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Minimizing bacterial biofilm in water using froth flotation and shock chlorination

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By

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DEDICATED TO THE SOUL OF MY BROTHER SALAH

Abstract

Microorganisms in potable water systems are either present in the planktonic “floating stage”, or attached to surfaces, usually as biofilm. Many hygienic and economic problems are attributed to this phenomenon. Through the last seven or so decades many trials were made to overcome biofilm problems. Unfortunately, biofilm prevention stills a premature field of science. Therefore, serious solutions with new horizons are needed.

The objective of this study is to provide a water distribution system which is free of microorganisms, both in the planktonic and biofilm stages, and biocides. The two components are interactive; biofilm formation is stimulated by the presence of low (residual) levels of biocides whereas removing the bacteria results in less or no biocide being needed.

The first part of the research investigates the use of chemical free froth flotation to remove microorganisms. Bacteria are hydrophobic and thus should be able to be separated by froths. A method for producing froth without using chemicals has been developed to avoid water quality deterioration and this froth is shown to hold bacteria without the need for biocide treatment or frothing chemicals. Experiments were undertaken to explore how far this process can purify a water stream. The froths formed were found to hold up to 2×10^{8} cfu ml^{-1} of bacteria without chemical collectors, and made a cfu/ml drop of 55% between the inlet and outlet streams.

The second area of research investigated shock chlorination and dechlorination to kill the microorganisms but almost immediately remove the biocide. Among all the dechlorination techniques, aeration was found to be the most suitable method. Investigations were undertaken to determine how fast and how practically acceptable the method of dechlorination by aeration is. Dechlorination design demand was found to be ($9\times10^{-4}\frac{lair}{lwater\ .\ ppm}$).

Around these two main areas of research supporting researches were undertaken. Studies found that dead bacteria were able to be removed by froth flotation as well as, or even better than live bacteria. Chlorination was faster than dechlorination under the same conditions by a factor of 25%. At low bacterial content, chlorine demand is directly proportional to cfu/ml. Zero bacterial content can be obtained through shock chlorination. The newly developed iPhone and iPad colony counting applications were investigated as a rapid way of measuring bacterial count.

These were found to be reliable and accurate and, with additional manual manipulation proved very suitable to use for counting bacteria.

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Nomenclature and Abbreviations

1- Nomenclature

C*= Gas maximum concentration that drives the mass transfer (ppm).

C= Gas concentration at time = t (ppm).

C= Chlorine concentration after time t (ppm).

C= Concentration gradient ($\frac{mol}{m^3}$)

C_o= Gas minimum concentration that drives the mass transfer (ppm).

C_o= Chlorine initial concentration (ppm).

D= Diffusion coefficient ($\frac{m^2}{s}$)

d_b = Bubble diameter (m).

J= Diffusion flux ($\frac{mole}{m^2.s}$).

K= Proportionality constant (dimentionless).

K_b= Bulk chlorine decay constant (1/time).

$K_l a$= Liquid phase mass transfer coefficient (1/time).

K_w= Wall chlorine decay constant (1/time).

N= Number of bacteria at time (t).

N= Number of microorganisms remaining at time t.

N_0 = Initial bacterial population.

N_o= Original number of microorganisms.

P= Pseudo read dots, these whose are not dots and counted as dots (or colonies).

R= Reading of iPad or iPhone by number of dots (or colonies).

Re_b= Reynolds number for a bubble rising in a liquid (dimensionless).

t= Time (time units).

tg= The generation time (time units).

t_o= The initial time (time units).

x = The number of bubbles that lined adjacently along the length of cube line (r). Or it is the cubic root of the bubbles inside the tubes.

x= The length vertical to mass transfer area (*m*).

μ_L: The liquid viscosity (*Pa.s*).

ρ_L: The liquid density (kg/m^3).

2- Abbreviations

cfu ml^{-1}= Colony Forming Unit per Milliliter.

CT= Concentration × Time.

NTU= Nephelometric Turbidity Unit.

rpm= Revolution Per Minute.

ppm= Part Per Million.

UV= Ultra Violet.

CHAPTER ONE

INTRODUCTION

Chapter One

Introduction

Since the attachment of microorganisms to wet surfaces was first described 70 years ago (Zobell, 1943), and first termed “Biofilm” more than 40 years ago (Buchholz *et al.*, 2010) huge hygienic and economic problems have been ascribed to its existence. Losses increase every day and there is little assessment of the loss. Threatened infrastructure elements include heating and air conditioning networks, medical implants, oil refineries, plumbing and food processing facilities (Kumar and Anand, 1998; Costerton and Stewart, 2001; Chmielewski and Frank, 2003). Assessments have been made of the effect of this phenomenon, in specific industries. Examples include the accumulation of bio-fouling and larger marine organisms on ship hulls which increases their fuel consumption up to 40% (Upadhyayula and Gadhamshetty, 2010). Medically, biofilms are responsible for many persistent infections including 80% of microbial cases, and consequently, 10% of hospital cases in the United States - 100,000 patients die annually (Davies, 2003; Schachter, 2003; Klevens, 2007). Drinking water systems can be an ideal environment for biofilms, and need particular attention, because the biofilms can contain bacteria such as *Escherichia coli*, *Aeromonas spp.*, and *Pseudomonas spp.* (Huq *et al.*, 2008), which represent a serious threat to human health.

Although many methods have been tried to decrease biofilm formation, none of them is perfect. They may need expensive capital or running costs, or frequent maintenance and shut down. However, fighting biofilm is still a developing field and needs more research (Simoes and Simoes, 2013).

In order to form, a biofilm needs certain fundamental elements such as the presence of the microorganisms themselves, water and a surface or interface. If one of these is eliminated the whole process stops. The formation of a biofilm is stimulated by certain factors, in particular stresses in the environment such as a lack of nutrients, presence of biocides, pH and temperature. Eliminating or minimizing such stimulating factors can minimize biofilm (Flemming, 2008; Simoes *et al.*, 2010).

This work seeks to investigate methods of breaking the biofilm formation chain to, ideally, produce bacterial free water and to produce water with low levels of stressors to minimize

subsequent biofilm formation. The first objective was to develop a process to purify water from microorganisms physically without stressing them and thus reducing any remaining from producing biofilms and minimize the subsequent need for biocides. The second objective was to completely kill all microorganisms with a high dose of biocides and then remove this dose to obtain water free of microorganisms and with minimal residual biocides and associated compounds. Both of these objectives aim to provide a drinking water distribution system with microorganism and biocide free water.

The method to physically remove bacteria utilizes the principles of froth flotation. Froth flotation purifies water from microorganisms using hydrophobicity as driving force as most microorganisms, especially bacteria, are hydrophobic. Shock (or "slug") biocide treatment using suitable concentrations and contact time of chlorine, followed by shock (rapid) biocide removal should kill all the microorganisms while leaving the water biocide free.

Over the last sixty years, froth flotation has been used to collect hydrophilic or less hydrophobic materials, for example the adsorption and subsequent flotation of metal particles (Smith *et al.*, 1993; Nagaoka *et al.*, 1999). Algae have been separated (harvested) from suspensions using hydrophobicity in bubble columns (Levin *et al.*, 1962). Bacterial strains have been separated in the laboratory using froth flotation (Boyles and Lincoln, 1958; Rubin *et al.*, 1966; Bahr and Schugerl, 1992; Rios and Franca, 1997). Japanese researchers succeeded in removing bacteria from fish sea farms using froth flotation columns using 1 mg/l milk casein as frother (Suzuki *et al.*, 2008). A recent trial for separating algae using micro-bubble flotation has given good results (Hanotu *et al.*, 2012).

The biggest limitation against using froth flotation in the drinking water industry is the need to use various chemicals to produce froth and keep the separated particle attached to a bubble for later transfer out of column. These chemicals may deteriorate water quality; for instance, taste and odour, as most of them are alcohols and polyglycols (Finch and Zhang, 2014). Therefore, the ideal froth floatation system for dealing with drinking water should produce froth without chemical frothers or collecting agents. In this work, three chapters deal with this aspect (Chapters 4, 5 and 6). The first describes the development of a new technique to produce froth physically without chemicals by using water and compressed air only. The second reports on whether, and

to what extent, this froth can hold bacteria like the chemical based froths. The third chapter reports on studies of the bio purification force of such a process.

The expected bio-purification from froth flotation may not give the highest quality bacteria free water. Therefore, it is suggested that a froth flotation treatment be followed by shock chlorination and shock de-chlorination. Chlorination is a well-known technique in water treatment but the de-chlorination is left to occur naturally and, in many cases, to leave an intended residual chlorine content at the point of use. Forced de-chlorination can be implemented by three main techniques, aeration, activated carbon, and chemical addition. The last two have many defects from the point of view of the drinking water industry. Aeration seems to be the best but it is described as a slow process because it performed stagnantly and naturally. There is little information in the literature on forced aeration de-chlorination and its engineering design and thus its acceptability to the industry. Chapter 7 of this thesis reports on an investigation into the use of de-chlorination by aeration. By using shock chlorination and de-chlorination the produced water should be with lowest chlorine level and no or low bacterial content. By this biofilm should be maintained at its minima.

Bacterial colony counting is an essential part in this work and chapter 3 describes the development and evaluation of a method using newly developed iPhone and iPad applications for rapid colony counting to support water quality analysis.

This thesis aims to answer the following questions with the objective to produce high quality drinking water free of microorganisms and biocides while preventing biofilm formation in the distribution system:

1- Is it possible to produce froth without chemical frothers?
2- If yes, can this froth hold bacteria without chemical collectors?
3- If yes, is it possible to continuously purify water from microorganisms by this method?
4- If further treatment is needed to give the desired purification, how fast, effective and safe is shock chlorination followed by shock dechlorination by aeration?
5- As part of developing the methodology, how effective and accurate are the iPhone and iPad colony counting applications?
6- How far do dead bacteria respond to the separation by froth flotation?

7- At low bacterial content levels, does bacterial concentration affects the chlorine demand?
8- How fast is the chlorination by chlorine gas in comparison with the dechlorination by aeration?

CHAPTER TWO

LITERATURE SURVEY

Chapter Two

Literature Survey

2.1 Biofilms

2.1.1 Definition of a biofilm

Many medical dictionaries like Mosby's Dental Dictionary start the definition of a biofilm with "Thick grouping…", while the Farlex Partner Medical Dictionary uses the contrary "Thin coating …" Most other sources avoid using these two words because neither is suitable to describe a biofilm, as the thickness of any layer is relative. Also, the words of both ("group" and "coating"), are not ideal. So, the most preferred word commonly used is "aggregates", but aggregates of what? The medical sources, especially the dental ones, uses "aggregates of bacteria", while some of studies prefer cells rather than bacteria especially when moving from medical to engineering fields (Chrysi and Rittmann, 2004; Kim *et al.*, 2012). But, the most common term is "microorganisms" because it is more inclusive.

The next commonly used expression is "attached to a wet surface", which indicates two facts, first, that the biofilm should be attached to a solid surface as opposed to "free planktonic" or "free floating" microorganisms. The second is this surface should be wet since it is extremely rare to find a biofilm on a dry surface (Iibuchi *et al.*, 2010).

Another part of the biofilm definition that is mentioned frequently is "secured by extracellular polymeric substances (EPS)"; EPS is made up of biopolymers produced by microorganisms and contains a wide range of proteins, glycolipids, and glycoproteins. In some circumstances extracellular DNA (e-DNA), can be found in its polymeric structure (Flemming *et al.*, 2007).

Finally, the main driving force behind biofilm formation is defensive strategy and metabolic needs (Chandra, 2001; Flemming, 2008; Simoes, 2010; Kim, 2012). Consequently, a complete statement that defines a biofilm could be, "an aggregate of microorganisms attached to a wet surface and secured by extracellular polymeric substances as a defensive strategy".

2.1.2 Biofilm formation stages

Regardless of the wide range of species that may form it, as well as the variety of theories to explain it, all biofilm formation can be described in three steps. The first is attachment, where planktonic microorganisms reach a surface and stick to it. Secondly is growth, in which the attached bacteria start to attract other ones to the main body of aggregates, and also begin reproduction. Finally, when the growth reaches a certain size or age, groups of bacteria and parts of the mature biofilm are detached to the flow stream (detachment). Figure 2.1 illustrates these three steps.

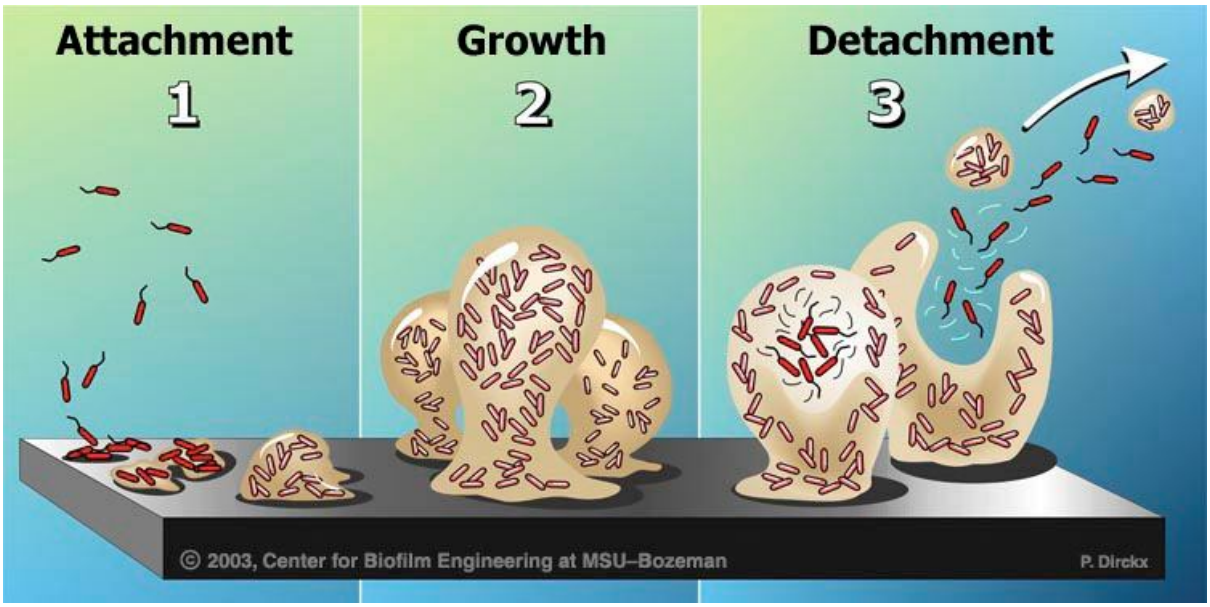

Figure 2.1: Biofilm formation stages. Source: (http://www.biofilm.montana.edu/node/2390)

2.1.3 Biofilm formation driving forces

Jefferson links biofilm formation to Darwin's theory of evolution, that the only driving force behind any change in any organism is reproductive fitness (Jefferson, 2004). Jefferson lists a number of advantages of biofilms; first, a defensive reaction against undesired environmental change, such as pH, disinfectants, oxygen radicals and antibiotics. Secondly, biofilm formation is a mechanism for remaining in a favorite niche, where the solid surface represents a better place to live. Thirdly, the biofilm community can be considered akin to multicellular organisms exhibiting cooperative behavior (Shapiro, 1998). Bacteria have the ability to adjust their metabolic activity to maximize the benefit of available resources as well as for protection and

gene transfer (Speranza *et al.*, 2011). Also, the biofilm may be considered to be the default mode of existence.

However, Darwin's theory deals with species not with colonies or communities, and finding an analogy between biofilm formation and a species is not a useful exercise. This was shown by testing the response of *Escherichia coli* strains to environmental cues, where they were found to respond differentially (Weiss-Muszkat *et al.*, 2010).

Hydrophobicity is also a significant force driving microorganisms to form biofilms causing bacteria to tend to leave the liquid phase and attach to the surface (Ruhs *et al.*, 2014). Unfortunately, the only known way to reduce bacterial hydrophobicity is by increasing the water NaCl concentration which makes reducing biofilm in drinking water using such technique unacceptable (Shephard *et al.*, 2010)

A poor nutritional environment has been found to drive microorganisms to form biofilms (Jefferson, 2004; Fujishige *et al.*, 2006). Phosphate, Magnesium, Calcium, D-Sorbitol, NaCl, Nitrate, and Sucrose are found to be the main nutrients that affect biofilm formation rate (Rinaudi *et al.*, 2006).

Furthermore, after reaching a certain biofilm thickness the upper part of biofilm layer tends to detach (Shirtliff *et al.*, 2002). The detached lumps present an indirect source of nutrients and provide a new growth surface for biofilm (Griebe and Flemming, 1998). The effect of nutrient reduction has a long range effect on both bulk and biofilm bacteria. In numbers, when applying Nano filters for nutrient reduction along time periods of 6 weeks and 1 year starting from a bulk bacteria concentration of 10^3 cfu/ml, the results were 259 and 50 cfu ml^{-1} for bulk bacteria and 1.2×10^5 and 1.9×10^5 cfu ml^{-1} respectively (Volk and LeChevallier, 1999). This clearly shows that low nutrient levels stimulate bacteria to form biofilms.

Most biofilms have a maximum formation rate around pH 7. Some show a sharp increase in formation rate as the pH comes near 7, while individual species have particular requirements, the biofilm produced by *Sinorhizobium meliloti* shows a sharp increase and then a sharp decrease in formation rate between pH 6-8, while *Burkholderia pseudomallei* shows little difference in formation rate between pH of 5 and 9 (Rinaudi *et al.*, 2006; Ramli *et al.*, 2012). Biofilms ideally

form around 30 C°, while raising or lowering the temperature reduces the formation rate (Rinaudi *et al.*, 2006; Di Bonaventura *et al.*, 2008; Ramli *et al.*, 2012). However, this may also depend on the nature of the attachment surface; more thermally conductive materials decrease the optimum temperature for biofilm formation and vice versa (Rogers *et al.*, 1994; Silhan *et al.*, 2006). Some metallic surfaces possess a toxic effect on bacteria in both, planktonic and sessile phases. Examples are Zinc and Copper (Labrie *et al.*, 2010).

2.1.4 Biofilm prevention

Simoes and Simos (2013) state that a biofilm can be minimized by one or more of the following techniques: optimizing the (Carbon, Nitrogen, and Phosphorous) concentration in the water, using chemical and biological stable pipes and fittings, designing the piping system to avoid sedimentation and stagnation; and frequent disinfection.

In the first technique, the aim is to achieve an optimized level of nutrients that avoids two limits, more nutrients needs more chlorine or other biocide treatment and less nutrients leads to more biofilm formation (Griebe and Flemming, 1998; Simoes *et al.*, 2010). In order to lower the organic matter, a wide range of pre-treatment is used like microfiltration, ultrafiltration, Nano filtration, reverse osmosis, activated carbon, and ion exchange. These methods are expensive and not effective in drinking water pipes, as bacteria can regrow in the oligotrophic medium (Momba and Binda, 2002; Srinivasan and Harrington, 2007; Zhou *et al.*, 2009). However, some European countries produce high quality drinking water without using residual chlorine by fine control of nutrient levels using chemical treatment like peroxide and ozone, rather than physical methods such as filtration and sedimentation (Simoes and Simoes, 2013). This was also emphasized during a visit to Mulheim- Germany drinking water facility as a part of DBP 2014 conference.

Some researchers found that a concentration of 0.05 ppm is sufficient to prevent biofilm growth (Lund and Ormerod, 1995) while earlier researchers noticed planktonic and biofilm bacteria even with 0.05 to 0.2 ppm but they found no biofilm at a free chlorine concentration of 0.5 ppm for 17 days (Characklis and James, 2009). Nutrient reduction and disinfection investigated recently has been evaluated as having “limited efficiency” especially when dealing with treatment after biofilm formation due to the cohesive nature of exopolymeric substance matrix (EPS) (Mathieu

et al., 2014). In other words once you have a biofilm it is more difficult to get rid of than preventing it forming in the first place.

Using the appropriate pipe material is the second prevention approach, as biofilms grow on some materials faster than on others (Silhan *et al.*, 2006). Unfortunately, there is no material that can stop the biofilm formation completely. Some iron based pipes react with chlorine and other disinfectants reducing their efficacy (Kerr *et al.*, 2003). On the other hand some are naturally biocidal, Copper being the most widely used example (Silhan *et al.*, 2006). But the conclusion is that, depending on pipe design can only be used as a supplementary method to other strategies. Furthermore, when designing a water piping system, many other concerns like cost, corrosion and pressure have to be considered in material selection before biofilm prevention.

The third strategy is a well-designed water transfer system. This may be achieved by avoiding dead ends and places of stagnation, since high rate biofilm formation and high bacterial numbers are associated with none or low-flow regimes (LeChevallier *et al.*, 1987; Maggy and Momba, 2002; Ayoub and Malaeb, 2006). Again, this does not prevent biofilm formation and deals only with some limited critical points, not with the whole system.

Disinfection represents the main strategy for fighting biofilm accumulation in drinking water facilities. Chlorine is the most used chemical disinfectant due to its suitable operational features; high solubility, low cost, stability, and ease of use. However, there are many disadvantages, one of them being the necessity to use excess amounts to achieve efficacy, which means stronger odor and taste and more organoleptic problems, as well as increasing the disinfection by-products such as haloacetic acids and trihalomethanes, which represent a serious threat to human health (Bull *et al.*, 1995; Nieuwenhuijsen *et al.*, 2000). The most negative concern of using chlorine for drinking and swimming pools is the direct relation of its by-products with bladder and rectal cancer (Morris *et al.*, 1992; Kogevinas *et al.*, 2010), pancreatic cancer (Do *et al.*, 2005), colon cancer (Hildesheim *et al.*, 1998) and even breast cancer. Ultra violet and Ozone techniques are also used for disinfection but not widely, because of high electricity consumption, hence, cost (Gomeza *et al.*, 2012). Table 2.1 summarizes the most used disinfectants in the drinking water industry.

Table 2.1: Used disinfectants in drinking water industry with their characteristics

Disinfectant	Effectiveness against	Inactivation mechanisms	Residual disinfection in DWDS	DBPs production
Chlorine	Bacteria Viruses	Capable of producing lethal events at or near the cell membrane, as well as affecting DNA. In bacteria, chlorine was found to adversely affect cell respiration, transport and possibly DNA activity. It causes an immediate decrease in oxygen utilization, damages the cell wall membrane, promotes leakage through the cell membrane and produces lower levels of DNA synthesis.	Yes	Trihalomethanes Haloacetic acids
Chloramines	Bacteria	It readily reacts with amino acids. The mechanism of inactivation is therefore thought to involve inhibition of proteins or protein-mediated processes, such as respiration.	Yes	Trihalomethanes Haloacetic acids
Chlorine dioxide	Bacteria *Cryptosporidium* *Giardia* Viruses	It reacts readily with amino acids, free fatty acids and nucleic acids, but not with viral ribonucleic acids. It inactivates viruses by altering the viral capsid proteins. In addition, it inhibits the protein synthesis and disrupts the permeability of the outer membrane of bacteria.	Yes	Chlorite Chlorate Haloacetic acids
Ozone	Bacteria *Cryptosporidium* *Giardia* Viruses	In bacteria, it attacks the cell membrane (glycoproteins, glycolipids, amino acids) and disrupts enzymatic activity by acting on the sulfhydryl groups of certain enzymes. Beyond the cell membrane and cell wall, it may act on the nuclear material and affect both purines and pyrimidines in nucleic acids. In viruses, it damages the viral capsid proteins and the nucleic acids.	No	Haloacetic acids Aldehydes Aldo- and Ketoacids Brominated DBPs Hydrogen peroxide
UV radiation	Bacteria Viruses Protozoa	Penetrates the microbial cell wall, disrupting the genetic material, making reproduction impossible.	No	No

Source: (Simoes and Simoes, 2013). DWDS= Drinking Water Distribution Systems, DBPs=Disinfection by-products.

In general, the desired approach to deal with a biofilm is prevention rather than treatment (Hori and Matsumoto, 2010). However, prevention is mostly dealing with the liquid, while treatment is working on the surface. Treatment of the liquid phase is mainly by disinfectants, which can be defined as the use of antimicrobials to kill the microorganisms that may form the biofilm. Their main aim is to decrease the number of viable cells that can attach to the surface. The efficacy of a biocide is increased in media that do not contain organic materials like fat, proteins and carbohydrates. However, this is not the only factor that may affect disinfectants: temperature, pH, chemical inhibitors, water hardness, contact time and concentration should all be taken into consideration when designing biofilm prevention systems (Cloete and Jacobs, 2001; Bremer *et*

al., 2002). The main limitation behind treatment is the need for frequent shutdown of the contaminated facilities which consumes time, labor and costs.

When adding any antibiotic to any liquid, both the nature of the residue materials as well as the microorganisms to be removed should be fully understood. Furthermore, safety, efficacy and ease of removal, should be also kept in mind. Also, even extreme treatments such as flushing continuously over 7 days with multiple biocides do not stop subsequent biofilm re-colonization (Anderson *et al.*, 1990). Some aggregates can survive in containers filled with iodine solution for 15 months (Panlilio *et al.*, 1992). Therefore, to combat bacterial resistance to conventional antimicrobials, new control strategies are required.

It is obvious from the above that prevention is a more logical strategy. Currently there is no known process that is able to completely prevent the formation of undesired biofilms without side effects (Simoes *et al.*, 2010; Simoes and Simoes, 2013). However, in some applications where operational conditions permit, cleaning and disinfection before the firm attachment of bacteria will considerably reduce biofilm formation (Midelet and Carpentier, 2004; Simoes *et al.*, 2006). Using the dairy industry as an example, Table 2.2 summarizes well-known disinfectants and the biofilms they are effective against.

Other prevention techniques have looked at material characteristics that can suppress biofilm growth (Simoes *et al.*, 2007). The effect of surface finish on stainless steel challenged by Bacillus stearothermophilus and Pseudomonas has been observed, and it was concluded that surface roughness is the most significant factor in the efficiency of surface cleaning (Frank and Chmielewski, 2001). Other attempts at retarding biofilm formation have been by incorporating antimicrobial products into the surface of materials (Weng *et al.*, 1999; Park *et al.*, 2004), or coatings (Tsibouklis *et al.*, 2000; Gottenbos *et al.*, 2001; Thouvenin *et al.*, 2003), or by manipulating the physicochemical properties of the surface (Whitehead *et al.*, 2004; Rosmaninho *et al.*, 2007). For instance, silicone rubber has been coupled covalently with quaternary ammonium coatings, or silver has been incorporated to prevent the attachment of bacteria. Some researchers have used both non-ionic and cationic surfactants to prevent bacterial adhesion. 90% inhibition of *P. aeruginosa* on stainless steel and glass has been found (Cloete and Jacobs, 2001).

Researchers demonstrated that surfactants affect the growth of flagella, which affect, in turn, the whole attachment process (Meylheuc *et al*., 2006; Pereira *et al*., 2006; Splendiani *et al*., 2006).

Table 2.2: Antimicrobial disinfectant for controlling biofilms in diary plants Source: (Simoes *et al.*, 2010)

Treatment	Biofilm type
Ozone, commercial chlorinated sanitizer	*P. fluorescens/Alcaligenes faecalis*
Benzalkonium chloride, hexadecyl trimethylammonium bromide, sodium hypochlorite, peracetic acid, hydrogen peroxide, o-cresol, phenol	*E. coli*
Chlorine, peracetic acid, peroctanoic acid	*L. monocytogenes* and *Pseudomonas sp.* mixed biofilms
Chlorine dioxide containing sanitizer	*B. cereus/P. fluorescens* single and mixed biofilms
Chlorine	*E. coli*
Chlorinated-alkaline solution; low-phosphate buffer detergent; dual peracid solution; alkaline solution; hypochlorite	*L. monocytogenes*
Sodium hydroxide; commercial alkaline cleaner	*P. putida*
Chorine; ozone	*P. fluorescens, P. fragi and P. putida*
Chlorine, hydrogen peroxide, ozone	*L. monocytogenes*
Glutaraldehyde, ortho-phtalaldehyde, hexadecyl trimethylammonium bromide, sodium dodecyl sulfate, chlorine solution sodium hydroxide	*P. fluorescens*
Sodium hydroxide; nitric acid	Mixed species
Chlorine; chlorine dioxide; commercial detergent	*B. cereus and Pseudomonas spp.*
Sodium hypochlorite	*S. typhimurium*
Peroxydes; quaternarium ammonium compounds; chlorine	*L. monocytogenes*
Hydrogen peroxide; sodium dichloroisocyanurate; peracetic acid	*Staph. Aureus*

During the last decade, new techniques of disinfection have been developed. For instance, direct electrolysis, combined with ultra-violet irradiation (Bergmann *et al.*, 2002). Acoustic and hydrodynamic cavitation, both producing areas of local high pressure and temperature leading to cellular damage (Mezulea *et al.*, 2009), however these again are high cost (Doosti *et al.*, 2012). Mixing cavitation with either hydrogen peroxide or ozone treatment has been reported (Jyoti and

Pandit, 2003; Chanda *et al.*, 2007; Doosti *et al.*, 2012). Engineered Nano-materials can also be used against bacteria (Klaine *et al.*, 2008; Fabrega *et al.*, 2009; Pelletier *et al.*, 2010). Two main limitations impede using these techniques widely; the first is the difficulty of attaching them to surfaces, while the second is that their effect on human health and the environment are yet to be fully understood.

