beads with a small size can be fluidized more easily compared to the larger beads which results in higher bead-bead collision and hence higher rate of cell disruption. Deters et al.(1976), however, found

that loading the mill with small beads can reduce the number of the ruptured cells of microorganisms.

The effectiveness of cell disruption in the bead mill technique is directly proportional with the agitation speed (Schütte et al., 1983), volume fraction of the beads (Tamer et al., 1998) and the concentration of microorganisms (Ricci-Silva et al., 2000). Nevertheless, in some studies the concentration of microorganisms had no influence on cell disruption during the bead mill process (Mogren et al., 1974). Effect of temperature on the cell disruption in the bead mill is similar to that of other disinfection techniques, as increasing the temperature of the suspension can weaken the resistance of microorganisms to any kind of treatment.

B.4 Micro-fluidizer

Micro-fluidizer is another mechanical technique of microorganism disruption. In this technique, two streams of microorganisms' suspensions are impacted at high velocity on a stationary surface, and disruption takes place at the impact point (Geciova et al., 2002). The disruption chamber of the micro-fluidizer is considered to have the highest temperature compared to other parts of the fluidizer, and therefore, requires a cooling process. The efficiency of cell disruption is affected by the concentration, the strain type and the growth rate of the microorganisms (Middelberg, 1995, Geciova et al., 2002). The fragments of the disintegrated cells increase with increasing pressure and the number of passes through the chamber. Increasing the fragments of microorganisms in the product can reduce the efficiency of the subsequent centrifugal separation (Geciova et al., 2002).

Appendix C

Historical pathway of ultrasound technology

Table C.1: Summary of the important events in the history of ultrasound technology from the early observation until recently

Date	Events
1895	The first observation of cavitational effects by Thornycroft and Barnaby (1895), who noted pitting and erosion in the propeller of the submarine.
1917	Lord Rayleigh published the first theoretical model to describe the collapse of spherical bubble in a body of incompressible fluid (Rayleigh, 1917).
1927	The first documented survey of the chemical effects of ultrasound conducted by Richards and Loomis (1927). In the same year, Wood and Loomis (1927) investigated, for the first time, the physical and biological effect of ultrasound.
1937	Brohult (1937) discovered the potential of ultrasound in degrading the biological polymers.
1944	Harvey et al.(1944) proposed the earliest model for heterogeneous bubble formation on cracks and crevices of solid surfaces.
1949	Plesset (1949) published the first study on the dynamics of cavitating bubble, which was integrated into Rayleigh's work (1917) on bubble collapse resulting in the well-known Rayleigh-Plesset equation that describes bubble dynamics in liquid irradiated by ultrasound.
1950	Noltingk and Neppiras (1950) conducted the first computer calculations to model a cavitating bubble.
1964	The first emergence of the terms transient cavitation and stable cavitation, introduced by Flynn (1964). In the same year Neppiras and Hughes (1964) investigated the effect of hydrostatic pressure on the rupture of *yeast* cell using ultrasound, which was the first exploration for the use of the combination of ultrasound and pressure or so-called Manosonication.
1972	Burgos et al.(1972) investigated the effect of ultrasound treatment on the heat-sensitivity of *Bacillus cereus* and *Bacillus licheniformis*, which opened the door to the use of Thermsonication tretement (ultrasound+thermal treatment).
In late 1980s-the early 1990s	The commercial eara of ultrasound technology began, as the menufacturers sarted designing and marketing ultrasonic devices to be used for sonochemical researches (Thompson and Doraiswamy, 1999).
Last decade until currently	Researchers have been trying to extend the use of ultrasound technology to cover various chemical and biological applications.

Appendix D

Effect of the initial radius of the bubble on collapse pressure

The effect of different values of initial bubble radius of 0.01, 0.1 and 1mm on bubble growth was investigated at constant ultrasonic intensity and frequency of 17.56 W/cm^2 and 55 KHz respectively, and under atmospheric conditions. The results are presented in Figure D.1. It was found that increasing the value of R_i decreases the extent of bubble growth which can be evaluated from the value of R_{max}/ R_i and this can adversely affect the intensity of bubble collapse. Gogate and Pandit (2000) showed that increasing the value of R_i from less than 0.1 to 0.5 mm can reduce the value of $P_{collapse}$ substantially from more than 40000 atm to less than 5000 atm. The low degree of growth for large bubbles could be attributed to the low natural frequency of these bubbles (Arrojo and Benito, 2008) as demonstrated in Figure 3.6 (the curve of bubble growth for R_i = 1mm). Moreover, the minimum radius of the bubbles with large initial size is significantly higher than that of the bubbles with small initial sizes. This effect can also decrease the value of $P_{collapse}$.