Ultrasonic irradiation in the presence of titanium dioxide and semiconductor photo-catalytic processes has also been reported but are not in general use due to their complexity. Although the use of disinfectants is accompanied by some health risks, these are small compared to the health risks of the absence of disinfection (WHO, 2011).

A work dealing with surface treatment was conducted in Harvard. Here, a coating fluid was used to provide a smooth, flat, lubricating layer, which forms a highly slippery surface to prevent bacterial attachment (Epsteina *et al.*, 2012). However, such surface modifications still need more testing to become an acceptable and practical solution.

Two apparently opposite viewpoints can be obtained from examining the literature; the first is that more biocide leads to less biofilm while the second is that more biocide leads to more biofilm growth as a defensive strategy. The answer is that both parties are right; that is, any bacterium exposed to biocide and is not killed will have a greater tendency to form a biofilm. On the other hand, when considering a group of bacteria treated with biocide most of them will die. The ones that persist will have a greater tendency to form a biofilm but because they are less in number the biofilm measuring techniques assess the general trend as a decrease.

To clarify take the following numerical example. Assume that every single bacterium without biocides exposure produce 1 µg of biofilm matrix per day. When exposed to biocides it produces 2 µg day^{-1}. If there are 10 bacteria they produce 10 µg of biofilm matrix per day when not exposed to biocides. When exposed, seven of them will die and the other 3 will produce 6 µg biofilm matrix per day. The biofilm measurement device reads the overall effect as a decrease while, when considered with respect to each surviving bacterium, it is an increase in biofilm activity.

Therefore, when thinking about decreasing biofilm from this point of view it is useful to consider two stages, first is exposure to biocides while the second is removing this biocide to have a real biofilm formation rate decrease. Or, to avoid bacterial regrowth, there is a need to avoid stressed bacteria overproducing biofilm. It may thus be best to overdose the biocide and then remove it by a de-biociding method.

2.2 Froth flotation

Froth flotation is a physical solid-liquid separation technique using differences in selective adherence of a particle to air bubbles in water/particle slurry. Particles which attach to air bubbles rise with the bubble to the water surface and can be continually discharged. Figure 2.2 shows a typical froth flotation cell.

While established for use in mineral upgrading, this separation technique has been adapted more recently for separating a wide range of micro and Nano scale particles such as separating carbon Nano tubes (Lertrojanachusit *et al.*, 2013; Jialun *et al.*, 2014) and harvesting of microalgae (Garg *et al.*, 2014) or using micro particles as an improver to main separated particles (Zech *et al.*, 2012; Hu *et al.*, 2014).

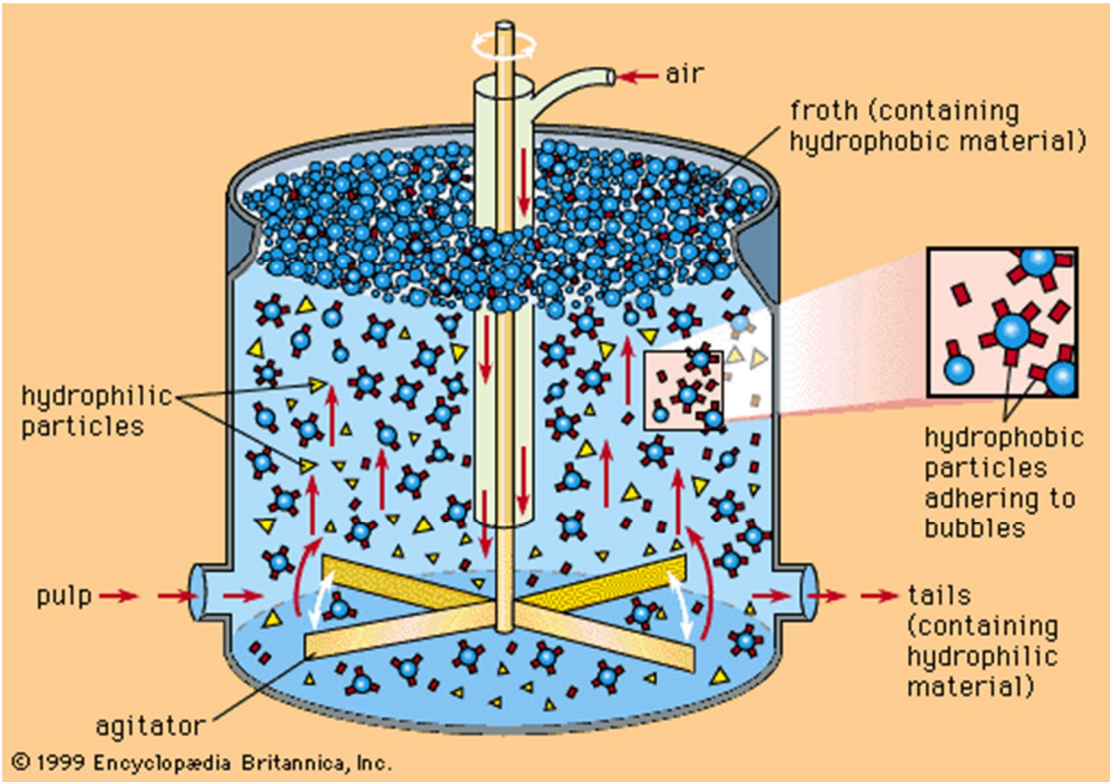

Figure 2.2: Typical froth flotation cell

This separation technique is widely used in industry. Historically, early use was in mining for upgrading mineral ores as a preparation to further purification techniques (Smith *et al.*, 1993; Nagaoka *et al.*, 1999). In the paper industry froth flotation is used to remove hydrophobic impurities such as printing inks and stickers from recycled paper (Finch and Hardie, 1999). Waste water can also be treated by this method. Fats, oils, grease and suspended solids are separated in the Dissolved Air Flotation (DAF) process (Edzwald, 2010). PVC can be separated up to 99.3% from mixtures with PET using bubble flotation (Marques and Tenório, 2000).

In biological science, bacterial strains have been separated in the laboratory using froth flotation principles for some sixty years (Boyles and Lincoln, 1958; Rubin *et al.*, 1966; Bahr and Schugerl, 1992; Rios and Franca, 1997). Sea water in Japanese fishing ports has been purified from bacteria using the same principles (Suzuki *et al.*, 2008).

Theoretically, both bubble and froth flotation can be used in purifying water from microorganisms as the majority of these species are hydrophobic (van Loosdrecht *et al.*, 1987; Stenström, 1989; Zita and Hermansson, 1997). Assuming bacteria are evenly distributed throughout the water column a bubble rising through the water column will attach one or more bacteria in its path and lift it to the water surface. When there is no froth, the bubble will burst when reaching water surface allowing the bacterium to return back to water again. The role of froth is to prevent the bubble from bursting and keep the bacteria attached to it long enough for it to be collected.

2.2.1 Froth flotation system components

Froth flotation is a perfect example of a highly inter-related engineering system as shown in Figure 2.3. Changing any factor will affect the others which have then to be compensated for.

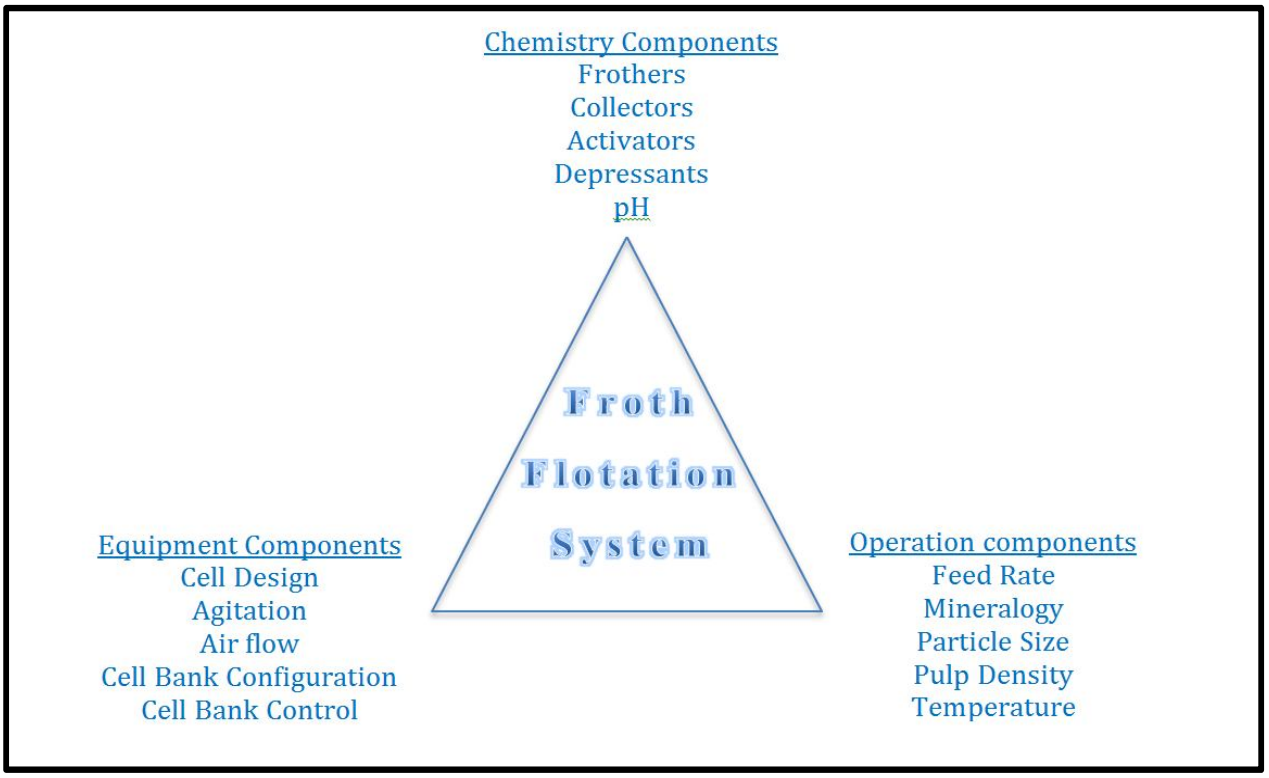

Figure 2.3: Froth flotation components. Source (Klimpel, 1995).

2.2.2 Dissolved air flotation (DAF)

The nearest technique used in the water industry to froth flotation is dissolved air flotation (DAF). It is a clarification process for water and wastewater, removing suspended particles like oil and solids by attaching them to a rising air bubble (Edzwald, 2010). It is a purely physical operation and depends on a simple design philosophy; The DAF tank consists of two adjacent zones separated by a partition. In the first one, the contact zone, the attachment of solid particles to air bubbles occurs. In the second part, known as separation zone, the floated matters is skimmed out using "flying" paddles driven by a moving belt over the tank (Kiuru and Vahala, 2001). As a pre-treatment, chemicals are added as coagulants and flocculants, rather than adjusting the pH. But the main feature of this process is the production of micro-bubbles, in which a pressurized air stream is mixed with water. The pressure increases air solubility and drives it into a super saturation state. Once this flow enters the DAF atmospheric tank, the air is released back as the pressure drops resulting in the generation of micro-bubbles. DAF is proven to be better than sedimentation and can reach 97% and 93% removal for algae and bacteria respectively (Shan-pei *et al.*, 2007); however it is still considered as a primary treatment and

needs further biocide and disinfection to bring the microorganism levels to an acceptable concentration for human consumption or to avoid biofilm formation. Figure 2.4 shows the main DAF components.

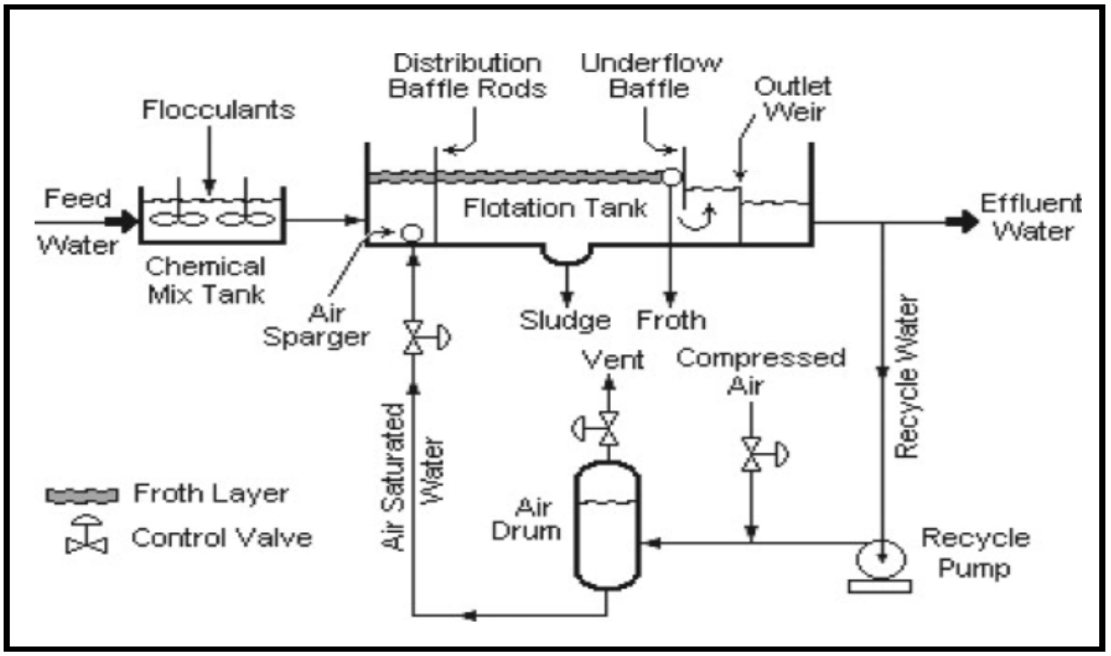

Figure 2.4: Schematic diagram of DAF unit. Source: (Bahadoria *et al.*, 2013)

Practically, DAF is a modified froth flotation process; the only difference is using fine bubbles and some minor changes in flow patterns through the system (Moruzzi and Reali, 2010). Larger bubbles have the disadvantage of relative high turbulence, which decreases the chance for attachment between the bubbles and particles. This drawback is eliminated with fine bubbles as the rising velocity is lowered, hence, Reynolds number, but the lifting force will also decrease. Both sizes actually give similar results (Boyles and Lincoln, 1958; Hanotu *et al.*, 2012), which leads to the conclusion that, for micro solid particles, bubble size does not matter, However Zimmerman's/Hanotu's work does indicate that the ideal bubble size is the same as the particle (bacterium) to be removed (Hanotu *et al.*, 2012). Also, in DAF, the solubility of air in water limits the number of bubbles, which in turn, reduces the probability of bubble-bacterial attachment. Recent studies have determined how much air can be held by liquid under certain pressures. At 621 kPa, 20 ml/l of air can be dissolved in water (Dassey and Theegala, 2012). In

froth flotation cells the normal air flow to liquid ratio is 10 m^3/m^3, while in DAF it is limited to 0.007 m^3/m^3 (Miettinena *et al.*, 2010).

Four kinds of forces can drive a particle to attach to a bubble, van der Waals, electrostatic, hydrodynamic repulsion, and hydrophobic forces (Bondelind *et al.*, 2013). All of them are inversely proportional to the distance between the bubble and the other species. Decreasing this distance will increase the attachment opportunity, or, logically, no distance leads to certain attachment. Neither froth flotation nor DAF ensure such closeness. Also, in general, higher rising velocity increases bubble–particle collisions (Miettinena *et al.*, 2010).

Therefore, an excess amount of air should be pumped through the system. However, for froth flotation, excess air is avoided because too much air will destroy the froth structure, while for DAF the whole matter is limited by air solubility in water.

Recently, many trials have been made to produce micro bubbles in a wider range than used in DAF to overcome its limitations. But all of them still under development and do not attain the desired massiveness needed in various applications.

The ideal system for purifying water using bubbles should be: high air/water ratio without increasing the turbulence, building a rigid froth without using any chemicals, and using a counter current flow pattern.

2.3 Bacterial regrowth inside a water distribution system

Bacterial regrowth in a distributing system is an important issue when designing high quality water facilities. Scientifically and practically the term “biological stability” is widely used to describe such phenomena and defined as the ability to reduce bacterial regrowth when water leaves treatment factory (Rittmann and Snoeyink, 1984). (WHO, 2006) reported that water in a distribution system should be microbiologically safe and biologically stable. Being microbiologically safe has been intensively studied and defined while biological stability remains less investigated and explained (Lautenschlager *et al.*, 2013).

While the traditional regrowth prevention technique is residual biocides, the realization of their possible side effects has stimulated the development of new methods. The alternative now most

used in Europe is to control nutrient levels (Lautenschlager *et al.*, 2013; Simoes and Simoes, 2013). However, apart from higher costs, nutrient control has another major problem; that is, more nutrient leads to faster and more regrowth but less biofilm formation and vice versa. Therefore, finding a point to optimize both factors is critical, sensitive and difficult to achieve in water industry.

The cfu ml^{-1} levels in various drinking water legislations are the source of the bacterial regrowth problem. None of these regulations mention that the cfu ml^{-1} in the produced water should be zero. The importance of obtaining absolutely bacterial free water comes from the fact that theoretically any single bacterium if it reproduces may reach a population of 2^{72} or 4.7×10^{21} within 24 hours if the generation time is assumed to be 20 minutes. To generalize, the equation that describes a single bacterium reproduction rate is (Koch, 2001):

$$N = 2^{t/tg} \quad \text{..... 2.1}$$

Where:

N: is the number of bacteria at time (t).

t: is the time when bacterial population measured.

tg: is the generation time.

Assuming there are two initial bacteria, then the time to produce the same number of bacteria is half of one bacterium. In general we get:

$$N = N_0 \times 2^{t/tg} \quad \text{..... 2.2}$$

Where: N_0 is the initial bacterial population.

Table 2.3: Generation times for some bacteria. Source: http://www.textbookofbacteriology.net/growth_3.html

Bacterium	**Medium**	**Generation Time (minutes)**
Escherichia coli	Glucose-salts	17
Bacillus megaterium	Sucrose-salts	25
Streptococcus lactis	Milk	26
Streptococcus lactis	Lactose broth	48
Staphylococcus aureus	Heart infusion broth	27-30
Lactobacillus acidophilus	Milk	66-87
Rhizobium japonicum	Mannitol-salts-yeast extract	344-461
Mycobacterium tuberculosis	Synthetic	792-932
Treponema pallidum	Rabbit testes	1980

According to this equation the initial bacterial concentration is very important factor to limit the regrowth of bacteria not only the nutrient level or residual disinfectants.

This suggests that unless the regrowth of bacteria can be stopped by residual disinfection or low nutrients, even a low bacterial concentration can reach danger levels within a few hours.

In real distribution systems bacterial regrowth is a slow process (Ollos *et al.*, 1998; Lautenschlager *et al.*, 2013). "Slow" here means the growth rate is not enough to raise the bacterial concentration to an unacceptable level between the water treatment plant and the consumers tap.

2.4 Chlorination and shock chlorination

Chlorination of drinking water has been used as a disinfection technique for more than a century or so to produce water that is safe from waterborne diseases. This method is preferred due to low cost, abundance, ease of use and less need of high technology equipment. However, during the last forty years disinfectant by-products "DBPs" have become a new branch of study in the water industry developing first by discovering the presence of Chloroform (Rook, 1974) and trihalomethanes "THMs" (Singer, 1994) in drinking water. To date some 700 or more DBPs have been identified, but understanding their effect on humans and the environment still needs more work (Brown *et al.*, 2011; Gonsior *et al.*, 2014; Richardson, 2014).

Therefore, new methods have been developed to replace chlorination. Unfortunately, most of them cannot compare with chlorination either economically or efficiently to challenge the ease of use of chlorination and the difficulty of replacing the chlorination infrastructure (Powell, 2010; Fawell, 2014).

Liberating the drinking water industry from chlorination side effects has been approached by developing new chemical, physical and hybrid techniques rather than novel methods such as plasma and solar disinfection (Kumar and Pandit, 2012). All of these strategies imply economical and health disadvantages which still gives disinfection by chlorine an acceptable reason for use in many places around the world.

Shock chlorination is mainly used in-situ in wells, springs, swimming pools or any other potable water sources. It is implemented by adding large amounts of sodium hypochlorite solution (common bleach 3-5% sodium hypochlorite) into the water (Mcdonald, 2011). Typical shock chlorination aims to reach 10-25 ppm of free chlorine in swimming pools or it may reach 200 ppm for highly contaminated wells. Time to reuse water again after shock chlorination varies from 8 hours to two weeks (Mcdonald, 2011).

In wells and swimming pools shock chlorination the water reservoir is kept out of use for a certain time for natural de-chlorination. The concentration of chlorine and contact time for killing all or most of the contaminants varies depending on initial and desired microorganism concentration and the chemical nature of water that may interfere with the chlorination process (LeChevallier *et al.*, 1981; Virto *et al.*, 2004; Virto *et al.*, 2005; Helbling and Vanbriesen, 2007). The lack of information about time-concentration needed may be due to the wide nature of reservoirs that make any generalized study difficult. However, the parameters can be measured in laboratory experiments.

One of the major advantages of chlorine is preventing bacterial regrowth if some residual level is maintained along the distribution system. Recently, some European water treatment facilities have prevented the regrowth; i.e. producing biologically stable drinking water, by optimizing nutrition levels in the water using pretreatment techniques such as sedimentation, filtration, UV disinfection, ozone, and peroxide (Lautenschlager *et al.*, 2013; Simoes and Simoes, 2013).

The recommended (CT) value (free chlorine concentrations (ppm) × resting time of the solution (min)) is the value that describes the time needed and chlorine combined for safe drinking water (Walker and Newman, 2011). “Safe” here does not mean an absolutely microorganism free water. It means it is within the acceptable biological contaminants level. Furthermore, this value is not a water property; it is a microorganism’s property i.e. every microorganisms has its own CT value. To unify this term (EPA, 2009) have identified a standard CT value as that which will reduce *Giardia lamblia* by three-log (99.9%) and produce a four-log of viruses inactivation (99.99%). Determining the CT is not that complicated a process and can be estimated practically in-situ as one of the tests needed for testing conventional design requirements.

Chlorine demand is known to be directly proportional to the bacterial concentration in the water to be treated (LeChevallier *et al.*, 1981; Virto *et al.*, 2004; Helbling and Vanbriesen, 2007). When the bacteria in water are doubled, the chlorine demand to treat this water will be doubled too. This doubling is valid for higher bacterial concentrations; for example, higher than 10^5 cfu ml^{-1} for *E-coli* (Virto *et al.*, 2005). Drinking water and in practice, most treated waters prior to disinfection, have total cell counts below this level which makes chlorine demand dependency on bacterial content negligible.

On the other hand, it is a "rule of thumb" in drinking water treatment to use more biocides for more bacterial concentration even in low bacterial contents.

Table 2.4: CT values for 99.9% reduction of *Giardia Lamblia*. Source: (EPA, 2009)

		Temperature °F (°C)					
Disinfectant	pH	33.8 (1)	41 (5)	50 (10)	59 (15)	68 (20)	77 (25)
Free Chlorine	6	165	116	87	58	44	29
	7	236	165	124	83	62	41
	8	346	243	182	122	91	61
	9	500	353	265	177	132	88
Ozone	6-9	2.9	1.9	1.4	0.95	0.72	0.48
Chlorine Dioxide	6-9	63	26	23	19	15	11
Chloramines	6-9	3800	2200	1850	1500	1100	750

Table 2.5: CT values for inactivation of viruses. Source: (EPA, 2009)

	pH (6-9)	Temperature °F (°C)					
Disinfectant	Inactivation	39.9 (0.5)	41 (5)	50 (10)	59 (15)	68 (20)	77 (25)
Free Chlorine	2	6	4	3	2	1	1
	3	9	6	4	3	2	1
	4	12	8	6	4	3	2
Ozone	2	0.9	0.6	0.5	0.3	0.25	0.15
	3	1.4	0.9	0.8	0.5	0.4	0.25
	4	1.8	1.2	1	0.6	0.5	0.3
Chlorine Dioxide	2	8.4	5.6	4.2	2.8	2.1	-
	3	25.6	17.1	12.8	8.6	6.4	-
	4	50.1	33.4	25.1	16.7	12.5	-
Chloramines	2	1243	857	643	428	321	214
	3	2063	1423	1067	712	534	356
	4	2883	1988	1491	994	746	497

The main limitation against using shock chlorination in drinking water is the need for long time periods to remove chlorine naturally. Such timescales are not available to the drinking water industry. When designing a water distribution system the chlorine should leave the purification facilities at a concentration not exceeding the maximum permitted level which is 4 ppm (WHO, 2006). However, consumers may live meters or tens of kilometers away from purification plant and the chlorine level has to be kept within the acceptable range along the distribution pipes. Therefore, if the shock chlorination is proposed to be used with drinking water, the chlorine level must be decreased by a suitable method before releasing the water to public.

It is not expected to be able to completely remove microorganisms from water using froth flotation. The only previous work found reporting the use of such technology reached 80% removal of bacteria using casein protein as frother and collector (Suzuki *et al.*, 2008). However, without using chemical frothers and collectors, any reduction around this ratio in bacterial content will cause a significant reduction in required chlorine dose, as chlorine dose is directly proportional to initial bacterial population (LeChevallier *et al.*, 1981; Virto *et al.*, 2004; Helbling and Vanbriesen, 2007). For instance, a reduction in the initial cfu ml^{-1} by a factor of half leads to reduction in chlorine demand of approximately the same factor (Virto *et al.*, 2005).

This conclusion is valid for high bacterial levels and low chlorine content. For example, data about the response of *E.coli* to various doses of chlorine is available over 10^5 cfu ml^{-1}. This concentration is considered as “the detection limit of the cell quantification assay” and there is little or no information available for bacterial densities below this level (Helbling and Vanbriesen, 2007).

It is a common practice to use a low chlorine dose with lower bacterial content when dealing with drinking water. Unfortunately, there is no reference data for this. Therefore, new techniques and methodology should be developed to discover such gap.

2.5 Water chlorination basic chemistry

The most common chlorination methods are using chlorine gas, sodium hypochlorite and trichloroisocyanuric acid. The first two are used in drinking water while the last is used mainly in swimming pools.

When using chlorine gas the following reactions occur:

$Cl_2 + H_2O \leftrightarrow HOCl + H^+ + Cl^-$

$HClO \leftrightarrow H^+ + ClO^-$

For the sodium hypochlorite the reactions are:

$NaOCl + H_2O \leftrightarrow Na^+ + HOCl + OH^-$

$HClO \leftrightarrow H^+ + ClO^-$

While for trichloroisocyanuric acid the reactions are:

$C_3Cl_3N_3O_3(s) + 3\ H_2O\ (l) \leftrightarrow 3\ HClO\ (aq) + C_3H_3N_3O_3\ (aq)$

$HClO \leftrightarrow H^+ + ClO^-$

Therefore, whatever the primary chlorine source is, the secondary acting source will be the hypochorous acid (HClO) which; in turn, reacts with hydrogen and chlorine reversibly to return back to chlorine molecule and water. According to Le Chatelier's principle, continuous withdrawal of chlorine should drive all the hypoclorous acid and chlorine monoxide to leave the water.

Hypochlourous acid is the main disinfectant. Therefore, it is used as a sanitation agent in bacterial and algal treatment while chlorine monoxide is used as an oxidizing agent (Harnvajanawong *et al.*, 2004).

Theoretically, Treatment with chlorine gas is perfect for chlorination/dechlorination processes because it does not leave any chemical residuals when completely evacuated from water. The main limitation for using chlorine gas for shock chlorination is the absence of known facilities for such a process. Most commercial dosing equipment deals with chlorination in a range of 0.2-4 ppm.

Most of the other chemicals that are used for chlorinating drinking water leave residuals when removing chlorine by any means including aeration. According to the balanced equations sodium hypochlorite leaves sodium ions in the water when chlorine is discharged continuously. For example, when it is aimed to produce a chlorinated water of 200 ppm and then withdrawing the chlorine, stoichiometry indicates that the residual sodium ion will be approximately 131 ppm. Fortunately, sodium is not considered a contaminant or a health risk and is seen as an essential

component that should exist at a defined level for acceptable water potability. In the drinking water production process it is not expected to exceed 200 ppm. Therefore; for example, when dealing with a dose of say 50 ppm it is acceptable to use sodium hypochlorite.

When considering trichloroisocyanuric acid, every 3 moles of evacuated chlorine produce a mole of cyanuric acid residual in the water. In part per millions, this is equivalent to 246 ppm of cyanuric acid for every 200 ppm chlorine. This level is out of the health regulations rather than taste and odor deterioration.

In practice, shock chlorination is used mainly when there is expected or measured high contamination in water reservoirs. 200 ppm is considered to be the highest shock chlorination dose. In swimming pools the maximum dose is 25 ppm in the most sever conditions because, above this concentration, the chlorine level will not return to an acceptable value fast enough for pool reuse.

In the drinking water industry there is no time for conventional dechlorinaton as the water moves directly from the chlorination facility to the distribution system. Therefore, if shock chlorination is to be used for drinking water it will have to be followed by shock dechlorination. To do this, two kinds of information are needed which represent a gap in designing such processes. The first is new tables for CT values that are able to kill all microorganisms completely or describing a simple and in-situ method to identify these values for any kind of water. The second is providing data for a fast, cheap and physical dechlorination method. In this thesis, methods for dealing with these two gaps are explored.

2.6 Dechlorination of water

Currently, dechlorination of water refers to two applications; first is removing chlorine from waste water treatment plants that use chlorine as a disinfectant before discharging into surface water while the second is dechlorinating domestic fish pools (Marion, 1994). For this purpose there are three main techniques:

1- Aeration
2- Activated carbon treatment
3- Chemical treatment

Aeration is described as slow and not able to remove all the combined chlorine; especially chloramines. Activated carbon is more efficient but has high equipment costs. Chemical additions are cheap and effective for waste water treatment and aqua life dechlorination. The most used dechlorination chemicals are, sulfur dioxide, sodium metabisulfite, sodium sulfite, sodium thiosulfate and hydrogen peroxide (Bagchi, 1991).

However, when dechlorinating drinking water as a part of purification process chemicals should be avoided as they lead to other known and unknown problems.

The aeration process described above as slow is stagnant aeration i.e. putting water in an open vessel and leaving it for the chlorine release naturally. The acceptable acceleration for aeration may be air bubbling which should be tested experimentally.

The level of chlorine is also regulated for minimum level at consumer taps. These regulations are hard to change especially in developed world countries. Therefore, even if a water system with no residual chlorine is proven to be hygienically safe, developing this method into practice will hindered by the legislation. For instance, in Japan the chlorine should be at least 0.1 ppm in normal cases and 0.2 when there is a risk of pathogenic organism contamination (Nagatani *et al.*, 2006).

2.7 Chlorine decay in drinking water distribution systems

Chlorine decays along a drinking water distribution systems mainly due to the following reasons (Castro and Neves, 2003):

1- Chlorine reaction with bulk chemical and biological contaminants
2- Chlorine reaction with pipe walls (Al-Jasser, 2007)
3- Natural evaporation.

Since a water distribution network can be considered a closed system, natural evaporation can be neglected. Bulk and pipe wall reactions are governed by the equation 2.3 (Castro and Neves, 2003; Warton *et al.*, 2006):

$$C = C_o e^{-(k_b + k_w)t} \quad \text{.....} \quad 2.3$$

Where:

C: is the chlorine concentration after time t (ppm).

C_o: is the chlorine initial concentration (ppm).

k_b: is the bulk chlorine decay constant (1/time).

k_w: is the wall chlorine decay constant (1/time).

Experimental values of k_b and k_w were found to be (0.28 - 0.3432) day^{-1} and (0.11-112) day^{-1} respectively (Hua *et al.*, 1999; Castro and Neves, 2003; Al-Jasser, 2007).

The variation of k_w described here depends on pipe diameter; that is, higher values are for smaller diameter and vice versa. A trial was made by measuring k_b in a bottle then measuring the overall constant (k_b+k_w) along the distribution net. The difference is assumed to be the chlorine decay constant k_w (Al-Jasser, 2007).

Factors directly proportional to k_b and k_w may decrease, stay constant or increase with time or along the piping system. Chemical contaminants and chlorine concentration are decreasing. Pipe material stays constant. Microorganisms are increasing. Therefore, any increase in the sum of (kb+kw) should be as a result of an increasing factor; that is, the microorganisms. This conclusion gives the importance of removing microorganisms from water for subsequent biological stability.

The reaction kinetics of chlorine with chemical and biological contaminants as well as pipe materials are not fully understood (Richardson, 2014). Some chemical reactions with chlorine have been found to be fast. The reaction constant is time and concentration dependent (Guntan, 2014). Therefore, shock chlorination may or may not increase the disinfectants by-products as concentration will increase while time will decrease and more investigation is needed to optimize these variables.

Another explanation can be obtained by studying the decay of chlorine inside the distribution system considering Bernoulli's equation for fluid flow and Henry's law. That is, Henry's law mentions that, as the partial pressure of a gas over a liquid increases, its solubility in the liquid also increases. Or simply, more pressure leads to more solubility of the gas in liquid. Whereas, and according to Bernoulli's equation, as the distribution net is a flowing system, the pressure at

the beginning is higher than the end. Under these hypothesizes chlorine concentration is expected to be high at the pumping station or the elevated head tank and low at the consumers tap.

2.8 Engineering considerations

2.8.1 Aeration and chlorination points in treatment plants

Aeration is a conventional activity in drinking water treatment used mainly for removing volatile organic chemicals (VOCs), gases, and oxidizing dissolved metals such as Iron (Albin and Holdren, 1985; Baylar *et al.*, 2010). Aeration can be implemented by two main techniques; the first is out of basin aerators such as packed tower, diffused-bubble or spray aerators. The second is in-basin aerators like fountain and sparger aerators (Jerry *et al.*, 2006). The latter is the most common in water industry as shown in figure 2.5.

Figure 2.5: Sparger aerators

Chlorine is added normally at the end of treatment process or at the beginning as a primary disinfection, depending on the plant design. Therefore, the chlorine processing point is flexible and open to any change in the purification process. In most of treatment plants the aeration process is located at the beginning of the production line.

2.8.2 Potable and drinking water bacterial quality variation

Most rivers worldwide vary in their cfu ml^{-1} for total coliforms (Obi *et al.*, 2003; Agbabiaka and Oyeyiola, 2012; Rajiv *et al.*, 2012; Sakai *et al.*, 2013). These variations are reflected in the design and operation of the water treatment plant to the extent that there are, effectively, no identical water treatment plants (EPA, 2009). Therefore, it is not recommended to take any set figures as a rule of thumb as the basis for design. Instead, there are a series of tests that can be made to give engineers the required data.

One of these tests that should be done is estimating the CT value for killing the bacterial spectrum of a water sample.

2.8.3 Construction materials

Chlorine is known as a highly corrosive substance. In current drinking water facilities the chlorine level is relatively low and affordable. When thinking of shock chlorination with high doses this fact should be kept in mind. Whatever the design chosen, every part that is in contact with shock chlorinated water should be constructed with corrosion resistant materials. For example, cast iron should be avoided and replaced with other materials such as concrete and plastic. If it is not possible to replace cast iron, coating the inside of any equipment by polyester, fiberglass, or any suitable non-toxic anti-corrosion paint should solve the problem.

In wells and swimming pools shock chlorination there is no need to anti-corrosion operations. In both applications the materials in contact with chlorinated water are not liable to corrode, in wells it is sands and muds while it is tiles in swimming pools.

2.9 Conclusions, aims and objectives of the literature survey

1- There are two main techniques to deal with a biofilm; treatment and prevention which is the most desired. It can be subdivided into two kinds of prevention, partial and complete. Partial prevention can be achieved by minimising the causes of biofilm such as avoiding biocides, minimizing bacterial content, or nutrient optimization. To prevent biofilm completely one or more of its essentials should be eliminated. These essentials are bacteria, liquid and surface. Bacteria seems to be the nearest to think about for removing to reach the desired goal.

2- The first research path for partial prevention of biofilm is to minimize the microorganism level without using biocides. Froth flotation principles can use microorganism’s hydrophobicity to purify water from them. Modification on this technique should be proposed to be competent for using in drinking water industry as a microorganisms remover such as avoiding the addition of any chemicals during the process.

3- The hypothesis relies on designing a system using ordinary bubble size and excess air to provide high bubble-particle attachment chances and lowering turbulence as possible.

This can be done by avoiding bubble burst on the surface of water. In mineral froth flotation, burst is avoided by using wide range of chemical frothers to ensure smooth transfer from liquid to froth phase.

4- For complete prevention, the primary suggestion is to kill all the bacteria before entering the distribution system using shock chlorination followed by shock dechlorination.

5- When dealing with drinking water shock chlorination, it is desirable to dechlorinate it using a method that is fast, physical, reversible, easy and cost effective. Aeration by air bubbling is a promising method but it needs more investigation to find how far, safe and cost effective it is and to identify its reliability.

6- After leaving the water treatment plant, bacteria start to reproduce again. Even though this phenomenon is slow, it has to be addressed. Residual disinfectant and nutrient limitation are well-known techniques to limit this regrowth and stabilize the water. The initial bacterial concentration is an important factor. Therefore, maintaining the bacterial level as low as possible should help to hinder the regrowth. Or, no bacteria leads to no regrowth.

7- Chlorine reacts with chemical contaminants, biological contaminants, and the pipe wall. Shock chlorination and dechlorination will eliminate such reactions and enhance pipe line durability.

8- Drinking water aeration basins can be modified to froth flotation facilities. This is more acceptable especially in cases of proximity of air pumping demand of the aeration system and the proposed flotation system. If the aeration system does not exist originally in the purification plant, they can be easily designed and installed.

CHAPTER THREE

DEVELOPING A COLONY COUNTING METHODOLOGY USING IPAD AND IPHONE APPLICATIONS

Chapter Three

Developing a Colony Counting Methodology Using iPad and iPhone Applications

3.1 Introduction

Manual colony counting is the standard method for determining the density of microorganisms (as colonies) on agar plates (plate counts) (Clarke *et al.*, 2010). Electronic plate counting using specially developed hardware and software goes back to the nineteen seventies (Coyne *et al.*, 1974; Goss *et al.*, 1974) Mechanizing the counting process saves time but must be reliable and accurate to gain acceptance. Mechanised counting has also tended to be expensive and task specific.

Automated methods using image processing and pattern recognition can be fast and simple (Dubuisson *et al.*, 1994). In 1995 this approach was used with a microcomputer (Dipti *et al.*, 1995). After 3 years, developments enabled greater accuracy (Olsztyn *et al.*, 1998). Such developments included moving the camera across the agar plate and taking successive images and adding fibre optic illumination. Putman suggested transferring an image via the internet to a centralised high quality counting system (Putman *et al.*, 2005). By 2008 Goyal was reporting differences as small as ±1.71% when comparing manual with machine vision counting methods (Goyal, 2008).

One of the main problems of electronic colony counting are clustered colonies and those close to edges (Bewes *et al.*, 2008). Other defects can be due to lighting and the digital camera feed (Chan and Zhang, 2009). Malik used a Wiener filter to aid edge detection (Malik and Grag, 2011). Brugger described the use of software under the MATLAB environment by processing the image using Top-Hot-filtering to get a uniform background and then segmenting the colonies to facilitate counting (Brugger *et al.*, 2012).

However, all these methods need expensive, multi-part and scarce equipment. iPhone and iPad are now easily available and relatively low cost. Their applications cover every detail in life including scientific measurement. During 2012 the first iPhone and iPad based application for electronic colony counting was introduced. Such apps have useful features but there are

questions about their accuracy, functionality, and relevance in using them instead of traditional techniques.

Before discussing the accuracy and functionality of any colony counter, an acceptable calibration method should be followed. Unfortunately, there is no known standard reliability technique for electronic colony counters. The only previous reliability test was comparing pre-counted plates with actual readings. The defects of such method are; first, it is not reproducible hence not a standard. Secondly, it does not have the ability to identify the source of error; that is, hardware, software or other. In this work a simple reliability test method is described and used to evaluate the iPhone and iPad applications. Multiple sizes and patterns of printed dots on white papers using AUTOCAD were produced as standards. This reliability test presents ideal working conditions to know whether the counter is well designed or not as black dots printed on a white background should be the best condition for any counter.

3.2 Materials and methods

3.2.1 ipad and iphone colony counters

The colony counting application (HGCOLONY) which is developed by (HyperGEAR,Inc) was used. It is available for both iPhone and iPad. Table 3.1 summarizes the specifications of the iPhone and iPad used.

Table 3.1: iPad and iPhone specifications

iPad	iPhone
iPad 4 with retina display 128 GB	iPhone 5 with retina display 16 GB
A6X chip	A6 chip
1 GB Memory RAM	1 GB Memory RAM
5 MP rear camera and 1.8 MP front camera	8 MP rear camera and 1.8 MP front camera
iOS 6 Operating system	iOS 6.1.2 Operating system

3.2.2 “Standards” for calibration and comparison

A pattern of black dots on white paper was produced using AUTOCAD (Autodesk Inc. 2013). These dots were spread equally inside an imaginary 80mm radius circle, (the most common agar plate size). The number of dots in any sheet is counted by shading the dots on computer screen as every dot in AUTOCAD program is considered as an object. Four variables were used, dot

diameter, background contrast, the number of dots per image, and dot size range in a single counting area.

For dots diameter, test sheets were prepared by printing 129 equally spaced dots in an imaginary 80 mm diameter circle. The diameter of these dots ranged from 0.15 to 1.4 mm. That is, dot diameter increases by 0.05 mm between one sheet and another. The dots in any one test were all the same diameter. Figure 3.1 illustrates the standard 129 dots, 0.25 mm diameter plot.

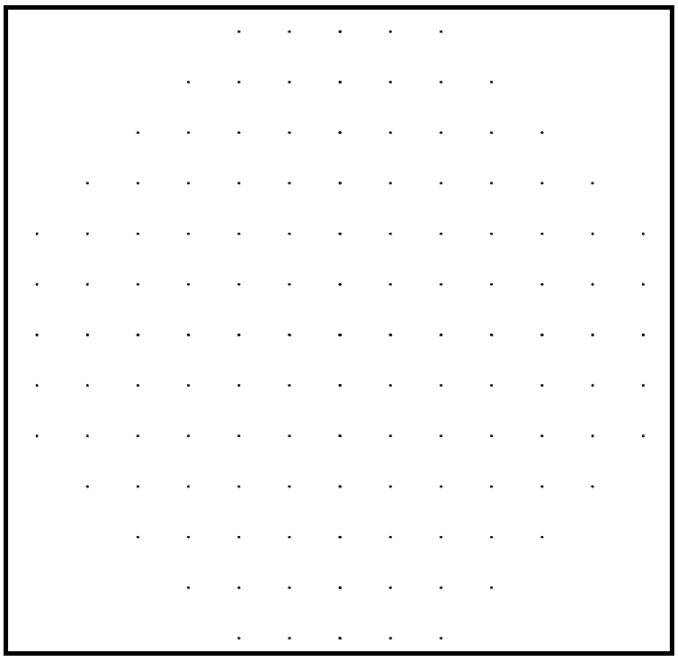

Figure 3.1: Standard 129-dot plot

For light/dark contrast, five values were evaluated. The highest contrast (100%) means that the dots are in the darkest black print against a white background while 0% would be for totally white dots, and the values in between represent grades of a grey scale. All dots were 0.25mm diameter.

For dots density, 5 sheets were produced with different distances between dots of the same size. For the 129 dots sheet the distance between dots is 6 mm. Other sheets were produced with distances between dots of 3, 1.5, 0.75 and 0.5 mm. These gave number of dots per sheet of 537, 2217, 8797 and 20049 respectively. All dots were 0.25mm diameter.

To produce a range of dot sizes in a single counting area, three sheets were produced with 13 concentric rings. Each ring contained 20 dots of the same size but all rings have different sized dots. In the first sheet the central circle consists of 20 dots of 0.15 mm diameter, the second circle consists of 0.2 mm diameter, and so on to 0.75 mm diameter for the outer circle. In the second sheet the size range started at 0.15mm then 0.25 mm, and continued to 1.35 mm diameter. The last set covered the size range from 0.15 to 2.55 mm with a difference of 0.2 mm between every circle. Figure 3.2 shows the sheet of the range (0.15-1.35) mm diameter.

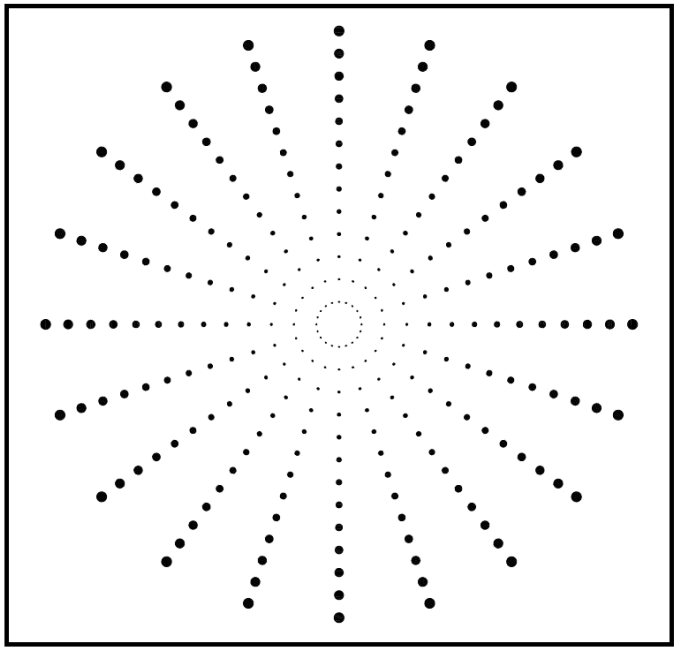

Figure 3.2: Standard plot using dot size range (0.15-1.35) mm diameter

3.2.3 Agar plates

A mixture of nutrient broth and agar (15g + 15g) (SIGMA-ALDRICH) was suspended in a litre of distilled water then boiled and mixed using a magnetic stirrer. When dissolved completely, the nutrient agar was autoclaved (121 C˚ and 1kg/cm^2) then cooled to about 50 C˚ and poured into plastic petri dishes. When empty, the plates were incubated for 24 h at 37 C˚ to confirm accurate sterilization.

The plates were cultured aseptically with K-12 strain *Escherichia Coli* bacteria (Texas Red®) and incubated for 24 hours at 37 C˚.

An inoculating suspension was prepared by mixing 15 g of nutrient broth (SIGMA-ALDRICH) in 1 litre of distilled water and autoclaved as described above. When cooled to room temperature, the broth was inoculated with the *E. coli* and incubated for 24 hours at 37 C˚.

Dilutions were prepared to get an approximate expected colony count of 1000, 500, 250, 125, and 63 per 100 µl. 100 µl being the amount of solution added to any agar plate. The actual colony count per 100µl obtained were 1054, 508, 246, 133 and 64 respectively according to manual counting.

3.3 Results

All results are expressed in percentage accuracy with respect to the drawn dots or the real colonies number as a Y-axis. X-axis represents the other studied variables.

$$\text{Percentage accuracy} = \left(\frac{R-P}{A}\right) \times 100 \quad \text{..... 3.1}$$

Where:

R= Reading of iPad or iPhone by number of dots (or colonies).

P= Pseudo read dots, these whose are not dots and counted as dots (or colonies).

A= Actual drawn dots or colonies.

3.3.1 Reading the drawn standards

The following are results of experiments to identify the reliability of both iPad's and iPhone's hardware and software using standard drawn sheets.

3.3.1.1 Dots of the same size in any one standard

Results for assessing dots of different diameter are summarized in Figure 3.3. The fixed parameters are 129 dots per image and 100% contrast. Results showed a perfect reading along the range of 0.2 – 0.6 mm while it is not readable for 0.15 mm. the decrease in reading accuracy starts to decrease from 0.8 mm till 1.4 mm to reach 59% for iPhone.

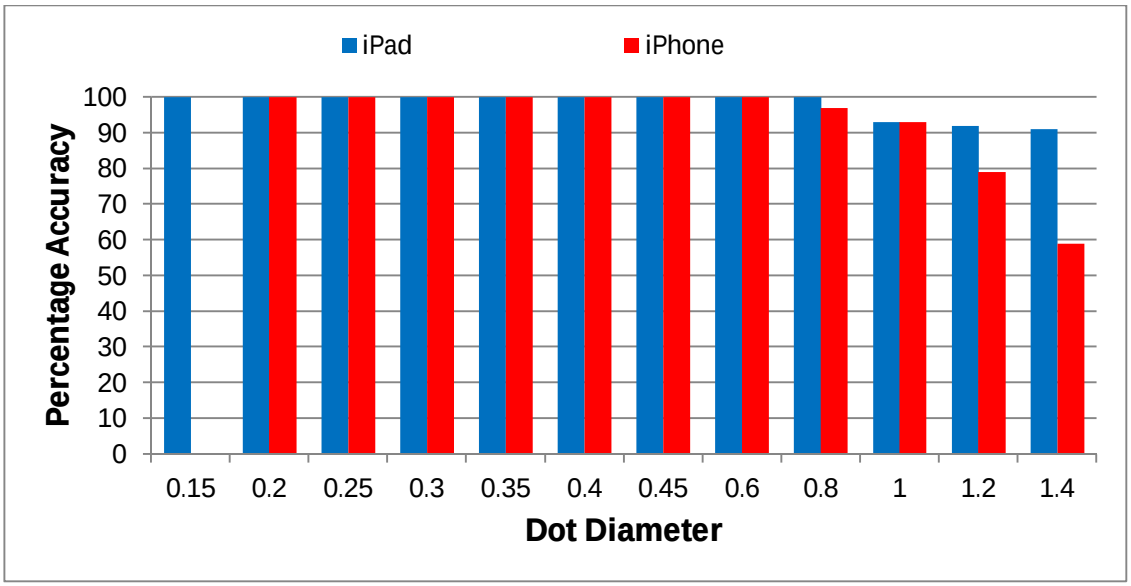

Figure 3.3: Effect of dots diameter on the reading accuracy

Note: Reading of iPhone for 0.15 mm does not appear because no consistent reading could be obtained

3.3.1.2 Effect of varying the dot diameter in a single image

Colonies grow at different rates and, therefore, will not be uniform in size. It was found that the greater the difference between the largest and smallest dots, the lower the accuracy. The only fixed parameter is 100% background contrast. The results are shown in Figure 3.4.

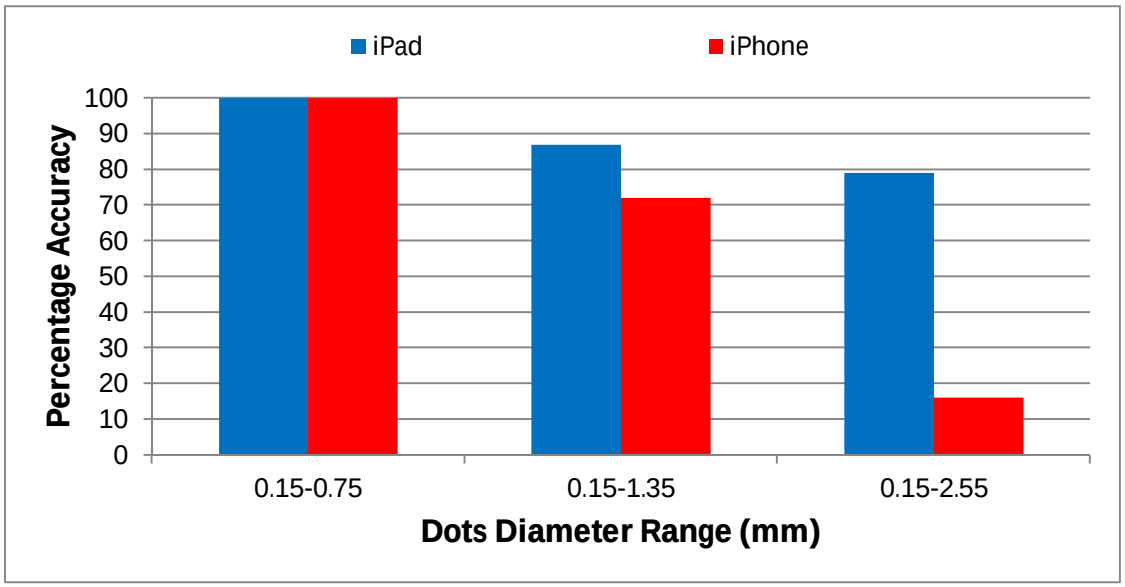

Figure 3.4: Effect of size range on reading accuracy

3.3.1.3 Effect of dot density

Most colony counters work in an optimum range of 20 to 300 colonies per standard (80 mm) agar plate. Experiments were conducted to find the maximum number of colonies a single agar plate that will give an accurate reading. The fixed parameters were 0.25 mm colony diameter and 100% colony-background contrast. Results are given in figure 3.5. It has been shown that iPhone and iPad applications count perfectly up to 8797 dot per plate. After that counting efficiency starts decreasing.

3. 3.1.4 Effect of background contrast

In these experiments the fixed parameters were 129 0.25 mm diameter dots. Five grades of contrast were considered, from 20% (lightest grey dots) to 100% (totally black dots) printed on white papers. Figure 3.6 shows the results. iPhone and iPad applications covers a wide range of contrasts. The readings were 100% correct from 40 % to 100% contrast.

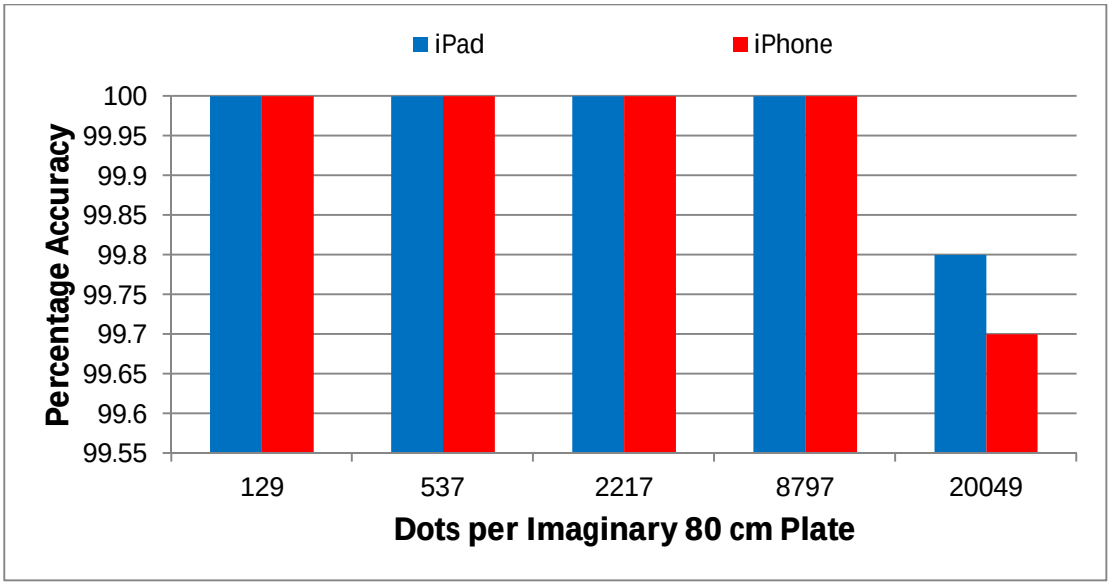

Figure 3.5: Effect of dot density on reading accuracy

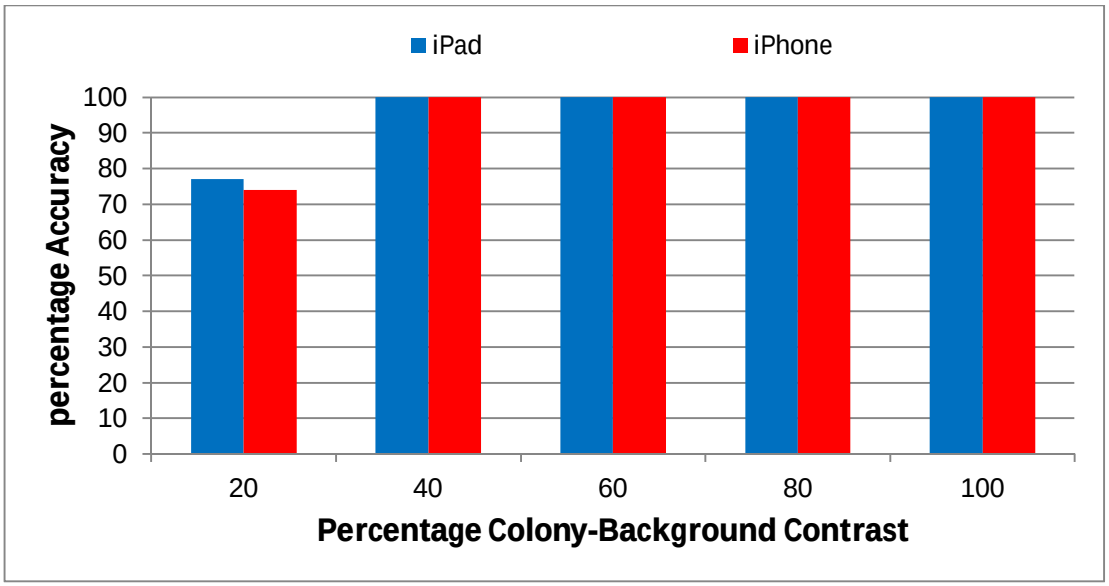

Figure 3.6: The effect of colony-background contrast on reading accuracy

3.3.2 Counting live colonies

In these experiments a set of agar plates were inoculated with bacteria to produce a known cfu/plate as described in (3.2.3). The known plates were compared with the iPad and iPhone readings. The percentage accuracies obtained using the app systems are summarized in figure 3.7. The best reading was obtained for iPad with 71% accurate and decreased as colonies number increase. The same is for iPhone but starts from an accuracy of 66%.