Although, the value of R_i is one of the important parameters in the numerical simulation that has a great effect on $P_{collapse}$ in the theoretical study of ultrasound, giving the exact value of R_i is difficult (Gogate and Pandit, 2000). However, the effect of initial bubble size on the reduction of microorganisms is outside the scope of the experimental work in this study.

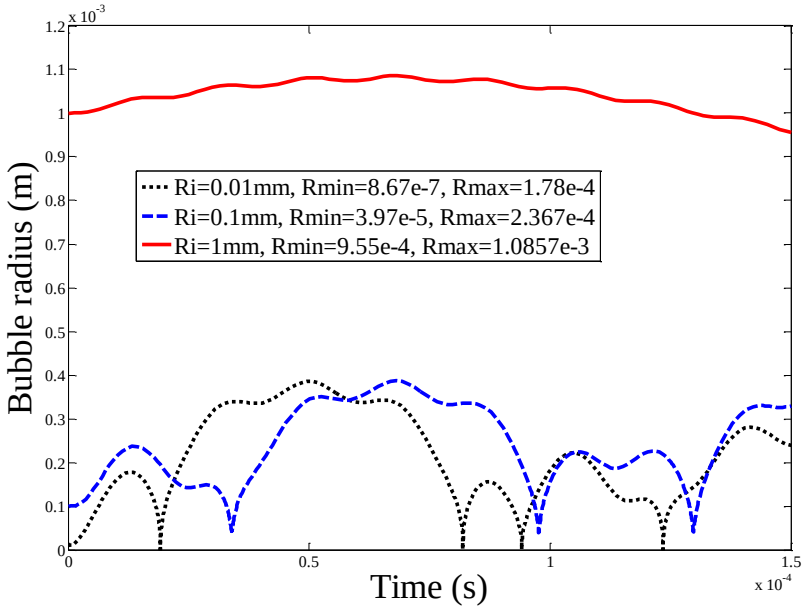


Figure D.1: Evolution of bubble with initial radii of 0.01, 0.1 and 1mm with ultrasonic intensity of 17.56 W/cm^2 and frequency of 55 kHz under atmospheric conditions ($P_\circ$ =101.3 kPa and T=25˚C)

Appendix E

Improving the performance of ultrasonic horn reactor

E.1 Introduction

An ultrasonic horn reactor was used in this study to deactivate *E. coli*. This type of reactor is the most used reactor of ultrasonic reactor types (Gogate, 2007). However, the mechanical and chemical effects of ultrasound in this type of reactor are restricted to the axial and radial distances away from the ultrasonic horn (Gogate et al., 2002). Another shortcoming of the ultrasonic horn reactor is the shedding and the erosion of its irradiating surface when using it with high ultrasonic intensity (Gogate and Kabadi, 2009). These two disadvantages of the ultrasonic horn reactor make the energy requirements associated with using this type of reactor high.

Immersing a corrugated surface as cavitation nuclei in the suspension of *E.coli* has been proposed in this study to enhance the ultrasonic deactivation for the ultrasonic horn reactor. Using this technique to improve the performance of the ultrasonic horn reactor in deactivating microorganisms is considered to be applicable for other ultrasonic reactors.

E.2 Preparing the corrugated surface

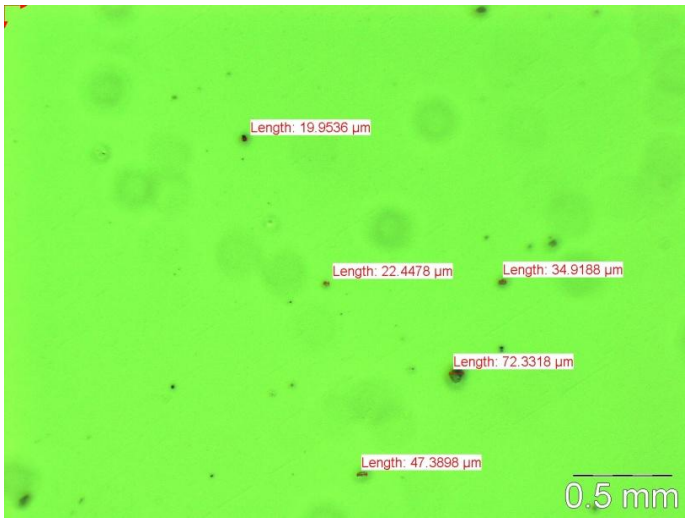
The corrugated surface used in this study was constructed from pebbles with an average length of 10 mm adhered to a circular plate with a thickness of 1 mm using commercial silicon.