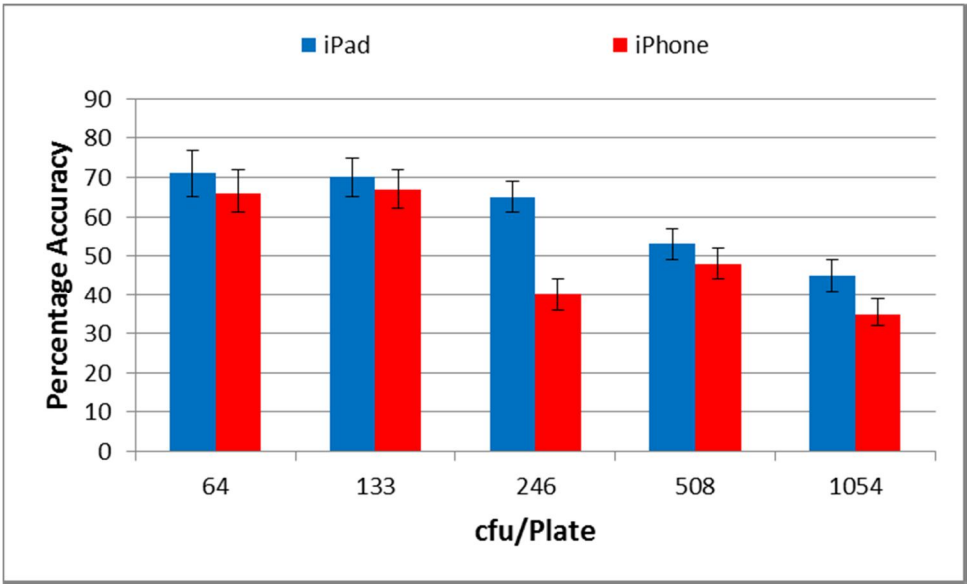

Figure 3.7: Counting with live colonies

3.4 Discussion

iPhone and iPad applications represent promising tools in the field of colony counting. They perform excellently against the "standards" (the printed sheets). Using these standards the counters can instantly recognise up to 20000 dots inside an 80mm diameter circle. Indeed in tests not reported here we found that the iPhone and iPad performance against the standards was much better than much more expensive automatic colony counters.

The iPad was found to be the most accurate equipment over the range of dot diameters despite it having a lower lens resolution that the iphone (5 MP for iPad rather than 8 MP for iPhone). Figure 3.3 shows three main trends. First the reading of 0.15 mm diameter dots for iPhone was very high due to reading voids on the paper (standard sheet) as dots. With diameters from 0.2 mm to 0.6 mm for iphone and from 0.15 mm to 0.8 mm for ipad, the readings are 100% correct i.e. the reading on screen is 129. The third trend is that dots larger than 0.6 mm for iPhone and 0.8 for iPad tend to be overestimated. This is because larger dots are being read as multiple colonies. Overall, this indicates that the application's software is well designed and covers a reasonably wide range of diameters.

For colony-background contrast, five grades of grey scale were used with a dot size of 0.25 mm in diameter. For the ipad and iphone, results were 129/129, except at 20% transparency (grey nearest to white), which read as 99/129 for the ipad, and 96/129 for the iphone. These results indicate that the influence of contrast on these colony counter readings is relatively low, and the smart applications software is effective when working across the range of contrasts.

To determine iPhone's and iPad's ability to count adjacent colonies, i.e. the distance between colonies (circumference to circumference), or colony crowding, a range of dot densities were tested. In the literature for most electronic counters, the highest recommended number of colonies for reading should not exceed 1000. However, by using iPhone and iPad, fully accurate readings were obtained for numbers around 9000 dots/sheet, and with more than 99.7% accuracy up to 20000 dots/sheet.

While there are many applications which are similar to the format of these printed sheets (for example in metallurgy and some aspects of ecology where photographs can be processed and

then “read”), the aim was to use the application to count bacteria colonies and the results are far less impressive.

For reading with live colonies; as shown in Figure 3.7, counting accuracy decreases as the number of colonies increases till reaching 45% for iPad and 35% for iPhone for 1054 cfu/plate.

Counting live colonies is very different from reading standard sheets. They have wide range of sizes, contrasts, shapes and layouts. Some colonies are smaller than 0.15 mm and greater than 0.8 mm in diameter. Also there are colonies very close to the background colour. Shapes vary from a single colony to a cluster of ten or more colonies. Layout can be crowded in some places while at low density in others. All of these factors affect the reading accuracy. However, the software can be easily switched to manual correction. In readings up to 500 cfu the electronic reading can be corrected by erasing the falsely read colonies and adding the missed ones. This manual adjustment makes readings reach 100% accuracy with relatively quick manipulation.

In addition to the (HGCOLONY); BFrontier has developed a completely manual counter for the iPad “ColonyCounter”. This is similar to the manual part of (HGCOLONY) and allows the user to manually count from a captured image. Four software features give them considerable versatility. First is the “scope”, where a user can choose the shape of the imaging area between circle, square or auto. These areas can be reduced or enlarged to occupy any part of agar plate. Second is zoom, where any part of agar plate can be zoomed in to see colonies more clearly and mark them. Third is the “add and erase” property where any falsely spotted colony can be erased and any miss spotted ones correctly pointed. Fourth is the ability to save the image and return back to it. “ColonyCounter” has extra features for marking colonies with different colour dots and sizes. Also zooming is unlimited to four quarters like (HGCOLONY) but it can be zoomed to any part of plate indefinitely (at least to the resolution of the image capture).

3.5 Conclusions

Colony counting using iPhone and iPad applications is a promising, quick and low cost technique. These devices are available for any person at any time. Their portability is a great advantage. Furthermore, software updates are readily available online and free and new developments rapidly launched. For example a new semi manual semi electronic application has features including the ability to tag colonies from different microorganisms or sizes.

However, for all their advantages, user familiarity and manual correction are still required when using these applications and manual correction was used in colony counting in the experiments described in later chapters of this thesis.

Reliability tests using pre-counted and pre-drawn standard sheet is an effective technique to evaluate the accuracy and functionality of any electronic colony counter. These sheets are easy to produce and print out with a minimum knowledge of using AUTOCAD.

CHAPTER FOUR

FROTH PRODUCTION WITHOUT CHEMICALS

Chapter Four

Froth Production without Chemicals

4.1 Introduction

Froth flotation is a physical separation method using the selective ability of particles to adhere to air bubbles rising in water (Alam and Shang, 2012). The process usually involves addition of chemical reagents to facilitate froth formation as well as attachment to the air bubble. The more hydrophobic materials are collected on the surface where a stable froth forms. The froth is skimmed to produce a "concentrate", leaving the less hydrophobic part to stay as a "tailing" in the bottom of the flotation cell. Chemicals are used for enhancing froth formation and quality, and to control the relative hydrophobicity of the particles (Alam and Shang, 2012; Zech *et al.*, 2012).

Using froth flotation for removing microorganisms from water could decrease the use of biocides in water treatment which would help minimize their side effects as the formation of Disinfectant by-Products (DBPs) which represents a serious threat to public health in the drinking water industry (Ngwenya *et al.*, 2013; Richardson, 2014). The other drawback of chemical disinfection is the formation of biofilm which is a defensive strategy of bacteria against biocides (Chandra *et al.*, 2001; Flemming, 2008; Simoes *et al.*, 2010; Kim *et al.*, 2012).

The use of froth flotation in drinking water and food industries is limited because of undesired taste and odor of chemical frothers even when in trace amounts, as the majority of them are alcohols and polyglycols (Finch and Zhang, 2014). Therefore, developing a method to produce froth without using chemicals will enable this separation technique to be used in a wider range of industries.

To observe how far air pumping will increase the oxygen solubility in water, mass transfer coefficient ($K_l a$) was calculated. It is well known that the saturation pressure of oxygen in water is 40 mg/l at 25C^{o} and 1 bar. Waters in nature brings their oxygen from atmospheric air. If air assumed to has 20% Oxygen, according to Henry's low the equilibrium concentration will be (40 × 0.2) 8 mg/l. Most of waters in nature in literatures found to be between 6 and 9 mg/l. Shallow

water fish need higher levels up to 15 mg/l (Kemker, 2013). At the lab conditions the obtained experimental value was around 5.5 mg/l. The reason behind reaching the value of 15 mg/l when aerating the water is the significant increase in mass transfer area and the access air (oxygen) pumped continuously. This pumping is a remarkable driving force to transfer all the gases from bubble to water. It is the same as of the ocean waves advantage for mixing the water with atmospheric air to increase the dissolved Oxygen that is necessary for aqua life.

4.2 Hypothesis

Froths are a liquid surface phenomenon formed as a result of lowering the water surface tension which otherwise prevents bubbles from forming by pulling their molecules to the water surface. Well-built bubbles can be formed near walls during boiling or pumping air into water. This indicates that when a rising bubble finds a support from one side it will not burst at the surface.

Assuming a layer of adjacent bubbles covering the entire cross sectional area of a contained column rises up through the liquid to the surface, the outer row will be attached to the container wall while the first inner row will be supported by the outer row and so on till the central bubble. Thus, the first layer of froth can be formed.

If a second layer of bubbles comes up through the water to the water surface, this second layer will displace the first upward. This will form a froth of two layers, and so on till a “stable” froth height controlled by operational variables is formed. Investigating the operational variables for a given column dimension should enable a maximum froth height to be determined.

4.3 Materials and methods

4.3.1 Froth flotation column

A compact froth flotation column system (Figure 4.1) consists of a 2 m long transparent Perspex (Poly methyl methacrylate) tube, 20 cm inside diameter. A ceramic sparger, 19 cm diameter, with a 50 micron pore size (from HP technical ceramics) is fixed 30 cm above the column base. The sparger is joined to a 15 mm diameter tube connected to a compressor with a rotameter (10-900 l/min). Note that the system in the picture is more complicated than the drawing because it is designed to be also used in other experiments.

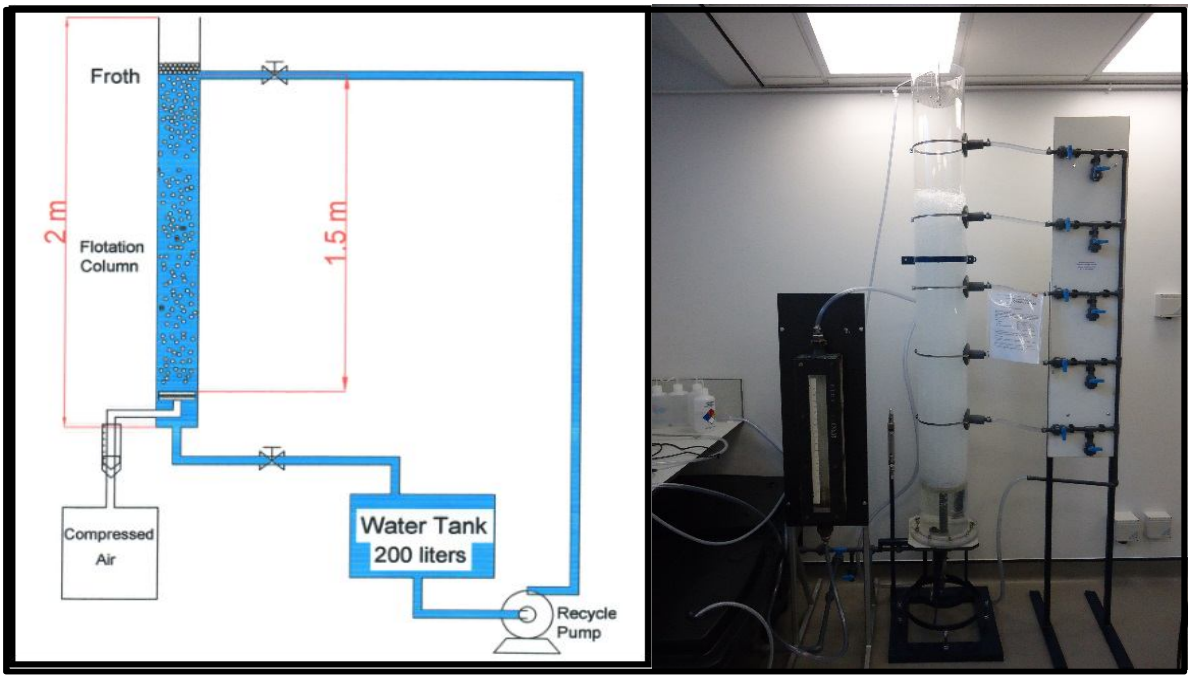

Figure 4.1: Froth flotation column

4.3.2 Oxygen concentration measurement

Oxygen concentration in the water was measured using an AZ-8403 Dissolved Oxygen meter produced by (AZ®) and calibrated daily according to the method mentioned in equipment manual.

4.3.3 Froth production methodology

The following steps were followed to produce froth of various heights in the column:

1- With an empty column, start air blowing at the rate of L min^{-1}.

2- Start water pumping at 1 L min^{-1}.

3- Once water level reaches 15 cm above the sparger, stop water pumping.

4- Measure for froth height.

5- Rise up air pumping to 30 L min^{-1}.

6- Measure for froth height. And so on till completing the full range of air flow rates to 210 l/min.

7- Start water pumping again at 1 L min^{-1} till reaching 30 cm above the sparger, and then stop it.

8- Repeat steps 4 through 7 for every 15 cm of water height over sparger till completing the full range of water heights from 15 to 120 cm in 15cm steps.

4.4 Results

4.4.1 Effect of air pumping on oxygen solubility

Air was pumped continuously through the water column with a water level of 45 cm. This is to estimate the increase of dissolved Oxygen as a result of pumping a large amount of air through the water column. The results are given in figure 4.2. The general trend of the oxygen concentration with time was a rise within the first 5 minutes then followed by a fluctuation between 5 and 30 minutes till reaching a plateau after 30 minutes. More pumped air led to higher dissolved oxygen levels.

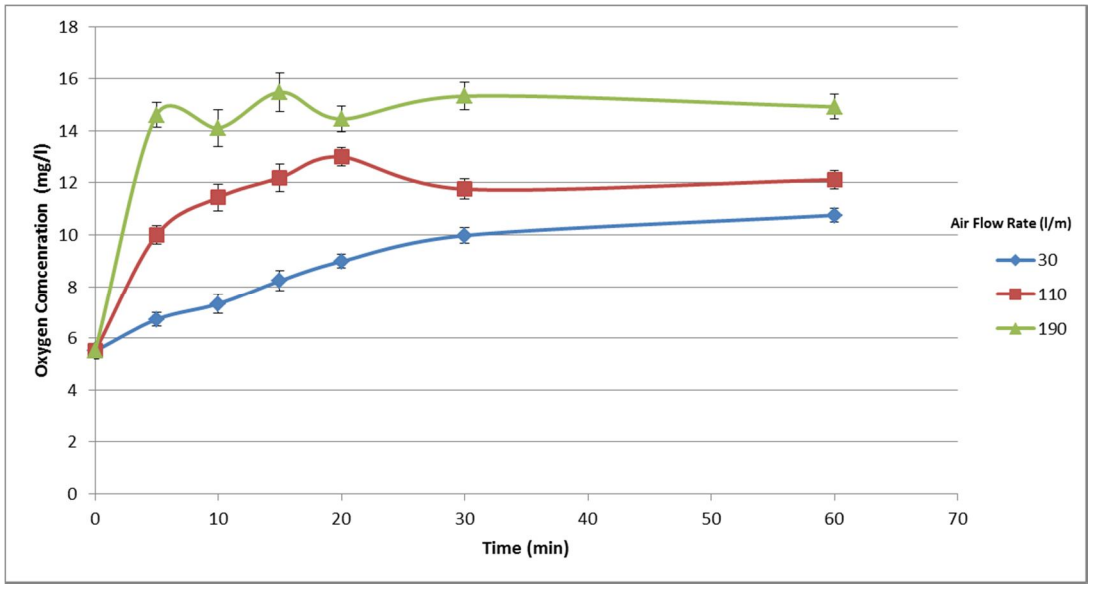

Figure 4.2: Effect of air pumping on Oxygen solubility

Following the same equations and calculations in 7.2 with taking the values of C^* of 40 mg/l and C_o of 5.5 mg/l with other values of time and concentration extracted from figure 4.2, K_la for oxygen dissolution in water can be presented by figure (4.3) below.

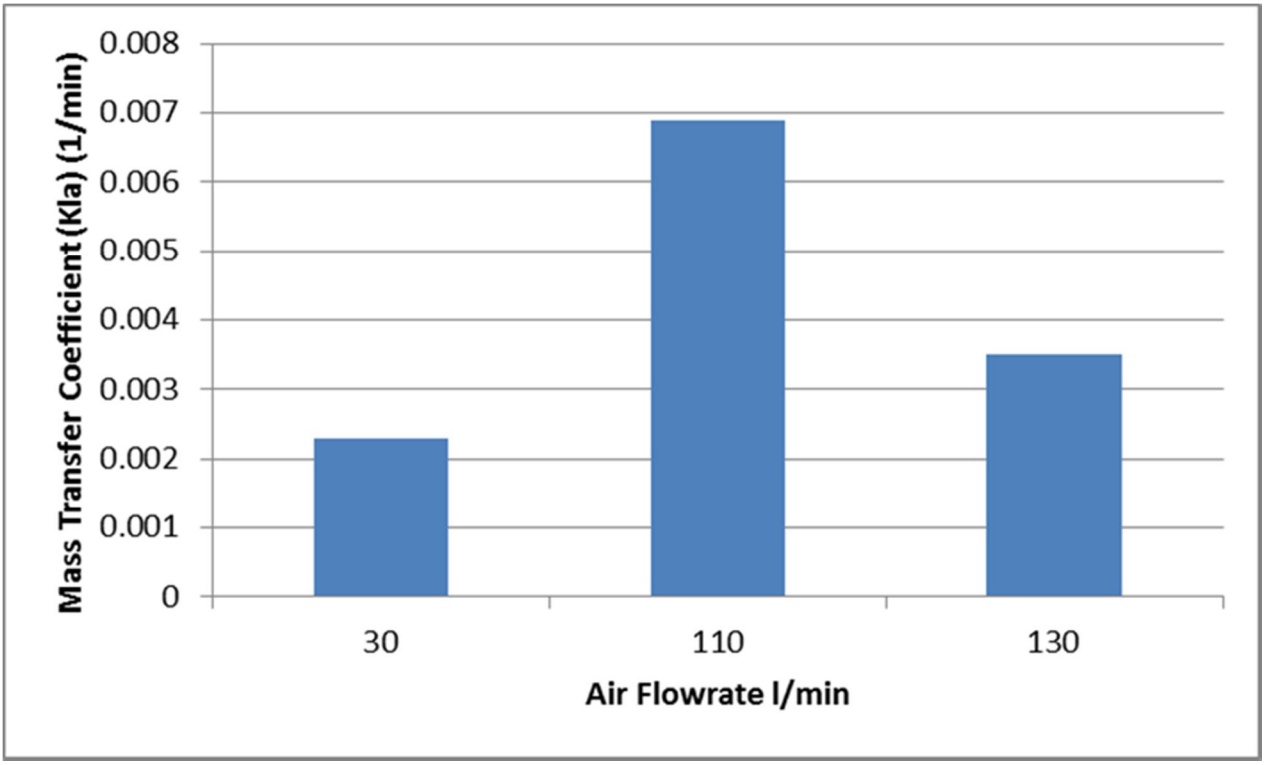

Figure (4.3): The relation between air flowrate and mass transfer coefficient (K_la)

4.4.2 Effect of air flow rate and water level on froth height

Figure 4.4 shows the variation in froth height with air flow rate and water height in a 20 cm (ID) column. Table 4.1 gives the error margins of figure 4.4 in centimeters. Froth height increases with increasing air flowrate to a maximum froth height of 27 cm at 130 L min^{-1} min then starts to decrease down as flowrate increases. Froth height decreased with water level to reach optimum value at 45 cm. After that (higher water levels) froth started to decrease.

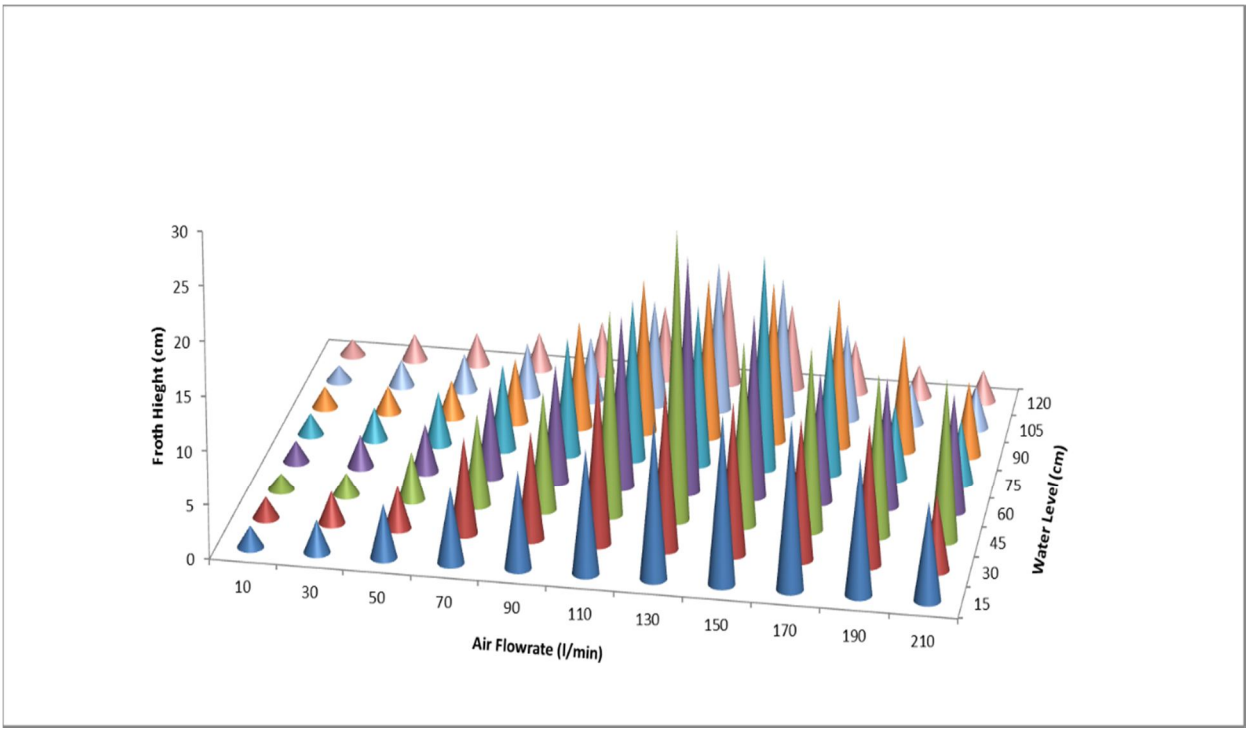

Figure 4.4: Effect of air flow rate and water level on froth height

Table 4.1: Error margins in (±cm) related to figure 4.4

		Air Flow Rate L min^{-1}										
		10	30	50	70	90	110	130	150	170	190	210
Water Level (cm)	15	±0.13	±0.18	±0.22	±0.25	±0.25	±0.25	±0.36	±0.41	±0.44	±0.45	±0.49
	30	±0.15	±0.23	±0.25	±0.33	±0.35	±0.36	±0.35	±0.55	±0.55	±0.57	±0.64
	45	±0.23	±0.25	±0.31	±0.35	±0.35	±0.35	±0.52	±0.73	±0.77	±0.81	±0.90
	60	±0.32	±0.35	±0.45	±0.45	±0.55	±0.55	±0.93	±1.12	±1.12	±1.21	±1.32
	75	±0.35	±0.43	±0.45	±0.57	±0.63	±0.68	±0.95	±1.15	±1.15	±1.34	±1.44
	90	±0.60	±0.65	±0.72	±0.75	±0.85	±0.85	±1.51	±1.59	±1.80	±2.05	±2.10
	105	±0.82	±0.85	±0.91	±0.95	±1.05	±1.05	±1.24	±1.43	±2.30	±2.45	±2.58
	120	±0.91	±0.95	±1.04	±1.05	±1.15	±1.15	±1.35	±1.91	±2.42	±2.50	±2.65

Figure 4.5: Left, froth in its optimum height (distance between belts is 30 cm). Middle, close view for the upper part of froth. Right, view for froth surface

4.5 Discussion

4.5.1 Oxygen solubility in water

In the early stages excess air leads to an increase dissolved air in the water. This increase is not a one way phenomenon. Once an air molecule (oxygen or nitrogen) passes into water, the tendency of an existing gas molecule to be released out of the water will increase. Forward action "solution" is fast; while the reverse "de- solution" is slow. As air pumping increases, there are fluctuations with increases and decreases with time until a steady state is reached (Figure 4.2).

The "de-solution" appears as fine bubbles generated from the liquid. It is similar to the release of gas bubbles in the well-known industrial application of DAF, "Dissolved Air Flotation". The importance of reaching air saturation can be demonstrated by suddenly pumping air into water filled column. The bubbles are spread in relatively large size in transparent water and no froth is formed. As time passes and the water starts to saturate with air, a white color (turbidity) starts to appear in column and a decrease in transparency is noticed. When air pumping is decreased, the "white smoke" disappears again and clarity returns back to water. This white color is the evidence of fine bubbles and the solution de-solution phenomenon.

These small bubbles have two main positive effects, first, in supporting the original big bubbles to form the desired froth, while the second is attaching and lifting micro-particles effectively, as the best bubble size for such techniques is close to the separated particles size (Hanotu *et al.*, 2012).

Figure (4.3) shows the relation between the mass transfer coefficient $K_l a$ and the air flowrate. It is shown that for the range (30-110 l/min) more air pumping leads to higher $K_l a$ as a result of increasing the mass transfer area. The range after (110-130 l/min) shows a decrease in $K_l a$ as a result of bubble crowd which leads to decrease in mass transfer area.

The increase in $K_l a$ along the range of (30-110 l/min) is not exactly proportional. The expected increase should be greater but it seems that the counteractive effect between $K_l a$ and air flowrate gives an advantage to air flowrate along this range but it decreases the expected $K_l a$.

4.5.2 Effect of air flow rate and water level on froth height

Air pumping parameters are the main factors leading to froth formation without chemicals. More air with less water leads to faster and richer froth building. While more water with less air leads to slow or no froth. This is because, at first, air is consumed by dissolving into the water. Air solution in water is relatively slow which makes froth formation nearly impossible when starting air pumping in a water filled column. To overcome this it is recommended to minimize water inlet and maximize air pumping. A water to air ratio of 1:130 was found to be optimum in the operating conditions in this work.

Higher air flow rates have two counteractive effects on froth height. More air builds a higher froth; but it increases water turbulence which destroys froth. Balancing these two factors the optimal flow rate was found to be 130 L min^{-1} for a column with an internal diameter of 20 cm using a water level of 45 cm. Larger diameters need greater air flow rates to keep the same air velocity across the column; that is, 0.069 m s^{-1}.

One of the purposes of this study is to avoid the limitations of two previous industrial applications, Dissolved Air Flotation (DAF) and froth flotation. In the water industry DAF is limited to the removal of solid contaminants rather than microorganisms. DAF depends on pumping air into water and keeping it under high pressure. Under this high pressure, solubility of air in water increases. When this water enters the DAF tank, the pressure returns to atmospheric and air starts to be released from water as a micro bubbles. The amount of micro bubbles is limited to 0.007 m^3 m^{-3} in best conditions depending on the applied pressure (Miettinena *et al.*, 2010); which in turn, limits the whole operation efficiency if it is desired to be used for removing microorganisms. In DAF, froth is not that important because the separated species will stay at

water surface by buoyancy. Furthermore, the flow regime in separation tanks is nearly laminar, so these species will not return back to water bulk body. Due to this, the main defect of DAF is its relative slowness; hence it cannot be used for further purification in drinking water industry.

In mineral froth flotation there is more freedom to use direct air pumping but this causes turbulence which remixes the separated particles with the water. This is where the importance of developing a stable froth comes from. However, more air pumping leads to froth destruction, which limits the air to liquid ratio to 10 m^3 m^{-3} (Miettinena *et al.*, 2010) and leads to the use of chemical frothers. The froth cannot be formed without chemical frothers because of the wide cross sectional area of flotation cells, which leaves the rising bubble without support when reaching water surface, hence bursting.

A large air to water ratio will help to produce more bubbles per volume of water; hence the probability for forming bubble layers that reach the water surface will increase. Thus a column with sufficient air pumping is able to form a stable froth.

Two variables were optimized in this work; first is air flow rate, where froth height increased as flow was increased until an optimum flow rate of 130 L min^{-1}. For air flow rates of 150 L min^{-1} and above, the froth height starts to decrease because of high turbulence.

For the second variable; water level, the optimum value was 45 cm above the air sparger. At lower water levels, air pumping from the sparger neutralizes the horizontal disturbance of the water surface. Above 60 cm the amount of water inside the column becomes too big to be neutralized with the amount of pumped air available. More air leads to more turbulence, hence low froth height.

4.6 Conclusions

A well-built froth can be produced in a column of suitable diameter and water level in addition to give the process enough time to saturate or oversaturate with air. This can be used to separate particles/bacteria by froth flotation without adding any chemicals that may affect water quality. By avoiding using chemical frothers hydrophobic particles can be separated in many industries like drinking water, food and pharmaceutical industries.

CHAPTER FIVE

THE ABILITY OF FROTH FORMED WITHOUT CHEMICALS TO HOLD BACTERIA

Chapter Five

The Ability of Froth Formed without Chemicals to Hold Bacteria

5.1 Introduction

Froth flotation is a solid-liquid separation technique that uses hydrophobicity as a driving force. Bacteria and other drinking water microorganisms tend to be hydrophobic and can be removed from water using this application. The biggest limitation against using froth flotation in the drinking water industry is the difficulty of producing a froth without chemical "frothers" and holding bacteria in this froth without chemical collectors. Both the frothing and holding chemicals can deteriorate water taste and odor. In Chapter four a method was described for producing froth using only water and compressed air. This enables froth flotation to be studied as an alternative to biocides for the removal of bacteria from drinking water.

Previously, it was believed that the optimum particle size for froth flotation is in the range of 88-500 microns (Zech *et al.*, 2012). However, the recent work by Hanotu et al. (2012), Lertrojanachusit (2013) and by Tang (2014) on carbon nano tubes indicates much smaller particles can be removed.

In an introduction to their work, Hanotu et al. (2012) state that separation efficiency is inversely proportional to bubble size because surface area is increased as bubble size decreases, increasing the probability of bubble-microorganism contact. Oppositely, as bubble size increases, buoyancy forces and hence rising speed reduces bubble particle attachment chances (Hanotu *et al.*, 2012). Therefore, the net effect of bubble size is still an area to investigate.

A series of experiments were therefore undertaken to investigate the ability of froth produced without chemicals to hold bacteria without using any chemical collectors.

5.2 Materials and methods

5.2.1 Froth flotation column

A compact froth flotation system was designed using a transparent Perspex (Poly methyl methacrylate) tube 20 cm diameter and 2 meters length. A ceramic sparger 19 cm diameter with 50 micron pore size (from HP technical ceramics) is fixed 30 cm above column base. The

sparger base is joined to a 15 mm diameter tube that is connected to a compressor via a rotameter of (10-900) L min^{-1}. Five side streams are attached to the column at 30 cm intervals above the sparger i.e. 30, 60, 90, 120 and 150 cm above the sparger. These side streams are used to collect froth samples. A 200 liter tank is installed beside the system to collects distilled water from a still and provides a reservoir to the column. The capacity of column is approximately 60 liters when totally filled. The assembly is fixed on a steel rig (Figure 5.1). The system was tested for not receiving bacteria from the outside by operating it with only distilled water for 8 hours and analyze for colony counting. The result was no bacterial interaction with the outside.

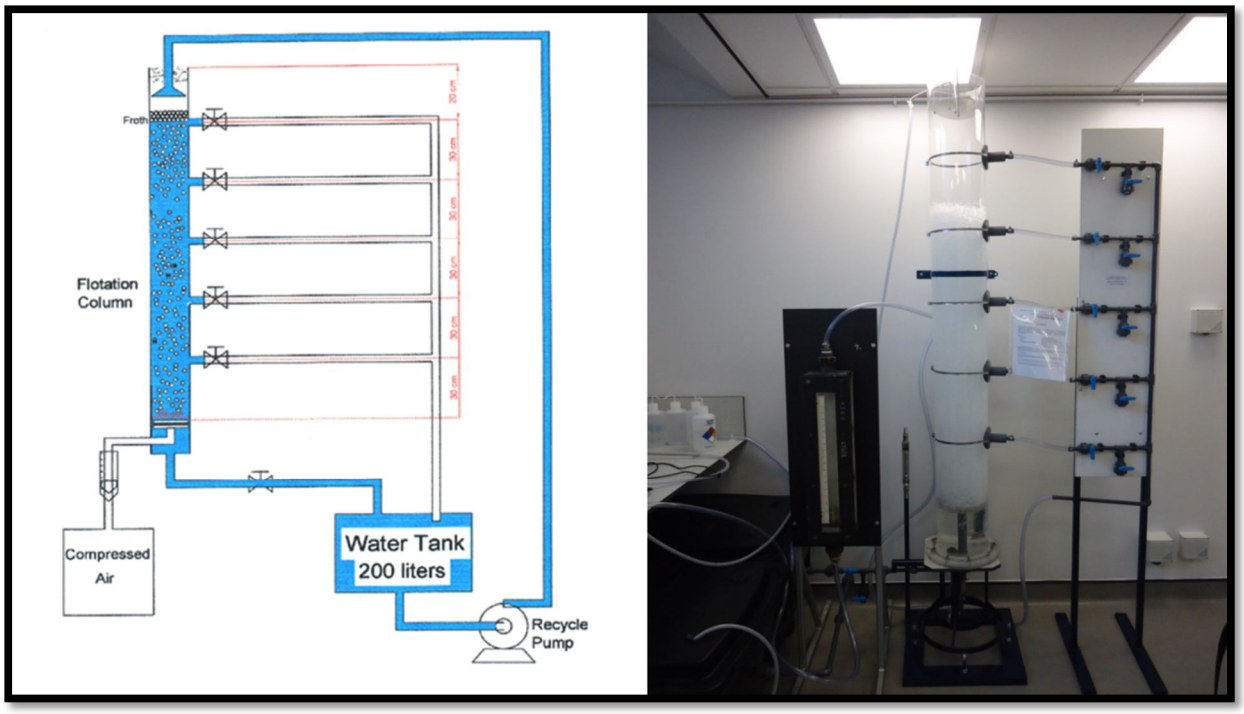

Figure 5.1: Experimental set up

5.2.2 Bacterial nutrient broth

Nutrient broth was prepared by mixing 15 g of nutrient broth (SIGMA-ALDRICH) in 1 liter of distilled water. When dissolved completely, it was autoclaved for sterilization. The broth was then inoculated with bacteria and incubated for 24 hours and 37 C˚. The bacteria used in this work were the K-12 strain *Escherichia Coli* (Texas Red). The mother bacteria were kept deep frozen and subcultured as required.

5.2.3 Turbidity measurement

A turbidity meter (TurbiCheck, from Lovibond water testing Co.) was used to measure turbidity as a function of total bacterial content. This device is equipped with four calibration standards, 800, 200, 20 and less than 0.1 NTU. Calibration of this meter was carried out daily before use.

A standardization test was carried out to check the meter readings and range. One liter of inoculated nutrient broth was prepared according to (5.2.2) and measured for turbidity (NTU). Dilutions in distilled water were prepared to obtain the following dilutions: 1/2, 1/4, 1/8, 1/16…etc. The turbidity of these dilutions was measured also for NTU. The obtained relationship, which shows a direct proportionality, is given in (Figure 5.2).

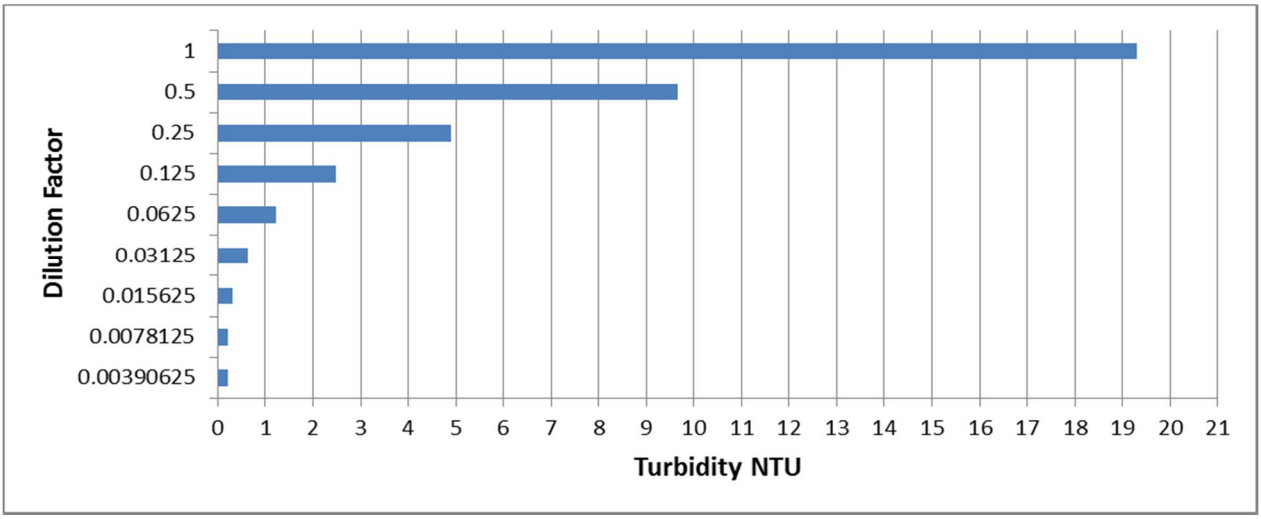

Figure 5.2: The relation between turbidity (NTU) and dilution factor

5.3 Operating methods

5.3.1 Batch system

1- Collect approximately 100 liters of distilled water in the tank.
2- Add gradually a suitable amount of cultured broth till reaching the desired turbidity (NTU) in the tank.
3- Start air pumping at a rate inside the column depending on run demands.
4- Start water pumping at a rate of 1 L min^{-1}.
5- When the froth reaches the 30 cm of column, stop upstream flow.
6- Start taking samples for turbidity reading every five minutes for 30 minutes.

7- When finished sampling for the selected time period, drain the flotation column. When fully empty, close the bottom stream.
8- Repeat steps 2 through 7 with every new air flow rate and column height.

5.3.2 Continuous System

1- Collect approximately 100 liters of distilled water in the tank.
2- Add gradually a suitable amount of cultured broth till reaching the desired turbidity (NTU) in the tank.
3- Start air pumping at a rate inside the column depending on run demands.
4- Start water pumping at a rate of 1 L min^{-1}.
5- When the froth reaches the 30 cm of column, open the bottom valve with the same flow rate as the upcoming stream.
6- Start taking samples for turbidity reading every five minutes for 30 minutes.
7- Stop upstream pumping to drain the column. When fully empty, close the bottom valve.
8- Repeat steps 2 through 7 with every new air flow rate and column height.

5.4 Results

Air flow rate, Time, initial (tank) turbidity, and water level (side stream height) were optimized for their effect on downstream turbidity. Each single graph represents the effect of air flow rate and time for different water levels (side streams height) or initial turbidity of (0.5, 1, 1.5, 2 and 2.5 NTU). Ranges for air flow rate were 10 to 170 L min^{-1} with intervals of 20 L min^{-1}. Samples were taken every five minutes.

Every graph represents the effect of air flow rate and time on froth turbidity. Then five graphs are developed each one is for a new water level starting from 30 cm above the sparger till 150 cm in 30 cm steps. The general trend showed an increase of froth turbidity with air flowrate till reaching the maximum level at 130 L min^{-1} then drop down suddenly to a value near the tank turbidity. Froth turbidity increased with time till reaching a steady level in a range up to 20 minutes. Higher water levels in the column gave greater froth turbidity. Finally, as the initial (tank) turbidity increases as the froth turbidity increases also.

5.4.1 Batch system

The effect of Air flow rate, Time, and water level on the froth turbidity in a batch system at an initial (tank) turbidity of 2.5 NTU following the steps in (5.3.1) are summarized in Figure 5.3.

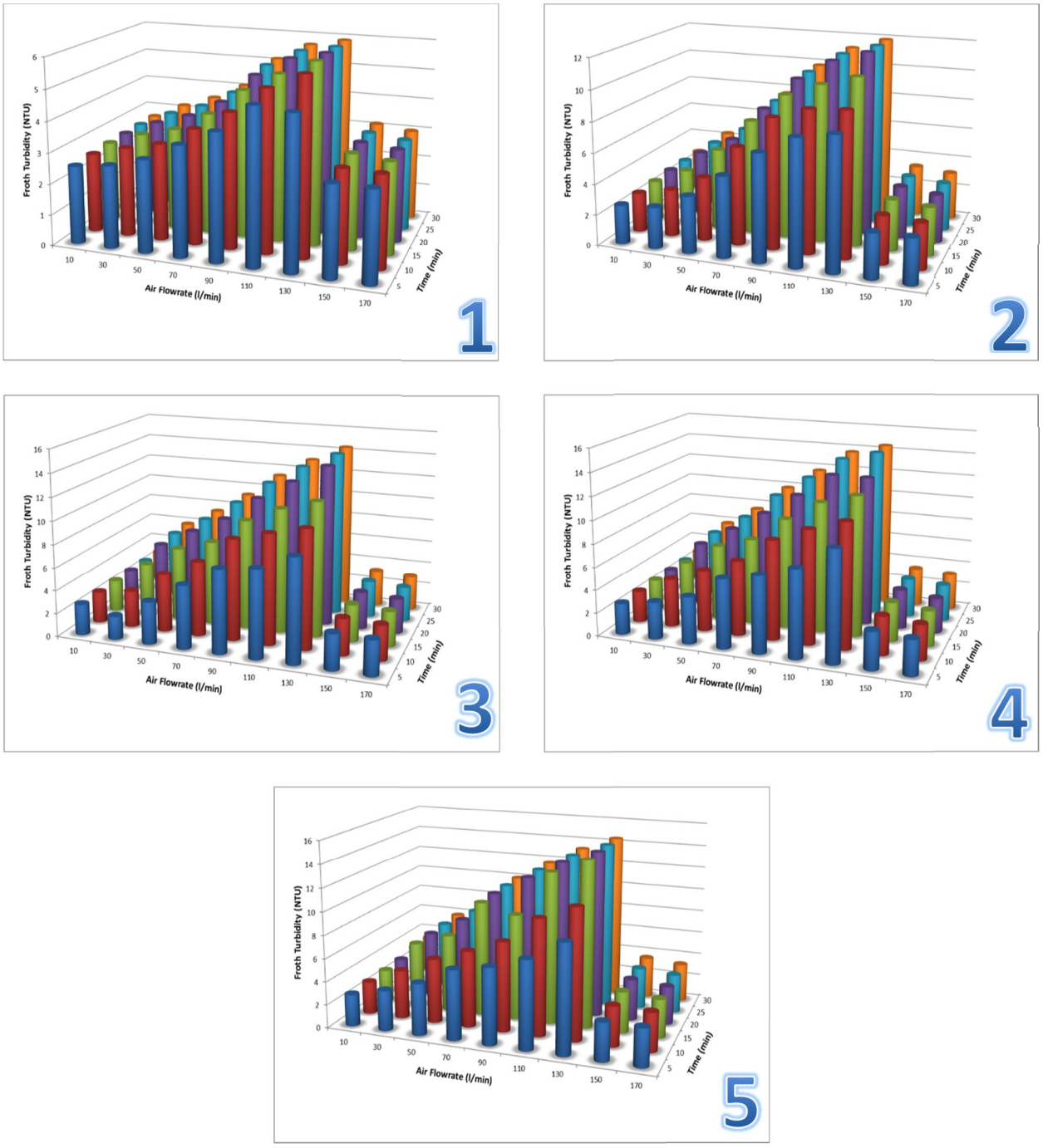

Figure 5.3: Effect of air flow rate and time on froth turbidity for a batch system (initial (tank) turbidity of 2.5 NTU) and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

5.4.2 Continuous system

The following sets were implemented according to section (5.3.2). Every graph represents the effect of air flow rate and time on froth turbidity. Then five graphs are developed each one is for a new water level starting from 30 cm above the sparger till 150cm in 30 cm steps. Figures 5.4 through 5.8 illustrate the initial turbidities of 0.5 to 2.5 with a step of 0.5 NTU respectively.

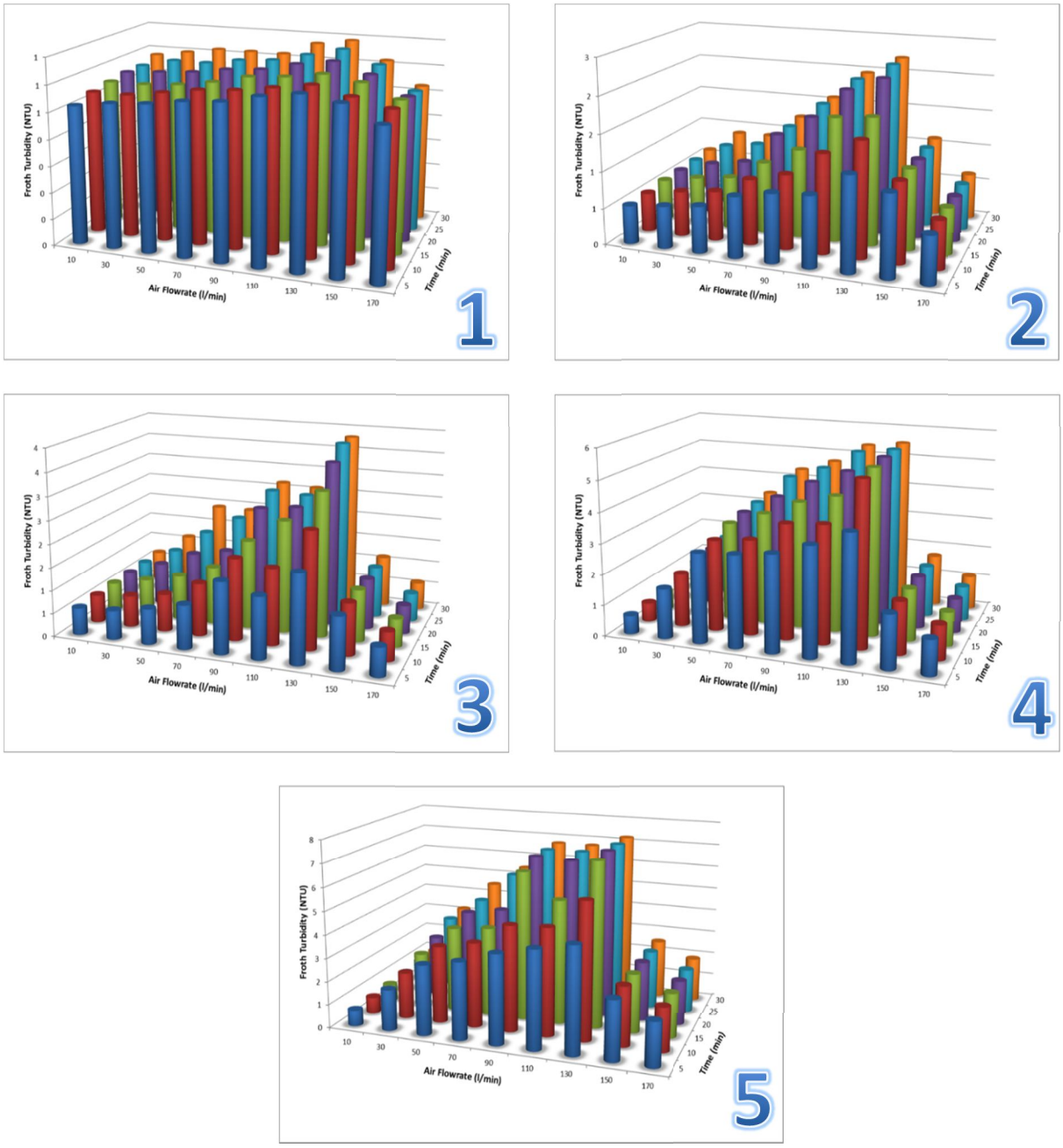

Figure 5.4: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 0.5 NTU in continuous system and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

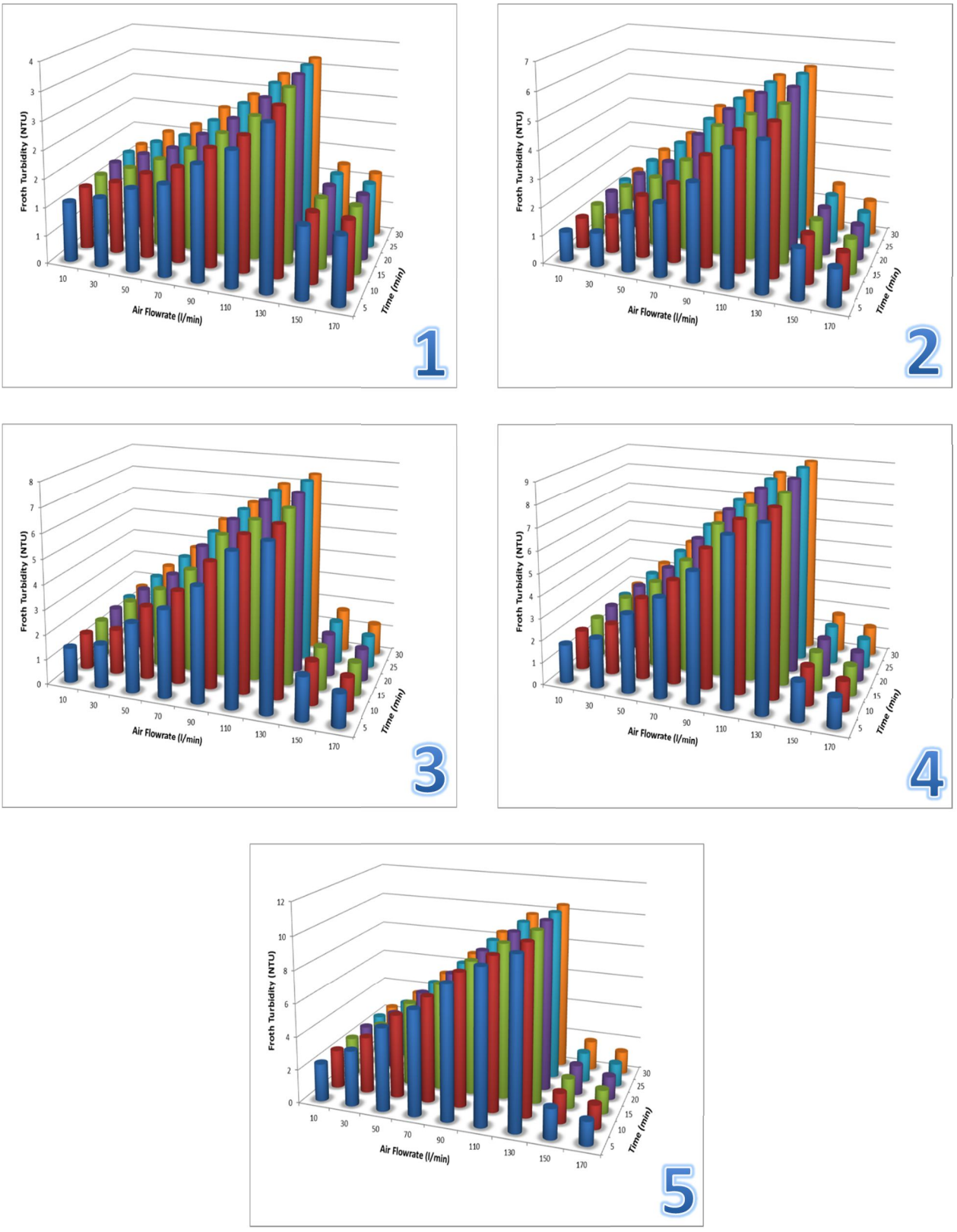

Figure 5.5: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 1 NTU in continuous system and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

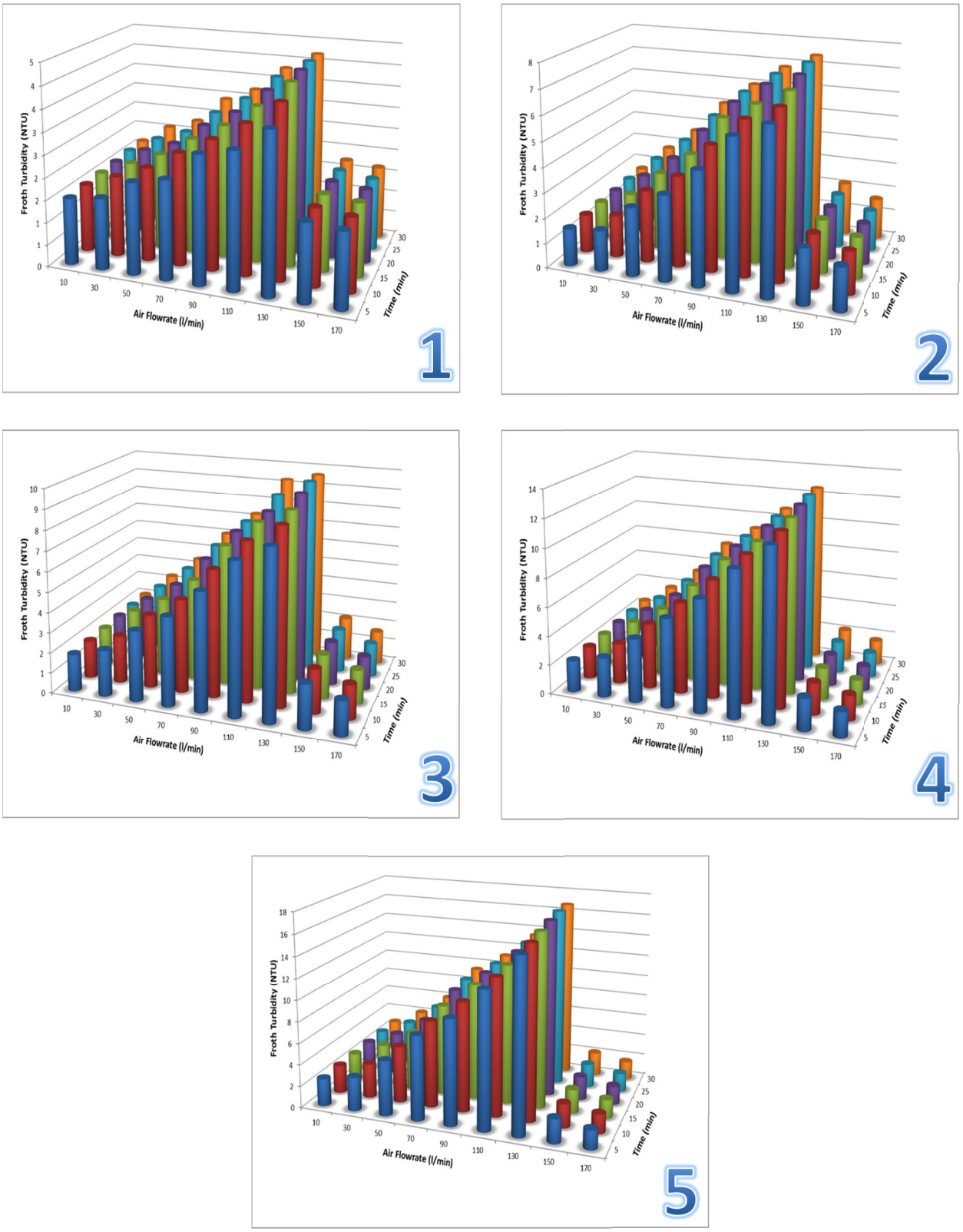

Figure 5.6: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 1.5 NTU in continuous system and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