E.3 Particle shedding

Irradiating *E. coli* suspension with the presence of a corrugated surface can cause particle shedding from the corrugated surface. To detect the occurrence of particle shedding during ultrasound treatment, the corrugated surface was submerged in 50 mL of nano-pure water and subjected to ultrasound irradiation with three intensities 17.56, 21.49 and 24.17 W/cm^2 for 4 minutes. Thereafter, the treated water was filtered using a flat-sheet RO membrane. This flat-sheet membrane was used due to its fine pores, so the particles with nano-scale size are captured. Later, the filtrate particles were visualized using a Motic Stereo-microscope. Photos of the eroded particles with the three ultrasonic intensities were captured using a CC12 camera which was attached to the microscope. The photos were analysed using *AnalySIS* software (as illustrated in Figure E.1).

It is shown in Figure E.1 that, the number of the eroded particles with ultrasonic intensity of 21.5W/cm^2 is higher than that of 17.56 and 24.2W/cm^2. These are expected results, as 21.5W/cm^2 has been identified as the optimum ultrasonic intensity for the ultrasonic device used in this study.

The effect of the eroded particles from the corrugated surface on *E. coli* reduction will be investigated in the following section.



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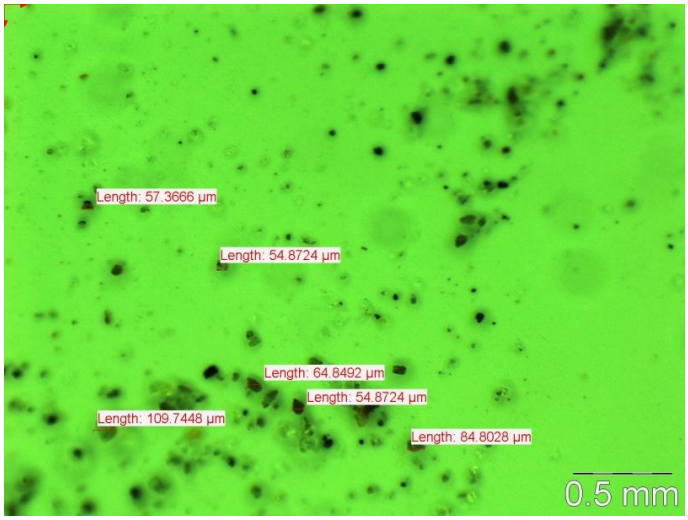

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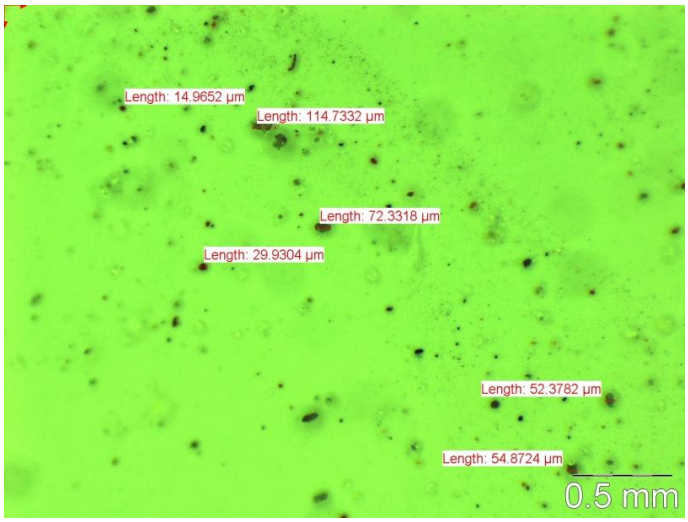


(c)

Figure E.1: Eroded particles from the corrugated surface with three different ultrasonic intensities (a) 17.6W/, (b) 21.5W/cm^2 and (c)24.2W/cm^2

E.4 Investigating the effect of the eroded particles on the viability of *E. coli*

The filtrate particles from the corrugated surface with three ultrasonic intensities 17.56, 21.49 and 24.17W/cm^2 were seeded in 50 mL of *E.coli* suspension and treated with their corresponding ultrasonic intensities for 3 minutes. The purpose of these experiments is to identify the effects of the eroded particles on the viability of *E.coli*. The results of viable cell count for these tests were compared to the results of the treated suspension with and without corrugated surface and are presented in Table E.1. Log reductions of *E. coli* in Table E.1 are expressed in the mean value of viable cell count for triple plates with the standard of errors of the mean.