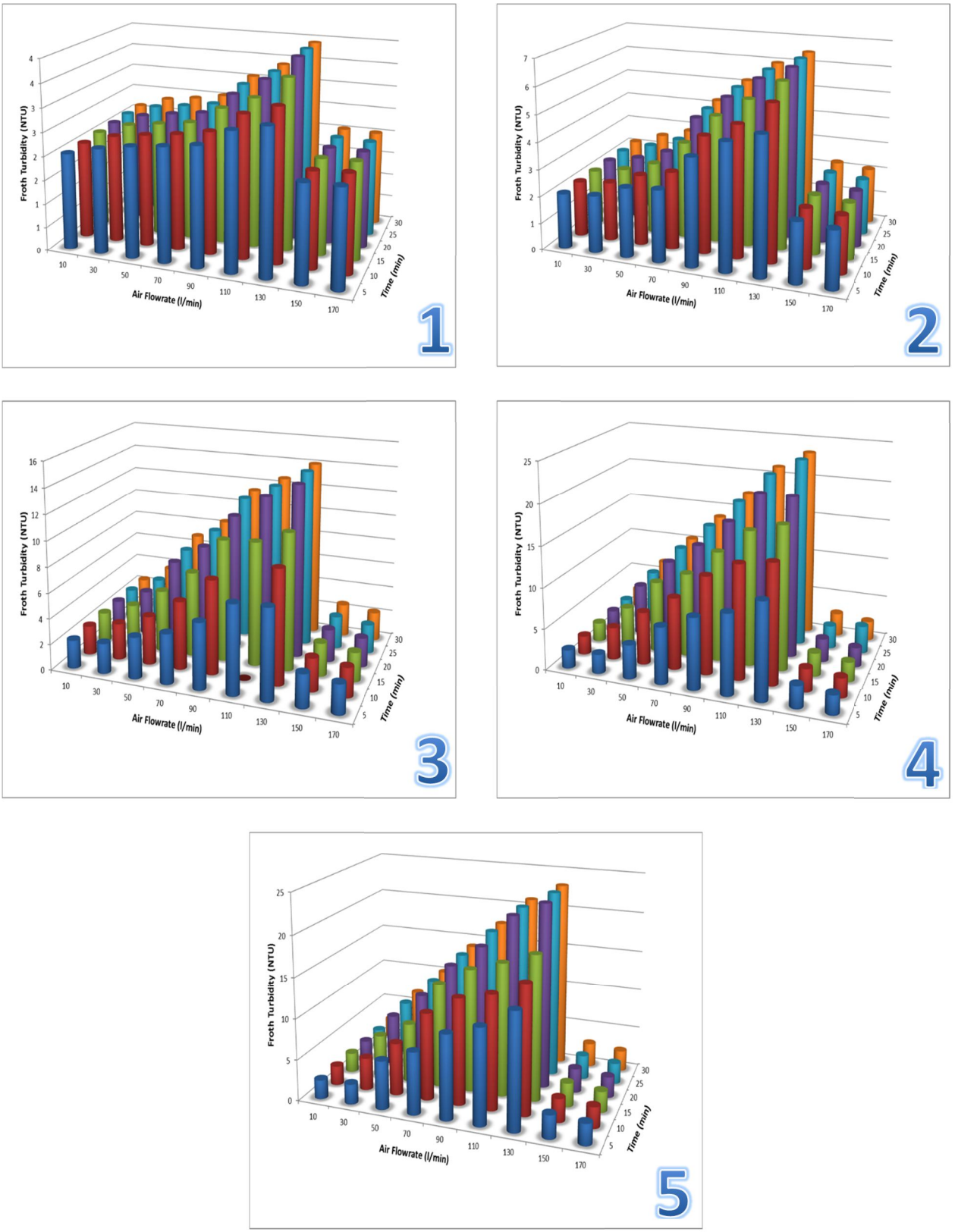

Figure 5.7: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 2 NTU in continuous system and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

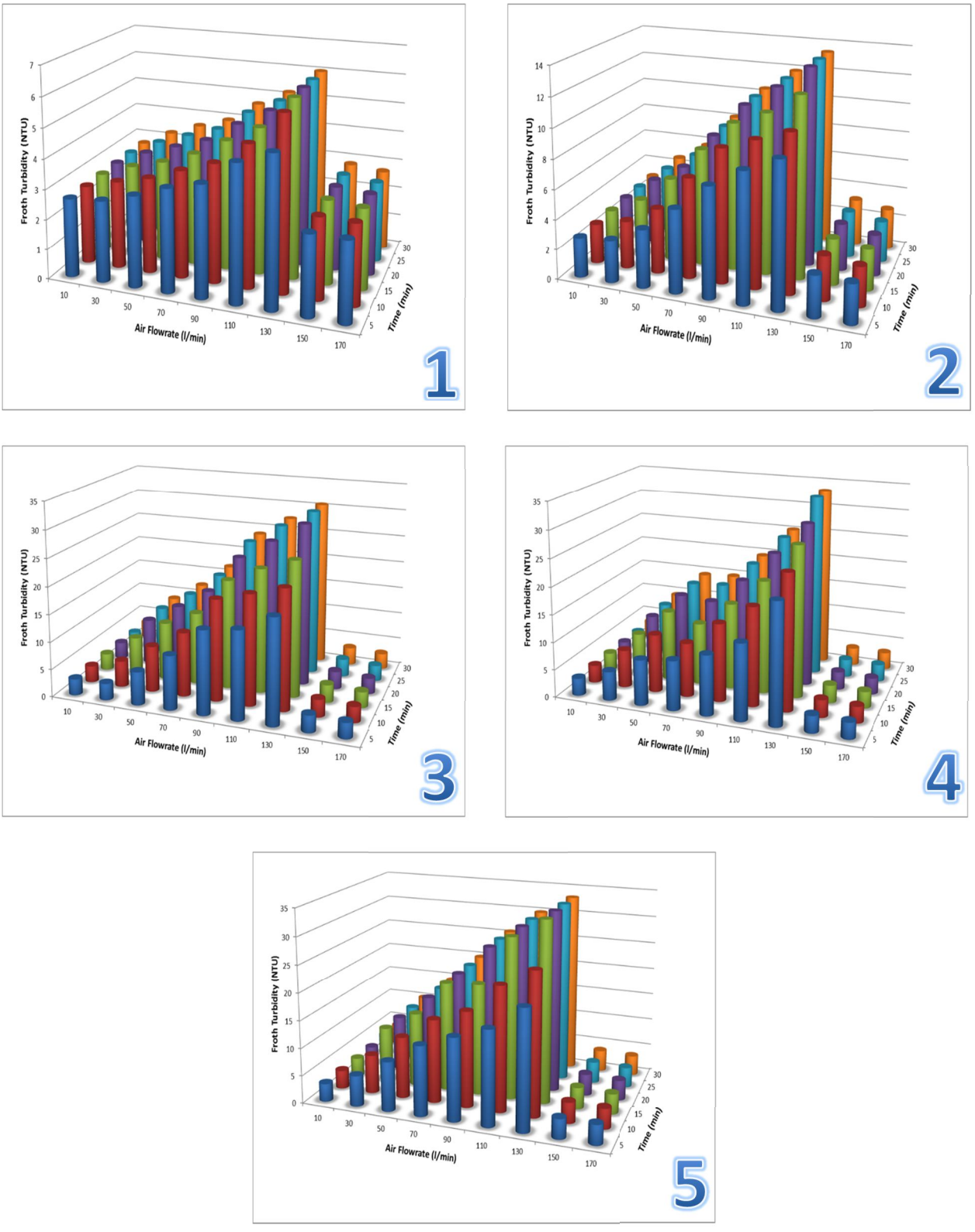

Figure 5.8: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 2.5 NTU in continuous system and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

5.5 Discussion

The main aim of this study is to determine whether froth formed without chemical frothers and collectors can function in removing bacteria from water.

In conventional mineral froth flotation with chemicals the optimum particle size is 88- 500 microns (Zech *et al.*, 2012). Particles of this size, if collected without chemical collectors, slip down the froth due to their weight. The weight of the attached particles works against the four forces, van der Waals, electrostatic, hydrodynamic repulsion, and hydrophobic (Bondelind *et al.*, 2013) which hold the particle to the froth. However, much smaller particles; such as bacteria, provided they do not agglomerate, may not need chemicals to help attach them to froth bubbles..

The effect of four variables on the turbidity (as a measure of bacterial content) of the froth was investigated. These were, air flow rate, time, water level in the column and starting (tank) turbidity. The results of these variables were analyzed using complete factorial analysis where all the possibilities across all the experiments ranges were taken into account (Collins *et al.*, 2009).

For air flow rate, froth turbidity increases proportionally along the range 10-130 L min^{-1}. Within this range, more air pumping leads to more bubbles and more chance for bubble-bacterial attachment. For the range 90-130 L min^{-1} the decrease is not sharp because of increased turbulence. At a rate of 150 L min^{-1} and more, froth is destroyed completely as a result of turbulence in the column. The mixing becomes very high and results in the bacterial concentration along the column being the same and similar to the tank concentration.

Froth turbidity is proportional to the height of the water column. When a bubble rises in a water column containing bacteria, a higher water level gives a bubble more time for bacterial–bubble attachment.

Five initial (tank) turbidities were used (0.5, 1, 1.5, 2 and 2.5 NTU). The general trend shows that the greater the initial turbidity the greater the obtained froth turbidity. These five starting values gave optimum froth turbidities of (7.23, 10.48, 16.77, 23.16 and 33.26 NTU) respectively at the optimum operating conditions for each set. These results show that the efficiency gets higher at higher initial (tank) turbidity. This is due to greater probability of bubbles to attach bacteria.

The last variable investigated was the time. Froth turbidity increases with time until reaching a constant value. Time to reach steady state is inversely proportional to air flow rate, initial turbidity and water level. The explanation of all these trends is the chance of bacteria to attach to a bubble and the continuous delivery of bacteria to the froth.

These results, without the use of chemicals, are, to some extent, similar to previous researchers findings, where chemicals were used. For instance, the first use of froth flotation with bacteria was isolating bacterial strains for laboratory purposes in the 1950's but its use disappeared with the development of more sophisticated techniques (Boyles and Lincoln, 1958; Rubin *et al.*, 1966; Bahr and Schugerl, 1992; Rios and Franca, 1997). In mineralogy some bacteria are recognized to have two functions, attaching to minerals and being highly hydrophobic. They are found to be ideal for mineral upgrading as some minerals are not hydrophobic and cannot be otherwise separated using froth flotation (Smith *et al.*, 1993; Nagaoka *et al.*, 1999). The most recent application is purification of sea water in fish farms. Sea water is sucked continuously into a froth column using casein as collector and frother and the bacteria removed to keep the environment healthy for the fish (Suzuki *et al.*, 2008).

The optimum froth turbidity obtained in this work is 33.26 NTU (2×10^8 cfu ml^{-1}). The initial (tank) water stream of 2.5 NTU; (10^7 cfu ml^{-1}), is inputted continuously to the top of the column. This gives a cfu concentration factor of 20. This means; theoretically, every 1 ml of froth can purify 20 ml of water.

Practically, this needs further research. For rivers and reservoirs bacterial content could be taken as 10^4 cfu ml^{-1} in average (Obi *et al.*, 2003; Agbabiaka and Oyeyiola, 2012; Rajiv *et al.*, 2012; Sakai *et al.*, 2013) which shows promise for further work. Next chapter investigates this.

5.6 Conclusions

Currently, chemicals are considered necessary as frothers, collectors, activators, depressants and pH controllers in standard froth flotation. In this study the ability of froth to separate bacteria without any of these associated chemicals is demonstrated. The results show that the separation force of froth alone is sufficient for bio purification. These results indicate the potential to move towards water treatment with lower or no biocides.

The findings of this work widen the horizon for many applications. The first is drinking water treatment. Bacterial and other solids can be lowered to an acceptable range either by this treatment alone or as an introduction to other purification steps. Food and pharmaceutical industries are other fields for such applications. It can be used as an alternative, or in series, with filtration and sedimentation for decreasing bacterial and solid load.

CHAPTER SIX

BIO-PURIFICATION OF DRINKING WATER BY FROTH FLOTATION

Chapter Six

Bio-Purification of Drinking Water by Froth Flotation

6.1 Introduction

The main technique for removing bacteria from water for various applications is chemical disinfection. However, this method has many disadvantages such as producing disinfectant by-products (DBPs), biofilm formation and either rendering the water unpotable (at high residual disinfection) or leaving a potential for lethal diseases such as Cholera (if the residual disinfection is too low).

Following the development of the a process for continuous removal of bacteria from water using the principle of chemical free froth flotation using compressed air only without any chemicals (as described in Chapters 4 and 5, and the work described in this Chapter). This work examines the extent to which chemical free froth flotation can purify drinking water.

Dead bacteria represent a considerable “sink” for the chlorine and will contaminate the treated water (Castro and Neves, 2003). They also represent a secondary source of nutrients to other bacteria in a water stream (Griebe and Flemming, 1998). This source of nutrients stimulates live bacteria to regrowth and decreases the biological stability of water inside the distribution system. Therefore, it is important to investigate how dead as well as live bacteria are removed by froth flotation in order to understand how to minimize such undesired contamination.

6.2 Materials and methods

6.2.1 Experimental set up

The experimental system consists of two Perspex (Poly (methyl methacrylate)) 20cm internal diameter columns of one and two meters length. Air is supplied through a ceramic sparger 19 cm diameter and pore size of 50 microns (from HP technical ceramics), fixed 10 cm above the column base. A water inlet is situated 15 cm underneath the column top. A tank of 200 litres is attached the system for two purposes, firstly as a reservoir for collecting distilled water from the still, and secondly as a recycle tank when an experiment is run. Figures 5.1 and 5.2 illustrate the experimental apparatus. Note that the system in figure 6.2 is more complicated than the drawing because it is designed to be used for other research as well.

6.2.2 iPad for colony counting

The iPad used for colony counting and the application (software) is (HGColony) developed by (HyperGear Inc.) and can be downloaded through the Apple app store. The calibration and best operating technique are described in chapter 3.

6.2.3 Peristaltic pump

A peristaltic pump from "Watson Marlow, Model - 505S", (range 2 - 220 rpm) was used for recycling and controlling the flow of the water between the tank and the column. The water flow rate produced by this pump depends on the pumping head. Since there are two column lengths, a calibration curve for each column was determined. For the two meters column, the pump range (2 - 220 rpm) gave a flow rate range of (0.024 - 2.35 L min^{-1}) while it was (0.029 - 3.15 L min^{-1}) for the one meter column. Both relationships are linear and directly proportional. Any flow rate could thus be calculated. For the one meter column every 1 rpm equals to 0.0143 l/min while it is 0.0107 L min^{-1} for the two meter column.

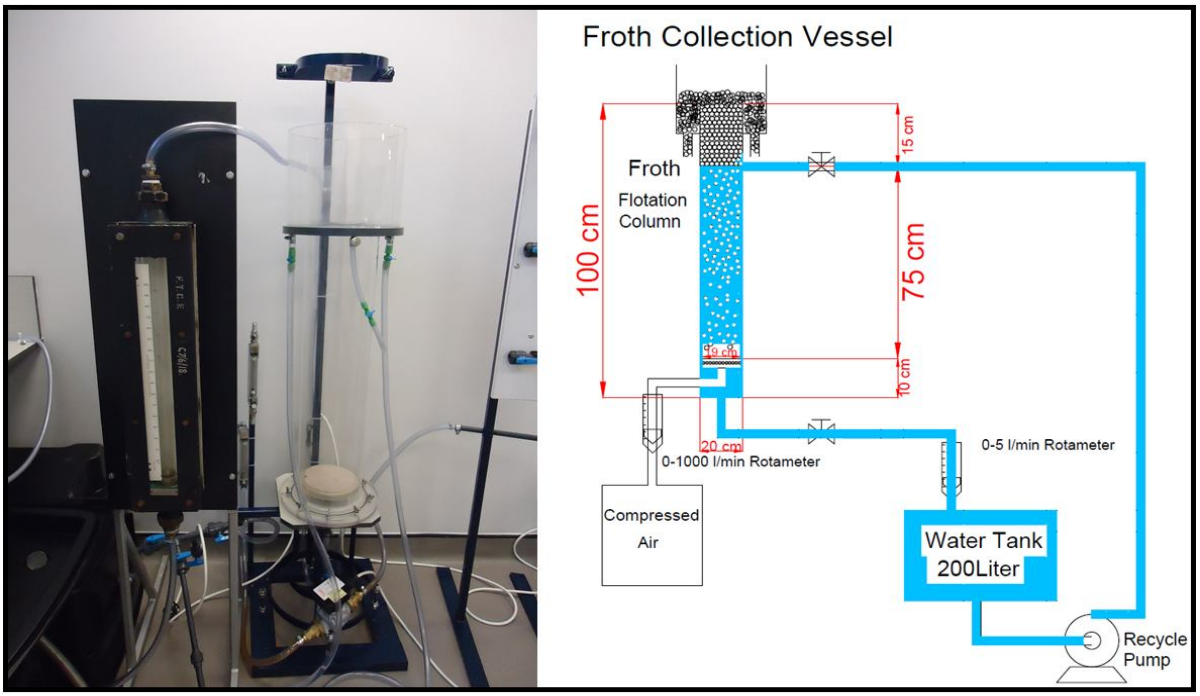

Figure 6.1: Experimental setup, 1m column

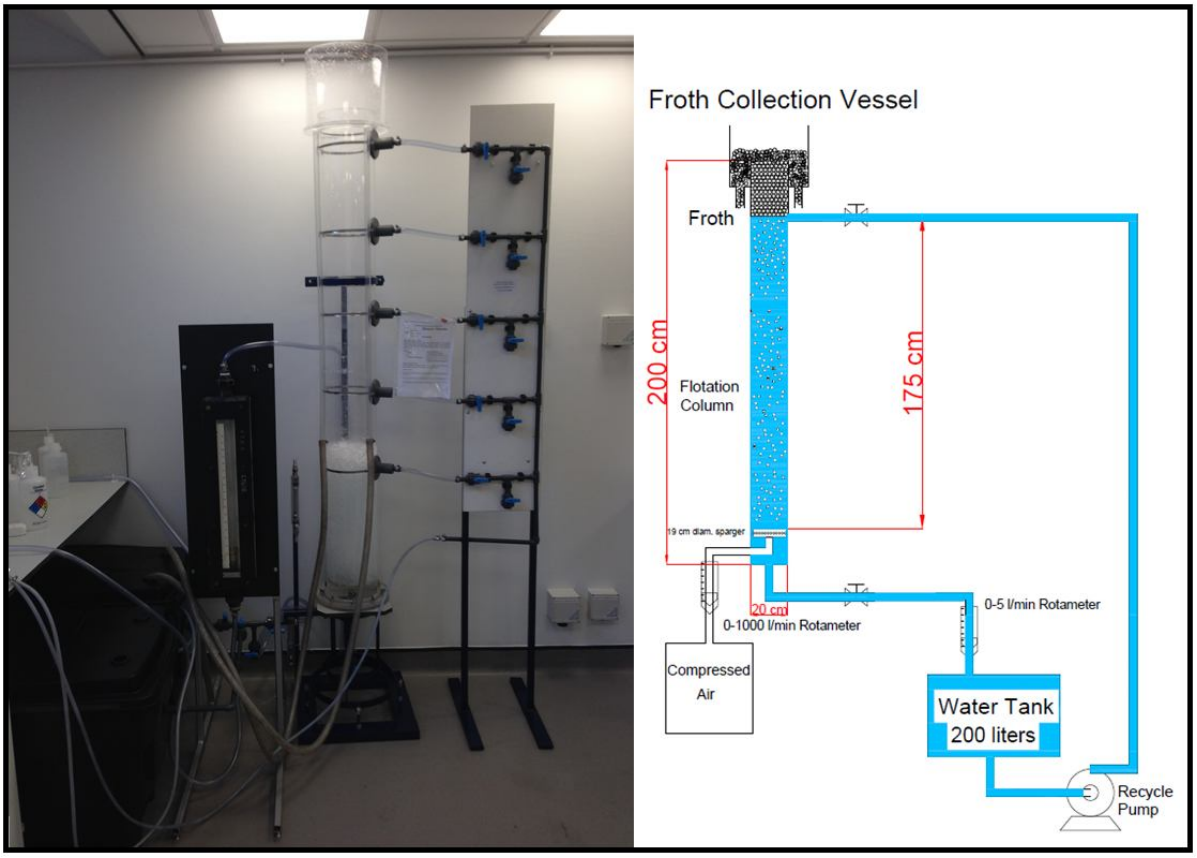

Figure 6.2: Experimental setup, 2m column

6.2.4 Agar plates

A mixture of nutrient broth and agar (15g + 15g) (SIGMA-ALDRICH) was suspended in a litre of distilled water then boiled and mixed using magnetic stirrer. When dissolve completely, the nutrient agar was autoclaved (121 C˚ and 1 kg cm^{-2}) then cooled to about 50 C˚ and poured in plastic petri dishes. When empty, the plates were incubated for 24 h at 37 C˚ to confirm accurate sterilization.

The plates were cultured aseptically with K-12 strain *Escherichia Coli* bacteria (Texas Red®) and incubated for 24 hours at 37 C˚.

6.2.5 Dead Bacteria preparation

Dead bacteria were obtained by treating a nutrient broth culture with a high dose of chlorine (1000-2000 ppm). One liter of distilled water highly saturated with chlorine produced by adding

trichloroisosyanuric acid was prepared and mixed with the same amount of fully mature bacterial nutrient broth. The time needed to kill 99.9% of the bacteria is less than one minute (EPA, 2009), the mixture is left for one hour to make sure of killing of all the bacteria. After that the mixture was tested for two criteria. The first was to measure the mixture turbidity to make sure that there is no reaction between the bacteria and the chlorine. Theoretically, if there is the same number of either live or dead bacteria in solution, they should have the same turbidity. The second test was making sure all the bacteria in the mixture were dead. One ml of the chlorine-bacterial nutrient broth mixture was added to a previously prepared and autoclaved nutrient broth and incubated for 24 hours and 37 C^o. If there is growth, the death of bacteria was incomplete. If the incubated nutrient broth showed no sign of growth the biocide process can be considered complete.

6.3 Experimental procedure

6.3.1 Preparing a water tank with known cfu/ml

1- Prepare one liter of inoculated nutrient broth as shown in section (5.2.2).
2- Collect 100 liters of distilled water in the tank.
3- Add one ml of broth to the water tank, then mix and measure for colony count using a triplicate of Agar plates.
4- Add a second ml of nutrient broth to the tank and repeat step 3.
5- Continue adding nutrient broth till reaching the expected cfu ml^{-1}.
6- Next day, check the produced Agar plates to identify the quantity of inoculated broth to produce the desired cfu ml^{-1}.

6.3.2 Measurement of percentage removal of bacteria

The following steps were followed to investigate the effect of studied variables (air flow rate and percentage of inlet water removed by froth) on the percentage removal of bacteria.

1- Collect 100 liter of distilled water in the tank.
2- Add the amount of inoculated nutrient broth obtained in section (5.2.2).
3- Take three samples for colony counting. These samples are for checking if the added amount of nutrient broth gave the desired cfu ml^{-1}. If not the whole set should be repeated.
4- With an empty column, start air pumping at the rate of 10 L min^{-1}.

5- Start water pumping at 1 L min^{-1}.
6- Once water level plus froth reach the column top, open the downstream valve with a flow rate of 900 ml min^{-1}. This will give a froth stream of 100 ml min^{-1}.
7- After 30 min, start taking samples for froth and downstream.
8- Stop the inlet flow. Then wait for the column for evacuating approximately quarter of its content.
9- Increase air pumping to 20 L min^{-1}. Then repeat steps 5 through 8, and so on for every next air flow rate.
10- Repeat steps 1 through 9 for every water downstream flow rates of (700, 600, 500, 400) ml min^{-1}.

6.3.3 Semi continuous flotation system

These experiments were designed to enhance froth performance and decrease the amount of water that is lost as froth. The same technique used in Section 5.3.2 was followed but the inlet water stream was stopped when it reached the column top. After that, the water level starts to decrease inside the column and froth should be built. When reaching an acceptable froth height, restart the water flow. This will push the froth out of column again, and so on. So, the following steps are followed:

1- Collect 100 liter of distilled water in the tank.
2- Add the amount of inoculated nutrient broth obtained in Section (5.2.2).
3- Take three samples for colony counting. These samples are for checking if the added amount of nutrient broth gave the desired cfu/ml. If not the whole set should be repeated.
4- With an empty column, start air pumping at the rate of 10 L min^{-1}.
5- Start water pumping at 1 L min^{-1}.
6- Once water level reaches the column top, open the downstream valve with a flow rate of 1 L min^{-1}.
7- Stop upstream water inlet.
8- Water level in column should start to decrease and the froth start to build.
9- Once the froth reaches its steady state height or moves away from column top edge, start upstream water inlet again at 1 L min^{-1}.
10- As the water level increases inside the column, it displaces the froth to froth collector.

11- When the froth disappears as a result of water level rising inside the column, stop water inlet again and so on.

12- Take samples for colony count every time the inlet upstream water is stopped.

6.4 Results

Measurements of cfu ml^{-1} were taken for the inlet, column bottom (after froth separation) and froth streams. The "purification force" depends on the difference between inlet and bottom streams:

$$\text{Percentage removal of bacteria} = \frac{Inlet\left(\frac{cfu}{ml}\right) - downstream\left(\frac{cfu}{ml}\right)}{Inlet\left(\frac{cfu}{ml}\right)} * 100 \quad \ldots\ldots 6.1$$

6.4.1 Effect of air flow rate and percentage of inlet water removed by froth on the percentage removal of bacteria.

In these experiments, two operating variables were investigated to determine their effect on the purification force of froth flotation, the air flow rate and the percentage of inlet water removed by froth. Figures 6.3 and 6.4 show the results for the 1 and 2 meter columns respectively. Percentage removal of bacteria started low at an air flowrate of 10 L min^{-1}, and then increased till reaching maximum value at 70 l/min followed by decrease for both of 80 and 90 L min^{-1}. For the effect of percentage of inlet water removed by froth on percentage removal of bacteria the trend was a slight inverse proportionality.

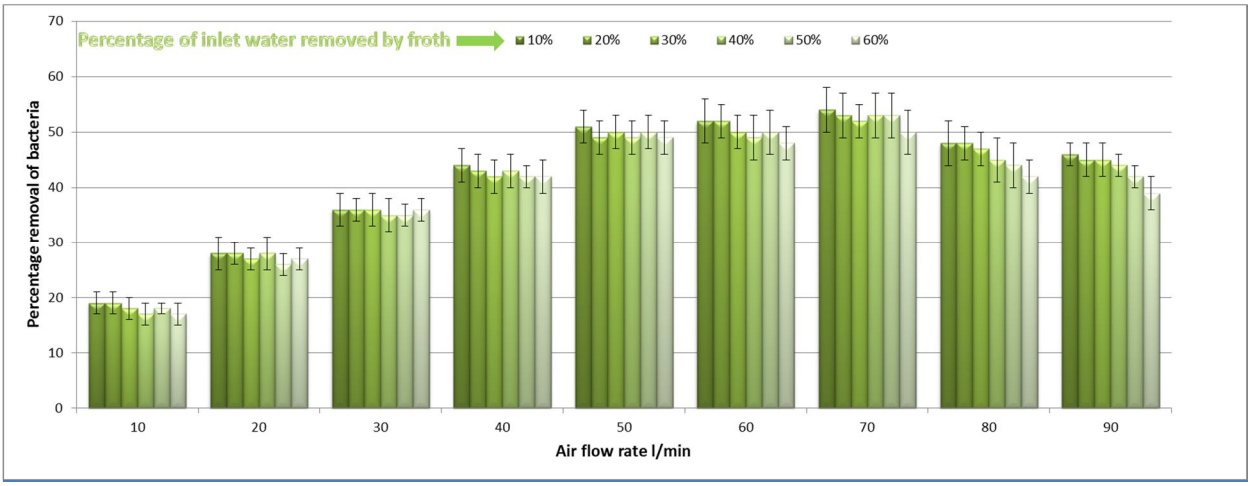

Figure 6.3: Effect of air flow rate and percentage of inlet water removed by froth on the percentage removal of bacteria (stage 1, one meter column length)

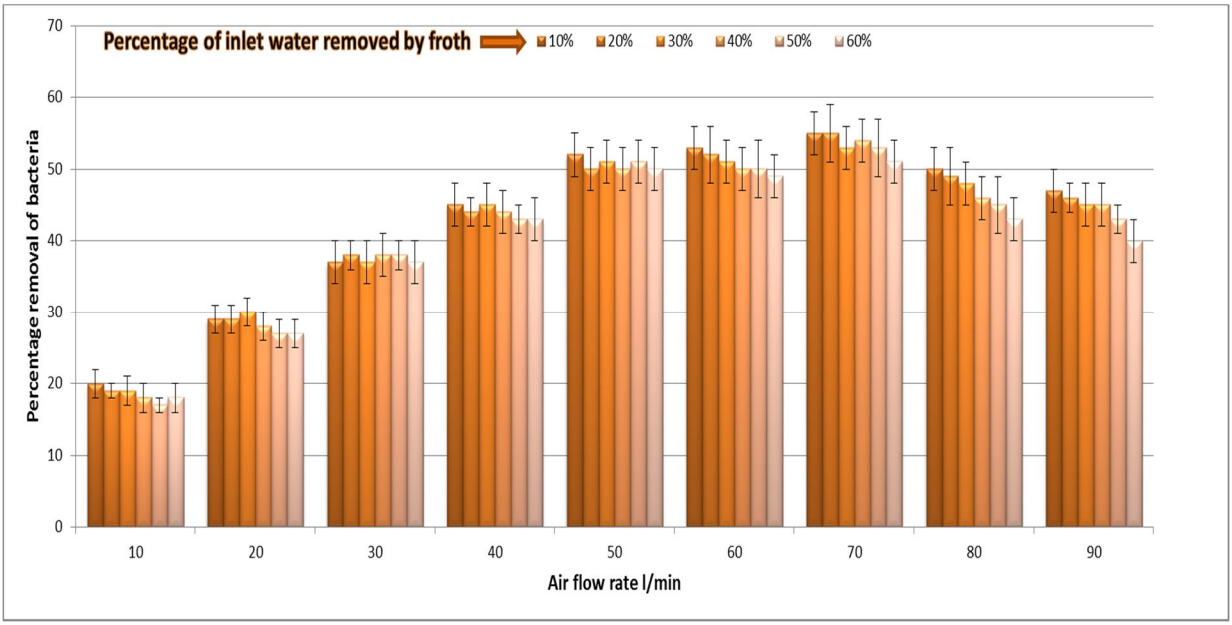

Figure 6.4: Effect of air flow rate and percentage of inlet water removed by froth on the percentage removal of bacteria (stage 2, two meters column length)

6.4.2 Semi continuous flotation system

Figure 6.5 represents the results obtained when running the experimental procedure, described in Section 6.3.3, for a semi continuous flow system. The studied variable was the air flow rate for both stages. The percentage removal of bacteria was determined. The general trend was approximately the same as trends in Sections 6.4.1 and 6.4.2.