Table E.1: Comparison between log reduction of *E.coli* of three ultrasonic intensities 17.6, 21.5 and 24.2W/cm^2 and treatment time of 3 minutes for three different treatments; without corrugated surface, with corrugated surface and with the eroded particles suspended in *E.coli* suspension

Ultrasonic intensity(W/cm^2)	**Log reduction of *E.coli* (CFU/mL)**		
	Without corrugated surface	**With corrugated surface**	**With eroded particles**
17.56	1.966527±0.12	2.685172±0.057	2.045417±0.073
21.49	3.104567±0.267	3.485542±0.042	3.205275±0.13
24.17	3.567778±0.192	3.71963±0.133	3.602783±0.097

Generally, the presence of the eroded particles in the suspension has a slight effect on *E.coli* reduction as compared to the overall effect of the corrugated surface. Submerging the corrugated surface in the suspension of *E.coli* led to an increase in the log reduction of *E.coli* by almost 0.72, 0.38 and 0.152 $\log_{10}$. In comparison, seeding the eroded particles in the suspension resulted in an increase in the log reduction by approximately 0.08, 0.1 and 0.035 $\log_{10}$ for the intensities 17.56, 21.49 and 24.17W/cm^2 respectively.

However, Table E.1 shows that the effect of eroded particles on *E.coli* viability is more sensitive to ultrasonic intensity of 21.5 W/cm^2 than the other two intensities (17.56 and 24.17 W/cm^2). This can be ascribed to the high loading of eroded particles of the intensity 21.49 W/cm^2 as opposed to the other intensities (as shown in Figure E.1). The eroded particles in the suspension act as beads in bead mill treatment, as increasing the loading of the beads in the suspension results in an increase in the disruption rate of the microorganisms (Tamer et al., 1998).

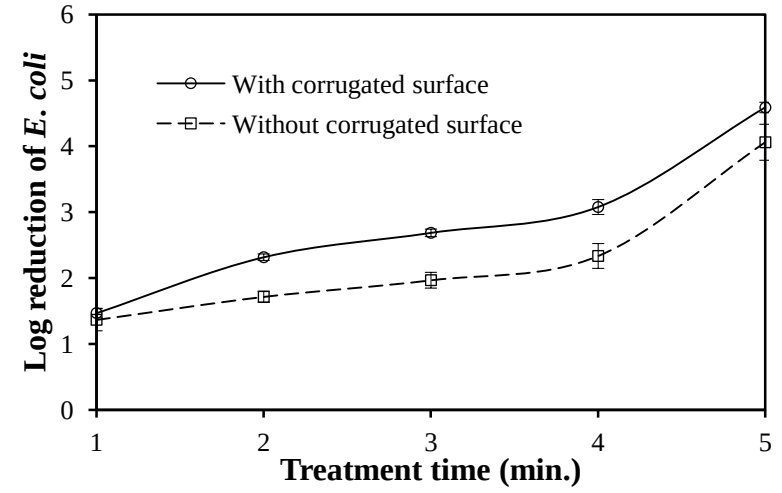
E.5 Effect of corrugated surface on log reduction of *E.coli*

Figure E.2 reveals the log reduction of *E.coli* under the effect of three different ultrasonic intensities 17.56, 21.49 and 24.17 W/cm^2 for two treatments, with and without corrugated surface. The improvement in the reduction of *E.coli* due to immersing a corrugated surface in the suspension could be due to the combination of the synergistic effects of the surface heterogeneity that served as cavitation nuclei and the effect of the eroded particles from the corrugated surface. The effect of the eroded particles was identified in Section E.4 as having a slight effect on the viability of *E. coli* as compared to the overall effect of the corrugated surface. Therefore, the increase in the log reduction is believed to be due to the heterogeneity of the corrugated surface that contributed to the formation of large number of bubbles and thus increased the cavitational events. Ince, N.H. and R. Belen (2001) found that the decrease in *E.coli* viability treated by ultrasound when seeding granules of activated carbon in *E.coli* suspension, is attributed mainly to the cavitational effects induced by the formation of bubbles on the solid-liquid interface. Similarly, Dadjour, M.F., et al (2005) used pellets of TiO_2 with the diameter of 2 mm to enhance the deactivation of *E.coli* using ultrasound treatment. They confirmed that the decrease in the *E.coli*

viability with presence of TiO_2 was attributed to the catalytic effect of TiO_2 and the surface heterogeneity of the pellets that contributed to the formation of additional bubbles.