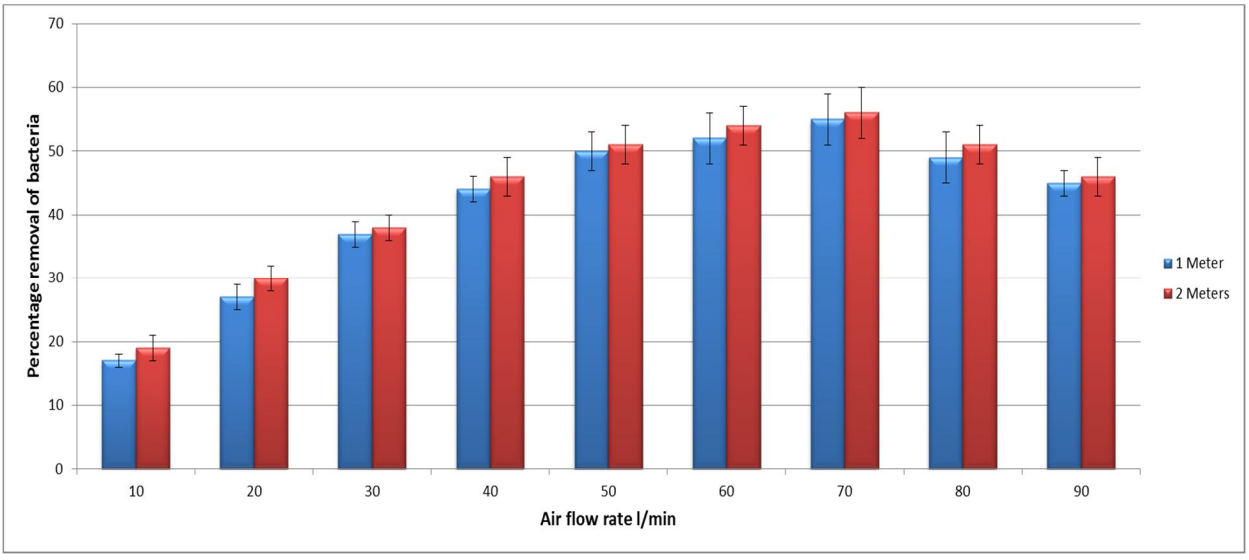

Figure 6.5: Effect of air flow rate on the percentage removal of bacteria in semi continuous system

6.4.3 Response of dead bacteria to froth flotation

Five optimum points from chapter four were selected to repeat with dead bacteria. The fixed operating parameters were, air flow rate of 130 L min^{-1}, operating time of 30 min, and the water level inside the column was 150 cm. The results are summarized in Figure 6.6. It has been shown that the average difference between chlorination and dechlorination rates was 5-10 % under the same circumstances.

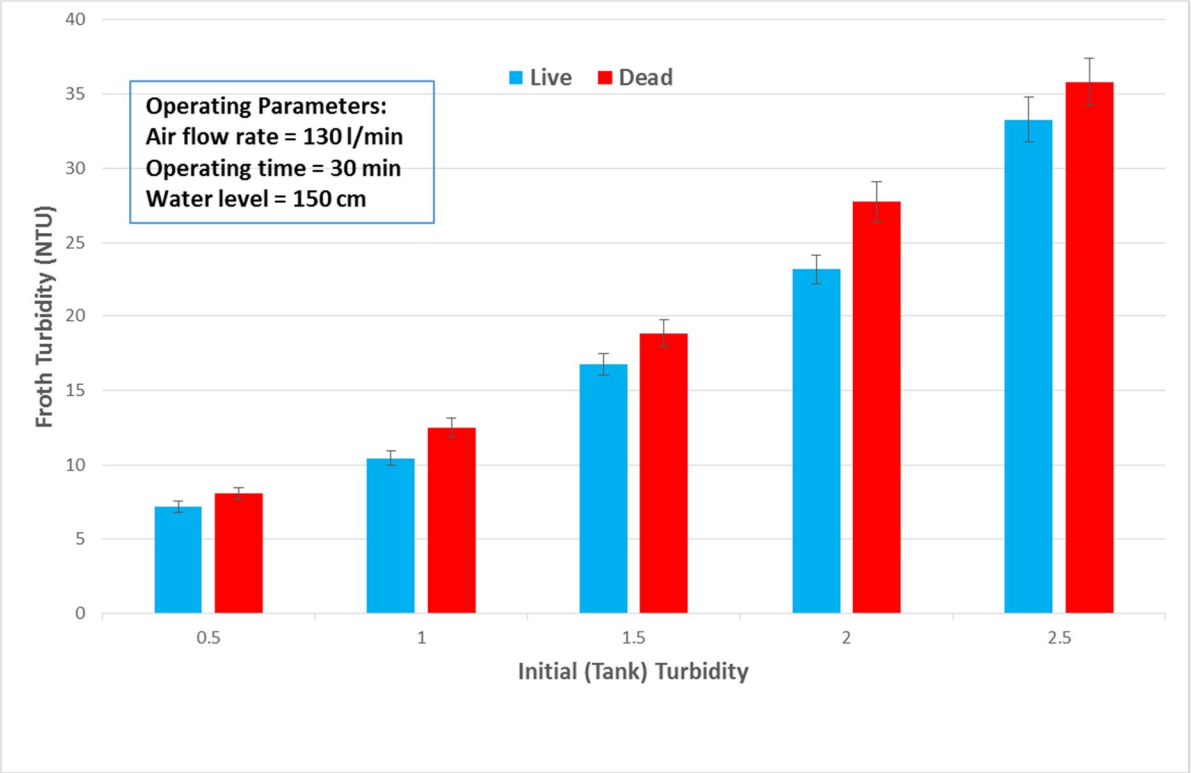

Figure 6.6: Comparison between using froth flotation with dead and live bacteria.

6.5 Discussion

6.5.1 Continuous purification process

The main aim of this work was to investigate whether it is possible to reach a suitable level of microorganism removal from water using chemical free froth flotation. The driving force for this process is hydrophobicity and most of the microorganisms in water are hydrophobic and should be suitable for removal by such a technique (Boyles and Lincoln, 1958; Rubin *et al.*, 1966; Bahr and Schugerl, 1992; Rios and Franca, 1997).

Three variables were optimized in this study, air flow rate, ratio of water removed by froth, and water column length. For the air flow rate the trend shows a decrease in downstream cfu ml^{-1} (increasing bacterial removal) with increasing air flow in the range from 10-50 L min^{-1}. This is because the increase of air pumping will increase bubbles which lead to the probability of more bubble-bacterial attachment. At higher air flow, in the range of 50-70 L min^{-1}, there was a near plateau state. This is due to the appearance of turbulence in the column and the remixing bubbles in froth back into the water bulk. Furthermore, water starts to move up and down into the froth. These "Waves" destroy the froth itself. Some water drops are not returning back to column completely but are transferred to froth collection vessel which ruins the froth concentration, hence the whole separation process. This phenomenon increases as the flow rate increases.

The turbulence is not taking place at the top of the column only but it is a bubble-water phenomenon and occurs along the whole column length. It affects the separation process directly and negatively because the process depends mainly on consolidating bubble-bacteria attachment and avoiding detachment. With high turbulence the detachment force will increase and this decreases the process efficiency.

The inlet upstream is divided into two outlet streams. The first is the purified downstream water while the second is water leaving the column top as froth. The second studied variable is the effect of the ratio of the inlet upstream that is discharged as froth on the downstream cfu ml^{-1}. If this ratio increases it will have two counteractive effects; that is, more discharged water but with less cfu ml^{-1}. The "more discharged water" should enhance the purification while the "less cfu/ml" lowers process efficiency. The sum of these two effects was tested and found to be nearly the same but opposite.

The third variable is the height of the column. It would be expected that the longer the column the greater the bacterial removal efficiency as there is more chance of bubble-bacterial attachment. The results endorse this hypothesis in direction but not in amount. Doubling the column length may be expected to double the froth bacterial concentration but in practice the longer column only slightly enhances the removal efficiency. It seems that every bubble has a certain holding capacity that cannot be exceeded. In chapter five it was found that a significant difference in froth bacterial concentration at different water column heights as the water column was increased in height. However, in that Chapter, there was no continuous discharge of the

froth. Therefore, the bacterial concentration in the froth was cumulative. In other words, if a certain bubble rises along the water column it will start attaching bacteria to some extent. Then it will rise up till reaching the froth. Once there, this bubble continues climbing up through the froth with the assistance of other bubbles which are rising up under it until it arrives at the froth top where it will burst leaving its bacteria on the froth. With no froth discharge from the column top the bacterial concentration increases within the froth. However, when there is a continuous discharge of froth there is no time for accumulating bacteria in the froth.

The result obtained here was reducing the cfu ml^{-1} by 55% of its original value. For example, for an input stream of 100 cfu/ml, the purified stream should be 45 cfu ml^{-1}.

In a previous work in Japan a separation of 80% was obtained in a 14l column which indicates that smaller columns can do the desired job as well as, or better than, larger columns (volumes in present study was 31.5 l and 63 l for columns 1 and 2 respectively) (Suzuki *et al.*, 2008). However, they were using froth flotation to remove bacteria from sea water in fish farms and not only added casein protein as frother, but seawater also contains self frothers like fish's mucus and salts. This may explain the better bacterial removal efficiency they found.

While not without cost, aeration is a conventional process used in the drinking water industry for various proposes such as the removal of volatile organic chemicals (VOCs), gases, and oxidizing dissolved metals such as Iron (Albin and Holdren, 1985; Baylar *et al.*, 2010). Depending on the purpose of aeration, in many industrial applications the air to water ratio used is close to that used in this study (Marjani *et al.*, 2009; Sales-Ortells and Medema, 2012) This suggests the economical limitations of using compressed air are already accepted in the water treatment industry. Indeed, some countries accept the higher cost of Ozonation to avoid the health issues of other biocides (EPA, 1999). Therefore, aeration basins could be modified in order to add the removal of microorganisms to the known duties of aeration. This method is clean and does not attack bacteria aggressively and drive them to produce biofilms which can harbor pathogens. Also it decreases the need for biocides, hence lowering their direct and indirect drawbacks such as disinfectant by-products. Finally, it is not a complicated technology and is easy to install and operate. The bacterial removal rate in this work reached 55% expressed as a percentage difference between input and purified streams. Using it as a solo technique for removing microorganisms is a controversial issue. Most of rivers worldwide vary in their cfu ml^{-1} for total

coliforms. Three rivers in India have been analyzed and give values between 100 - 120 cfu ml^{-1} (Rajiv *et al.*, 2012). The Foma River in Nigeria gives counts ranging from 2700 to 12300 cfu ml^{-1} (Agbabiaka and Oyeyiola, 2012). River water sources in rural Venda communities in South Africa gave a minimum and maximum of 600 cfu ml^{-1} and 37000 cfu ml^{-1} respectively (Obi *et al.*, 2003) In Myanmar samples from deep wells and dams in two urban areas; namely, Nay Pyi Taw and Yangon gave 3 to14 cfu ml^{-1} (Sakai *et al.*, 2013). For drinking water, the colony count does not necessarily equate to the health risk because humans have immunity to many bacterial species. However, for example, German drinking water regulations consider 100 cfu ml^{-1} as an acceptable limit for tap water (Bartram *et al.*, 2003). Also, an upper limit is reported to be 500 cfu ml^{-1}, though the range of 100 to 500 cfu ml^{-1} is still "questionable" (Edstrom, 2003). Therefore; industrially, the acceptance of such methodology as non-chemical froth flotation depends on the source water and desired water quality.

6.5.2 Semi continuous purification process

In Chapter four, the froth was found to be stable up to a flow rate of 130 L min^{-1} but in the experiments described in this chapter it starts to collapse after L min^{-1}., this is because the froth is working at the column top (upper edge). Here the froth structure losses the wall support and collapses into the froth collector. Also, continuous operation drives a lot of water to exit from the column top with the froth bubbles.

Some operational modifications can be suggested to avoid this problem. When the upstream inlet is shut off, the water level inside the column goes down. When this happens the column wall helps the froth to build up again. This froth will continue collecting bacteria at the same rate of continuous process but accumulatively. If the upstream inlet is only restarted when the froth reaches its maximum sustainable height, the water level will increase again and push the froth up to be discharged with minimal additional water. Once the froth is pushed out the top of the column completely the inlet can be stopped again and the most efficient cycle repeated.

The effect of this optimization on bacterial removal efficiency may not be great. However it is very useful for minimizing the ratio of disposed water with the froth. Therefore, it is recommended to use an optimized semi continuous process when water is valuable.

6.5.3 Dead bacteria separation

The aim of this experiment is to study the separation of dead bacteria by froth flotation. The results show that the dead bacteria can be separated to a greater extent that of live bacteria by a factor of 5-10%.

Live bacteria do have some independent motility, depending on the species, and have active attachment/detachment mechanisms. These results could indicate that a small proportion of live bacteria may be able to avoid attachment to bubbles.

Using froth flotation to purify water from dead bacteria can decrease the amount of any additional disinfectant required and decrease disinfectant by-products and increase water biostability. Therefore, the use of froth floatation has the double advantage of removing both live and dead cells (and, by implication, removing other particulate contaminants) (Griebe and Flemming, 1998; Castro and Neves, 2003).

The destiny of dead bacteria has received little attention in the drinking water industry. In conventional chlorination most of bacteria will die and the water is considered safe, but this is not the final word. The role of dead bacteria in the drinking water system requires further research.

6.6 Conclusions

Froth flotation is a promising technique in water industry. This study shows that some 55% of bacterial cfu ml^{-1} can be reduced by froth flotation without chemicals. Semi continuous flow gave slightly less purification efficiency but with much less water discharged with the bacterially laden froth.

Dead bacteria are removed by froth flotation in greater proportion than live bacteria. This is very important in producing clean drinking water as dead bacteria represent a secondary nutrient source which decreases drinking water biostability.

CHAPTER SEVEN

CHLORINATION AND DE-CHLORINATION OF DRINKING WATER BY FORCED AERATION

Chapter Seven

Shock Chlorination and De-Chlorination of Drinking Water by Forced Aeration

7.1 Introduction

Shock chlorination is a well-known practice in swimming pools and domestic wells. One of the limitations for using this technique in drinking water purification facilities is the difficulty of quickly removing high chlorine concentrations in water distribution systems or production facilities without side effects. A shock de-chlorination method should be introduced for producing microorganism and biocide free water.

De-chlorination using natural stagnant aeration (leaving the water to lose the chlorine naturally) is the safest known method if compared with chemical and charcoaling methods. Unfortunately, stagnant aeration is a slow process. Therefore, developing a process for accelerated de-chlorination by aeration would pave the way for using shock de-chlorination in the drinking water industry.

Forced air bubbling is a possible technique for de-chlorination but there is lack of data supporting such a process. The theory is that air bubbling has the advantages of higher mass transfer area, higher Reynolds number across the bubble water interface, and higher mass transfer concentration gradient as the bubbling presents a continuous stream of fresh bubbles. All of these factors accelerate aeration to various extents.

In this Chapter the ability of pumped air for de-chlorinating drinking water was investigated. Such information is needed for the process design of drinking water facilities. The information should help in calculating the dimensions and operating conditions of continuous de-chlorination columns.

When dealing with shock dechlorination equipment design, the designers will need both of the dechlorination and chlorination rates. Unfortunately, the chlorination rate is also an industrial gap. In conventional chlorination processes, chlorine gas is injected into a water stream. This process is sufficient as the chlorination level is low and there is a relatively long time for it to dissolve. However, shock chlorination does not have that time. This makes a comparison between chlorination by chlorine gas and dechlorination by forced aeration. More specifically,

which is faster under the same circumstances chlorination by chlorine gas or dechlorination by aeration and by how much.

The method was to select some dechlorination levels previously measured and compare them with the chlorination rate using the same experimental circumstances. When the average difference between the chlorination and dechlorination is known, it can be generalized to all points.

The final investigation is the required CT value is the value for completely killing all the microorganisms in water, not for a log reduction. A set of experiments was undertaken to collect primary data for the effect of initial bacterial concentration; in low levels, on the concentration of chlorine biocides needed to completely kill all the microorganisms. Rather than that, this will help assess the benefit of using froth flotation in water industry as a pretreatment process for reducing the bacterial concentration and hence chlorine demand.

7.2 Hypothesis

According to Fick's law:

J= -D $\frac{\partial C}{\partial x}$ …… (7.1)

Where:

J: is the diffusion flux ($\frac{mole}{m^2.s}$)

D: is the diffusion coefficient ($\frac{m^2}{s}$)

C: is the concentration gradient ($\frac{mol}{m^3}$)

x: is the length vertical to m^2 in D and C above (*m*)

Mass transfer coefficient can be expressed as (Kazim, 2012; Karimi, 2013):

(rate of mass transferred) = K (interfacial area) (concentration difference)

Or in symbols:

$Na = K_l a\ (C^*-C)$…….. 7.2

Where Na is the mole flux at the interface.

Since $Na = \frac{dC}{dt}$ 7.4

Then: $\frac{dC}{dt} = Kla\ (C^*-C)$..... 7.5

In integral form: $\int_{Co}^{C} \frac{dC}{(C^*-C_\circ)} = K_l a \int_{t_\circ}^{t} dt$.....7.6

We get: $ln\ \frac{(C^*-C)}{(C^*-C_\circ)} = K_l a\ (t-t_o)$ 7.7

Where:

$K_l a$: Liquid phase mass transfer coefficient (1/time).

C*: Gas maximum concentration that drives the mass transfer.

C: Gas concentration at time = t.

C_o: Gas minimum concentration that drives the mass transfer.

t: Time at concentration C.

t_o: Initial time.

Units should be constituent. Any concentration units can be used as the left term is dimensionless. The term $(C^* - C)$ represents the concentration difference of the gas in liquid due to bubbling process along the time (t) while the term $(C^* - C_\circ)$ is the driving force along the mass transfer interface. In the current work it is aimed to decrease chlorine concentration dissolved in water by fresh air. The proposed chlorinated water with 200 ppm Chlorine will be aerated with an assumed 0 ppm air. Also it will be assumed that the bubble climbs the water column fast enough to ignore the chlorine concentration increase due to Chlorine mass transfer from water to bubble. Therefore, the term $(C^* - C_\circ)$ will be 200 ppm in later calculations.

Therefore, to increase the moles of chlorine transferred, both of mass transfer area (a) and the concentration gradient which also represents the driving force $(C^* - C_\circ)$ should be increased. Forced aeration is suggested as a way to increase them both.

7.2.1 The role of aeration in increasing the mass transfer area

Chlorine is transferred from water to air through the contact area between air and water. In stagnant aeration the chlorine is only transferred from the upper surface of the water. Increasing

the air water interface will increase chlorine transfer and decrease the time to reach the desired level of dechlorination.

Consider a cube of chlorinated water with a length of (r).

Assume that the upper face of the cube is the mass transfer area for chlorine; which equals to $A=r^2$ as happens in stagnant aeration.

Assume also there is a spherical air bubble of radius (r) inside the cube as in figure (7.1A). The surface area of this bubble will be $4\pi r^2$.

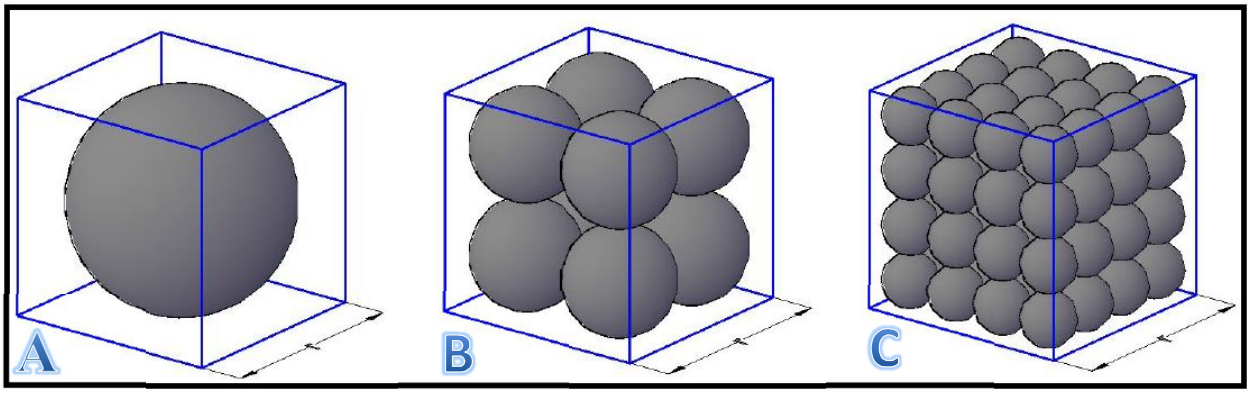

Figure 7.1: Hypothesis graphical illustration

The total surface area available for mass transfer will be the sum of upper face plus the bubble surface areas ($r^2 + 4\pi r^2$). The percentage increase in surface area can be expressed as:

$$\% \text{ surface area increase due to aeration} = \frac{New\ mass\ transfer\ area}{Original\ mass\ transfer\ area} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots 7.8$$

$$\text{Or } \% \text{ surface area increase due to aeration} = \left(\frac{r^2 + 4\pi r^2}{r^2} \times 100 \right) = 1357\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 7.9$$

Assume also there are 8 bubbles of a radius (0.5r) inside the cube as in figure (7.1B). The total percentage increase in surface area will be:

$$\% \text{ surface area increase due to aeration} = \left(\frac{r^2 + 8(4\pi\ (0.5r)^2)}{r2} \times 100 \right) = 2613\% \quad \ldots\ldots\ldots\ldots\ldots 7.10$$

Finally, assume there are 64 bubbles of radius (0.25r) inside the cube as in figure (7.1C). The total percentage increase in surface area will be:

% surface area increase due to aeration = $\left(\frac{r^2 + 64\left(4\pi\,(0.25r)^2\right)}{r2} \times 100\right) = 5127\%$ …….. 7.11

Or in general, $\%\ surface\ area\ increase\ due\ to\ aeration = \frac{r^2 + x^3\left(4\pi\left(\frac{r}{x}\right)^2\right)}{r2} \times 100$ ……..7.12

Where: x is the number of bubbles that lined adjacently along the length of cube line (r). Or it is the cubic root of the bubbles inside the tubes.

Simplifying by eliminating (r) to get:

$\%\ surface\ area\ increase\ due\ to\ aeration = 1 + x^3 4\pi\left(\frac{1}{x}\right)^2 \times 100$ ……..7.13

Or:

$$\%\ surface\ area\ increase\ due\ to\ aeration = (1 + 4\pi x) \times 100 \quad \text{…7.14}$$

Equation 7.14 is valid for fully packed water cubes with bubbles. As spaces among bubbles increases the error margin increases. Also it is valid for bubbles of equal volume; hence, every variation in bubble size range will also increase the error margin.

This equation can be represented graphically to get the plot in figure 7.2.

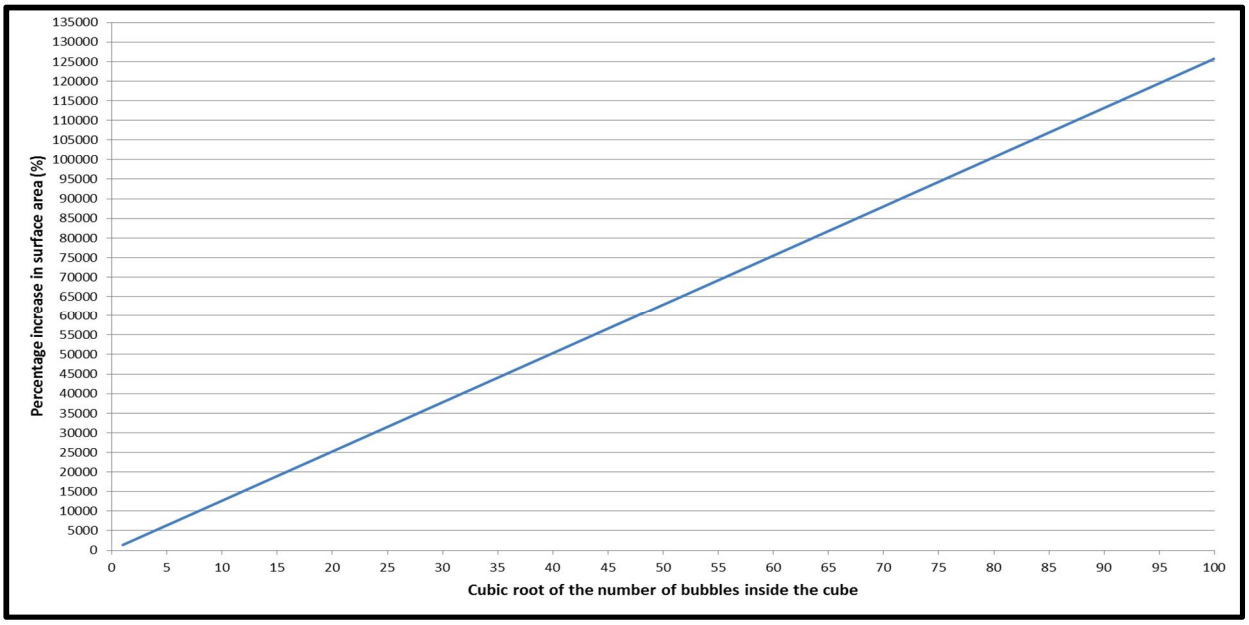

Figure 7.2: The graphical representation of the relation between the number of bubbles inside a cube and the percentage increase in surface area

(Lu *et al.*, 1999) made a set of experiments investigating the effect of the ratio of surface area to volume (S/V) of chlorinated water on chlorine decay "figure 7.3". They suggested that a 1500% increase in surface area will reduce chlorine level to a value of approximately one fifth of its original level. These tests as well as the conclusion in figure 7.2 encourage the use of air bubbling for dechlorination.

7.2.2 The role of aeration in increasing concentration gradient

When a fresh air bubble rises through chlorinated water, chlorine is transferred from water to air bubble driven by the concentration gradient (dC). The bubble at the surface of the sparger has the least chlorine concentration, hence the greater concentration gradient.

The average velocity of a rising bubble in water column is approximately (20-30) cm/sec (Sharifullin and Luebbert, 2001; Chen, 2004). Therefore, as the chlorine will not have the time to reach saturation in the bubble then the concentration gradient continues to be near its maxima. The greater the bubble velocity the greater the concentration gradient during its travel.