From Figure E.2, it can be said that the presence of a corrugated surface in the suspension is more effective with low ultrasonic intensities than higher intensities. This could be because the irregularities of the corrugated surface acted as pockets to accommodate the heterogeneous cavities. This in turn helped the low ultrasonic intensity to form additional bubbles at negative pressure less than the required pressure to overcome the cavitation threshold of the water (Bremond et al., 2005). Similar observations were reported by many studies. For instance, Broekman et al.(2010) reported that the presence of micro-bubbles in the medium can reduce the cavitation threshold and stimulate the low power ultrasound to generate cavitation in that medium. Likewise, Mahulkar et al. (2008) injected steam bubbles into an ultrasonic bath to enhance the cavitational yield. They succeeded in increasing the efficiency of acoustic cavitation in the non-degassed and degassed water by 4 and 16 times respectively. However, the case of higher ultrasonic intensities is different, as these intensities may have sufficient negative pressure to rupture the water and generate bubbles. Hence, the effect of the corrugated surface was not as pronounced as it was with low ultrasonic intensity.

Over all, immersing a corrugated surface or constructing a cavitation chamber with a corrugated internal surface, can increase the reduction of microorganisms for the same ultrasonic intensity. This technique could be even more feasible with low ultrasonic intensities than high intensities.



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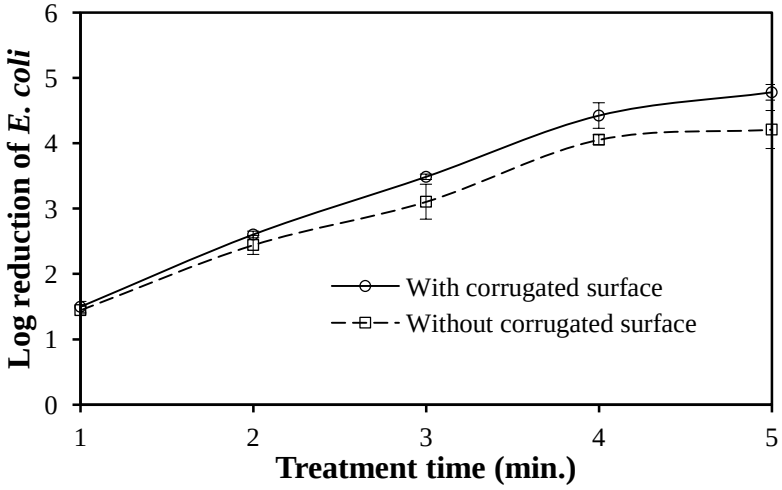

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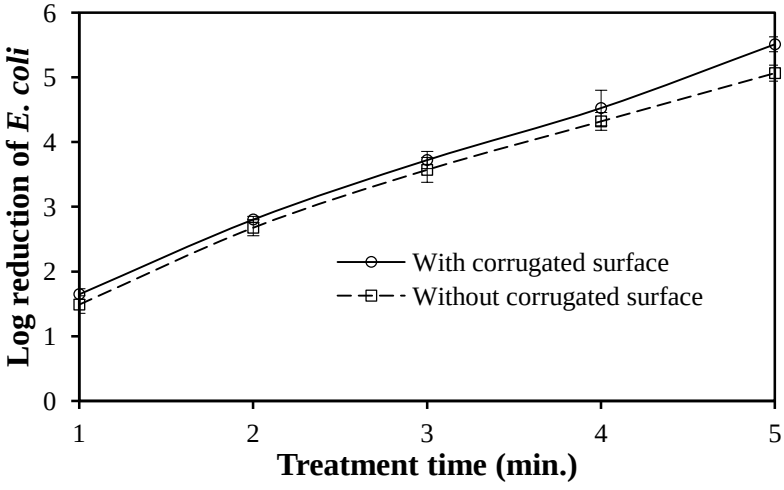

(c)

Figure E.2: Log reduction of *E.coli* for three ultrasonic intensities a) 17.6, b) 21.5 and c) 24.2W/cm^2 using corrugated surface (solid line) and without corrugated surface (dashed line)