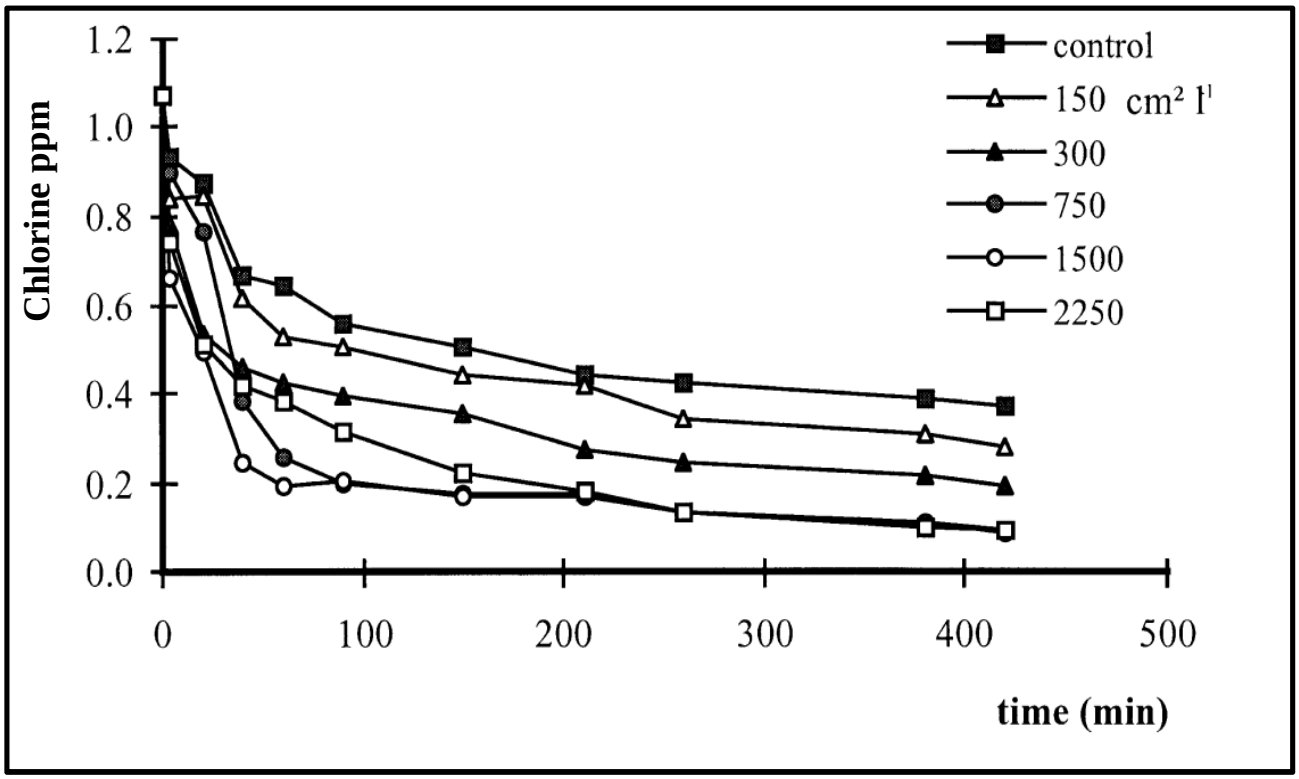

Figure 7.3: Effect of surface area to volume on chlorine mass transfer rate. (Lu et al., 1998)

7.2.3 Effect of turbulence

At higher Reynolds number the boundary layer thickness increases, hence lower resistance to mass transfer. The higher decrease in mass transfer resistance occurs when the Reynolds number jumps from a laminar to turbulent flow regime. Therefore, many mass and heat transfer (such as car radiators and air conditioners (Bird *et al.*, 2007; Incropera and Dewitt, 2007).

Neglecting the jet effect and turbulence resulting from air leaving the sparger, for an air bubble rising in a column by buoyancy only, its Reynolds number can be expressed as (Scheild *et al.*, 1999):

$$Re_b = \frac{\rho_L u_b d_b}{\mu_L} \quad \text{..... 7.15}$$

Where:

Re_b: is Reynolds number for a bubble rising in a liquid (dimensionless).

ρ_L: is the liquid density (kg/m^3).

d_b: is the diameter of bubble (m).

μ_L: liquid viscosity ($Pa.s$).

Values of Re_b over 500 are considered as turbulent (Lau *et al.*, 2012). Assuming the average bubble size along the column is 1 cm and the average velocity is 25 cm s^{-1}, Re_b gives the value of 2500. With this Reynolds number there is a big leap in chlorine transfer from water to bubble when using forced aeration instead of conventional stagnant aeration.

7.3 Materials and methods

7.3.1 Aeration system

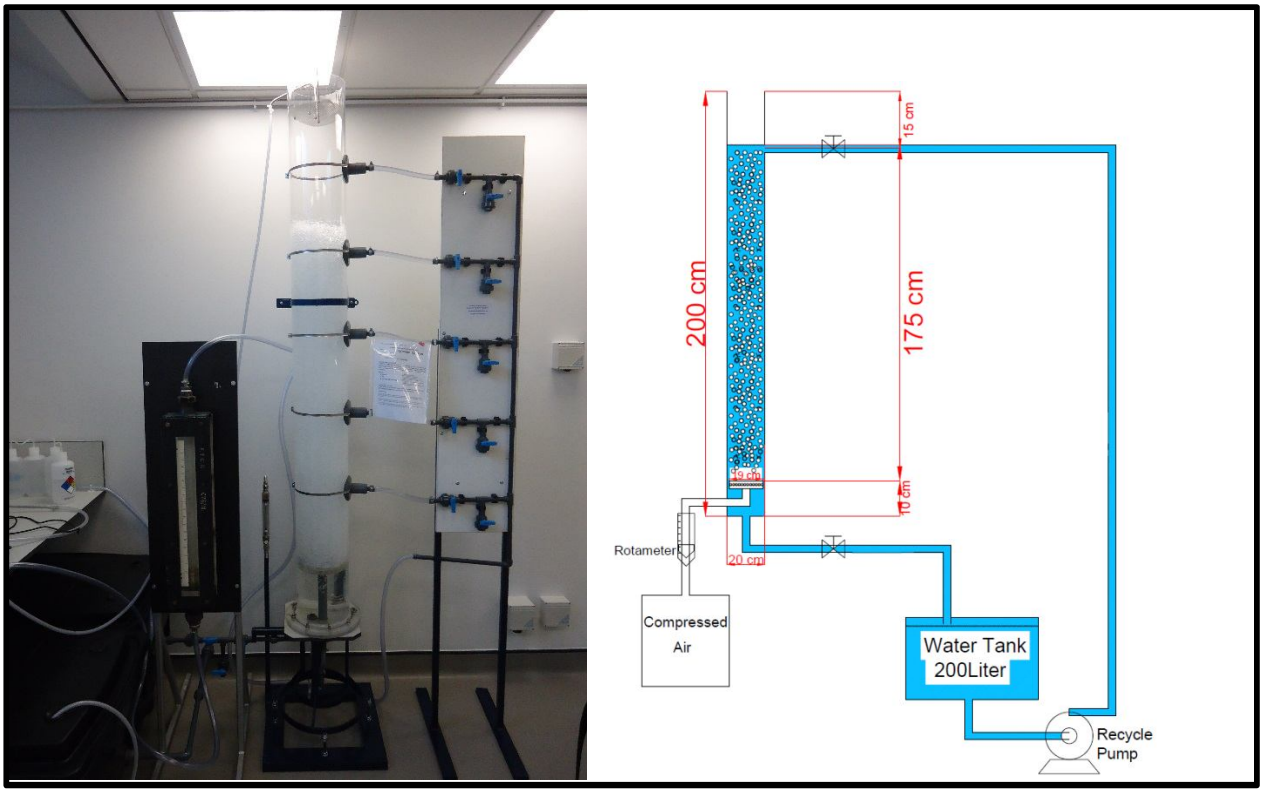

Figure 7.4: Experimental set up

A compact aeration column system 1 m long was designed and built. The main part is a transparent Perspex (Poly (methyl methacrylate)) tube, 20 cm inside diameter. A ceramic sparger, 19 cm diameter, with 50 micron holes size (from HP technical ceramics) is fixed 10 cm above the column base. The sparger is joined to a 15 mm diameter tube connected to a compressor with a rotameter (10-900 L min^{-1}) or (0-10 L min^{-1}) depending on run demands. A 200 liter tank is installed beside the system to collect distilled water from a still and provide a reservoir to the column. The working capacity of column is approximately 50 liters and 63 liters when totally filled. The assembly is fixed on a steel rig (Figure 7.4).

7.3.2 Chlorine

Three chlorine sources were used in this work:

1- Trichloroisocyanuric acid tablets obtained from Acti.

2- Sodium hypochlorite obtained from SIGMA-ALDRICH (assay of 10%).
3- Chlorine gas: This is synthesized in the lab through reaction between trichloroisocyanuric acid and hydrochloric acid (37 M); obtained from SIGMA-ALDRICH, according the following equation:

$$C_3Cl_3N_3O_3 + 3HCl \rightarrow C_3H_3N_3O_3 + 3Cl_2$$

The produced chlorine gas is dissolved in distilled water using the system in figure 7.5. A peristaltic pump (Watson Marlow, Model - 505S, range 2 - 220 rpm) was used for transferring chlorine gas from the reaction bottle to saturation bottle then circulating it back to the first bottle. The reaction is implemented under standard atmospheric conditions. 100 grams of trichloroisocyanuric acid tablets were placed in the reaction bottle then 5 litres of distilled water was added. Then 20 ml HCl was added to the mixture and left till the water turns yellow. Using more or less amounts of the chemicals is not dangerous as the reaction is not fast especially with the presence of this relatively large amount of water. Finally, the peristaltic pump is started to transfer the released chlorine gas from reaction bottle to saturation bottle. While he increase in chlorine concentration in the saturation bottle can be estimated roughly by yellowish water, samples were taken every 10 minutes for analysis until the desired concentration was reached.
To compare between chlorination and dechlorination rates, chlorination samples were taken every 15 minutes till reaching the first value over 200 ppm.

For the dechlorination rate the same should be followed but with changing the input gas from chlorine to air. Also, samples every 15 minutes should be taken to reach zero ppm chlorine.

7.3.3 Chlorine meter

Two chlorine meters were used in this study. The first was exact®Z Obtained from Industrial Test Systems, Inc. (ITS). The second was ExStik® obtained from EXTECH instruments, model number of CL200. Both meters are self-calibrated. In addition of that they are provided with standards for external calibration.

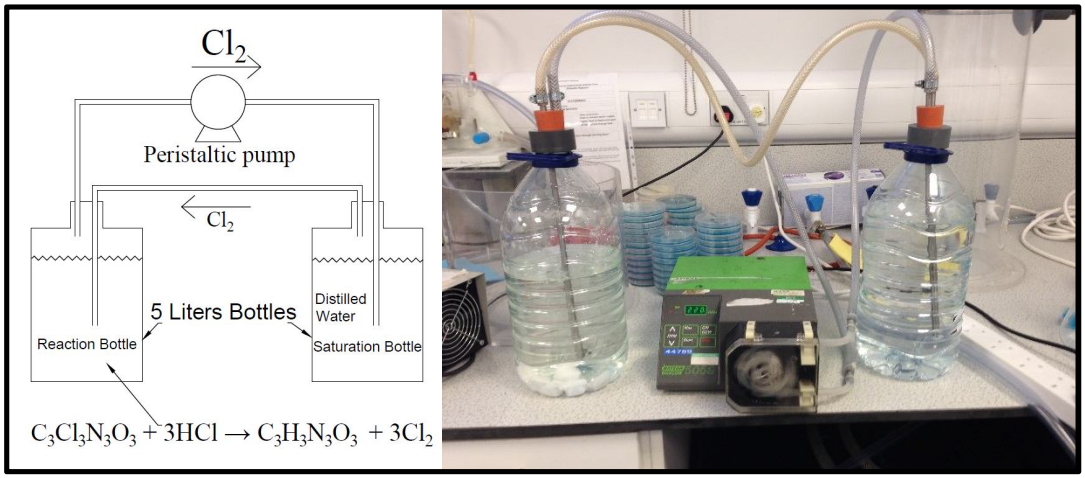

Figure 7.5: Chlorine production and water chlorination system

7.3.4 pH meter

The pH meter used in this work obtained from AZ® with the model number of 8685. The device was calibrated using three pH standard buffer solutions of the values of 4.0, 7.0 and 10 obtained from VWR international and manufactured by MERCK MILLIPORE.

7.3.5 Experimental procedures

7.3.5.1 De-chlorination

1- Prepare 5 litters of 2000 ppm chlorine water solution in a separated bottle.
2- Fill the column with 45 liters of distilled water.
3- Add saturated chlorine solution obtained from step 1 gradually to the column with mixing to get 200 ppm.
4- Start air pumping at 1 l/min.
5- Start taking samples every 1minute for low range or 2 minute for high range, for 30 minutes for analysis.
6- Stop air pumping and drain the system.
7- Repeat steps 1 through 6 for every new air flowrate.

7.3.5.2 Effect of initial cfu ml-1 on chlorine demand

All the experimental materials and equipment such as turbidity meter, nutrient broth, Agar plates, *E-coli K-12* strain, and saturated chlorine solution are the same as described in chapters 4, 5 and 6. The experimental method is as follows:

1- Prepare one liter of nutrient broth cultured with *E-Coli* and incubated for 24 hours and 37 C^o. Check for turbidity to make sure that the cfu ml^{-1} is approximately 10^8.
2- Prepare 10 dilutions of this nutrient broth (100000, 50000, 12500, 6250, 3125, 1562, 781, 390, 195 and 97). Each dilution is with 10 replicates, 100 ml each.
3- Using the procedure and chlorination system in 6.3.2 Prepare an approximate 400, 200, 100, 50, 25, 12, 6, 3, 1.5, 0.75 ppm chlorine solution. Each one is with 10 replicates, 100 ml each.
4- Prepare 100 flasks every one containing 1L of autoclaved nutrient broth.
5- Mix every sample in step 2 with a sample in step 3 as in the table 7.1. Note that when the mixing is occurred, both the concentrations of nutrient broth and chlorinated water will drop to half as a result of mixing.

Table 7.1: Samples mixing, every cell represents ($\frac{Chlorine\ ppm}{Bacterial\ cfu/ml}$)

400/100000	400/50000	400/25000	400/12500	400/6250	400/3125	400/1562	400/781	400/390	400/195
200/100000	200/50000	200/25000	200/12500	200/6250	200/3125	200/1562	200/781	200/390	200/195
100/100000	100/50000	100/25000	100/12500	100/6250	100/3125	100/1562	100/781	100/390	100/195
50/100000	50/50000	50/25000	50/12500	50/6250	50/3125	50/1562	50/781	50/390	50/195
25/100000	25/50000	25/25000	25/12500	25/6250	25/3125	25/1562	25/781	25/390	25/195
12/100000	12/50000	12/25000	12/12500	12/6250	12/3125	12/1562	12/781	12/390	12/195
6/100000	6/50000	6/25000	6/12500	6/6250	6/3125	6/1562	6/781	6/390	6/195
3/100000	3/50000	3/25000	3/12500	3/6250	3/3125	3/1562	3/781	3/390	3/195
1.5/100000	1.5/50000	1.5/25000	1.5/12500	1.5/6250	1.5/3125	1.5/1562	1.5/781	1.5/390	1.5/195
0.75/100000	0.75/50000	0.75/25000	0.75/12500	0.75/6250	0.75/3125	0.75/1562	0.75/781	0.75/390	0.75/195

6- After one minute take 1 ml sample of every bacterial broth-chlorinated water mixture and add it to the 1 L of the nutrient broth prepared in step 3 then incubate for 24 hours and 37 C^o.

7- Every sample has bacterial growth is failed. Every sample continues being clear is successful.

8- The whole experiments are repeated 3 times for statistical purposes.

7.4 Results

Results were expressed as chlorine decay in ppm as a response of pumping time in minutes and air flowrate in L min^{-1}. Two ranges of air flowrates were investigated; the first was a high range (10 – 50) L min^{-1} with an interval of 10 L min^{-1} while the second was (1-9) L min^{-1} with an interval of 1 l/min which represented the fine tuning of the first range.

7.4.1 Chlorine decay without air pumping (control)

For the control chlorinated water was put in the column at the same concentration used in the full experiments and left to evaporate naturally. The results are summarized in Figure 7.6. Chlorine ppm was decreased with time for the three chlorine sources used.

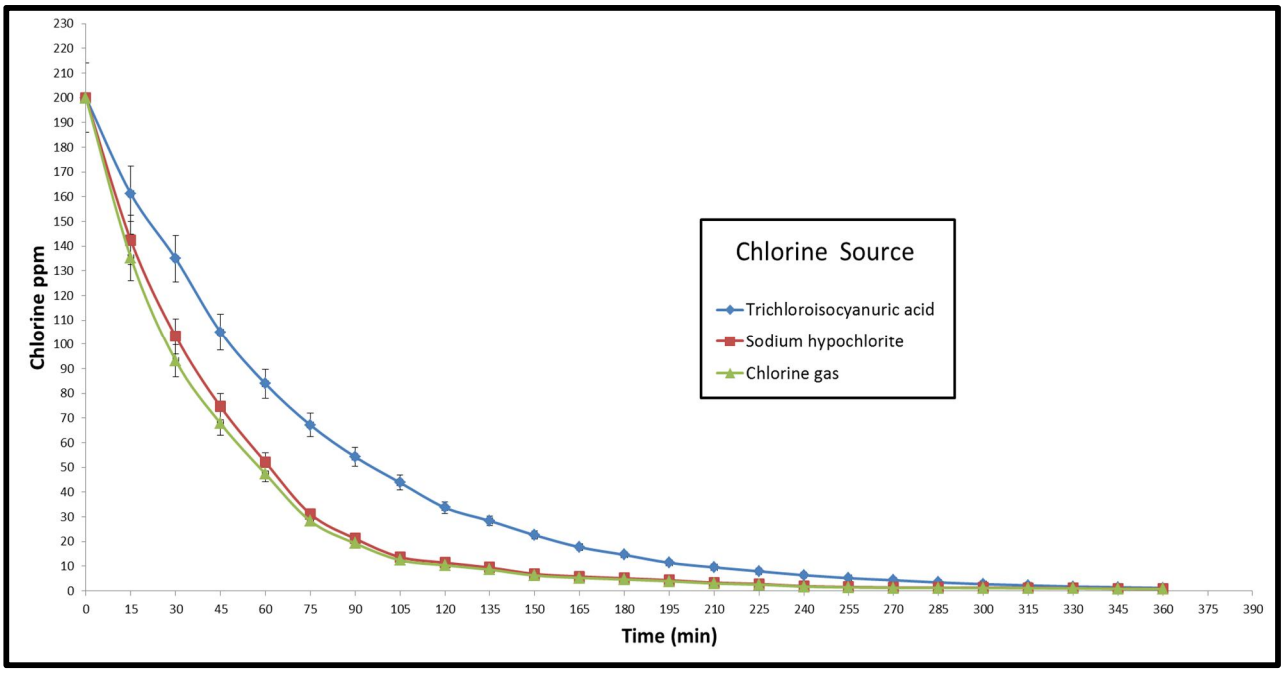

Figure 7.6: Natural stagnant aeration rate (control)

7.4.2 De-chlorination of chlorine obtained from trichloroisocyanuric acid

In these experiments, the saturated chlorine solution in step 1 in Section 7.2.5 was prepared using trichloroisocyanuric acid (200 ppm). The results are summarized in figures 7.7 for low and 7.8 for high ranges of air flowrates. The general trend of the chlorine ppm showed a decrease with time for both low and high flowrates ranges. Chlorine ppm depleted faster as air flowrate increased. These trends are the same for all the studied chlorine sources.

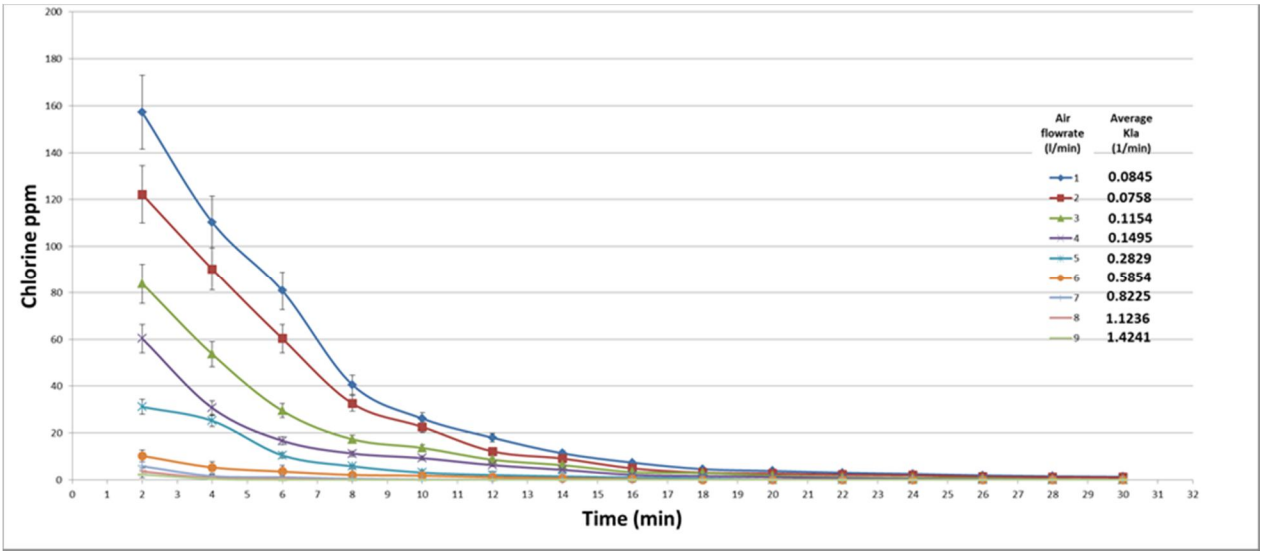

Figure 7.7: Effect of low range air flowrate on chlorine decay when using trichloroisocyanuric acid as chlorine source

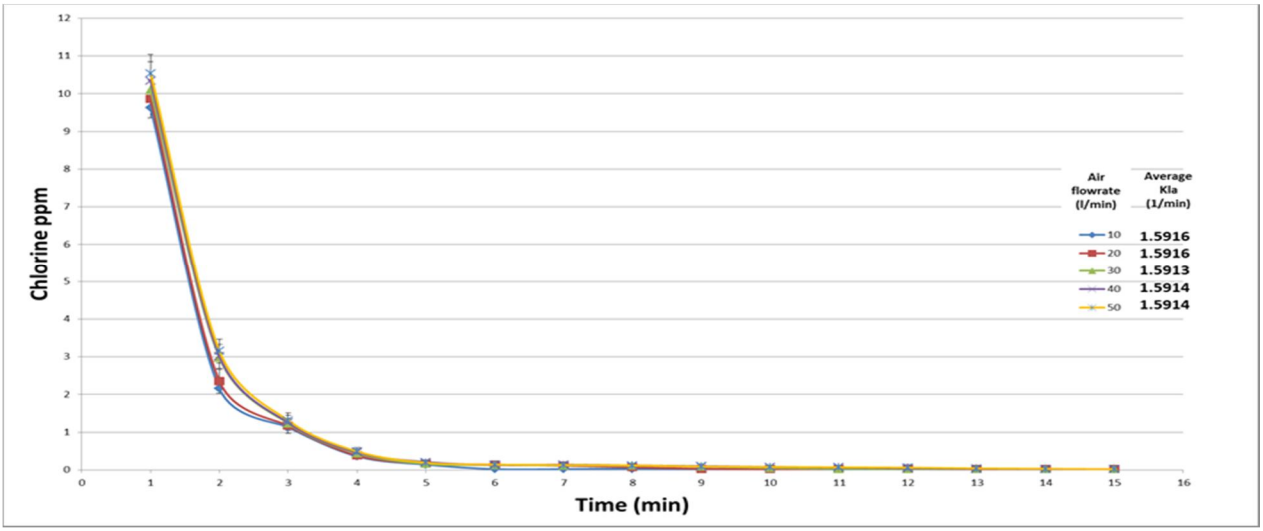

Figure 7.8: Effect of high range air flowrate on chlorine decay when using trichloroisocyanuric acid as chlorine source

7.4.3 De-chlorination of chlorine obtained from Sodium hypochlorite

In these experiments the saturated chlorine solution in step 1 of the method given in section 7.2.5 was prepared using sodium hypochlorite (200 ppm). The results are summarized in figures 7.9 for high and 7.10 for low air flowrates.

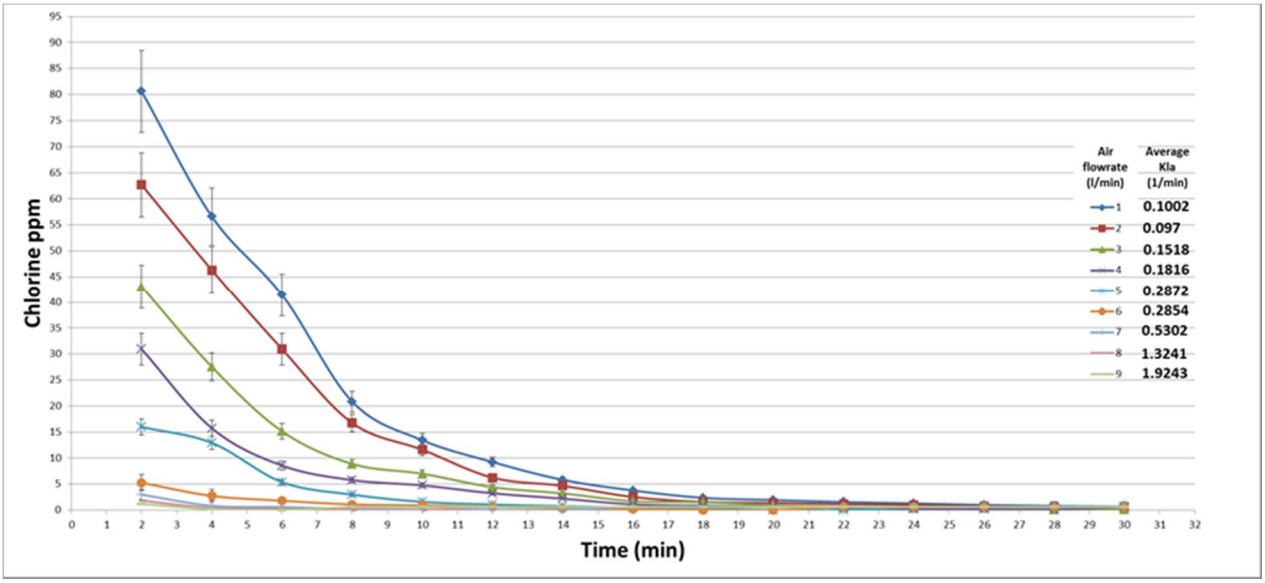

Figure 7.9: Effect of low range air flowrate on chlorine decay when using sodium hypochlorite as chlorine source

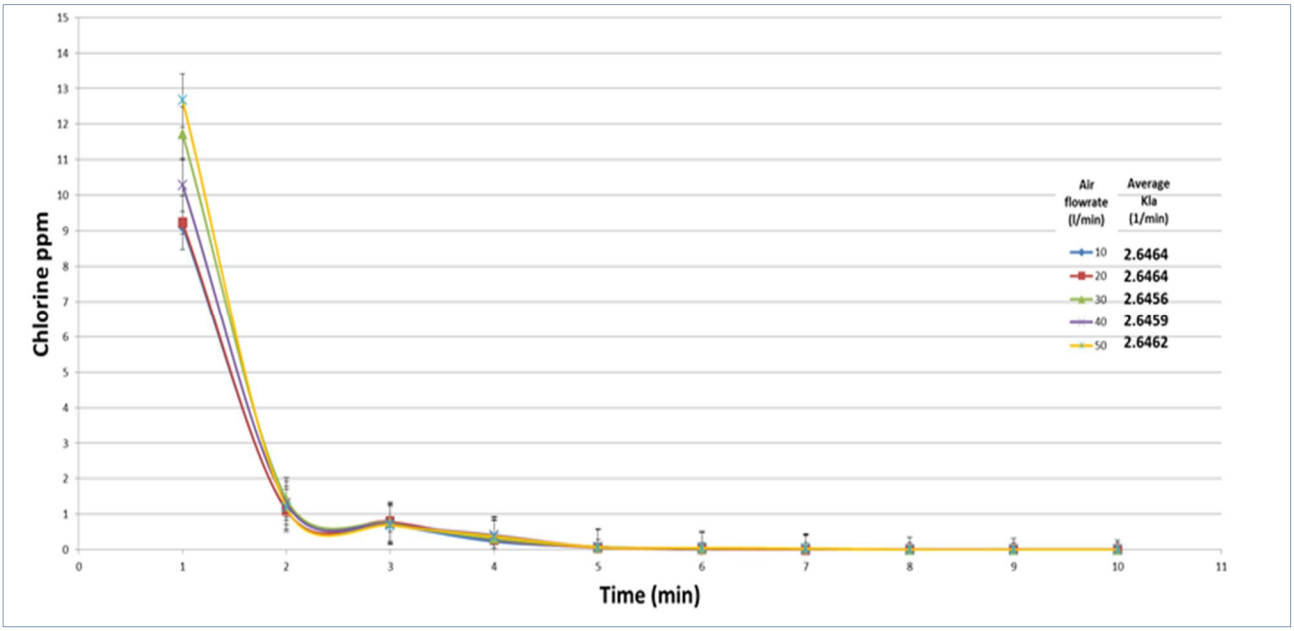

Figure 7.10: Effect of high range air flowrate on chlorine decay when using sodium hypochlorite as chlorine source

7.4.4 De-chlorination of chlorine obtained from Chlorine gas

In these experiments the saturated chlorine solution in step 1 of the method shown in section 7.2.5 was prepared using chlorine gas produced using the system in figure 7.4 and following step 3 in 7.2.2 (200 ppm). The results are summarized in figures 7.11 for low range and 7.12 for high air flowrates.

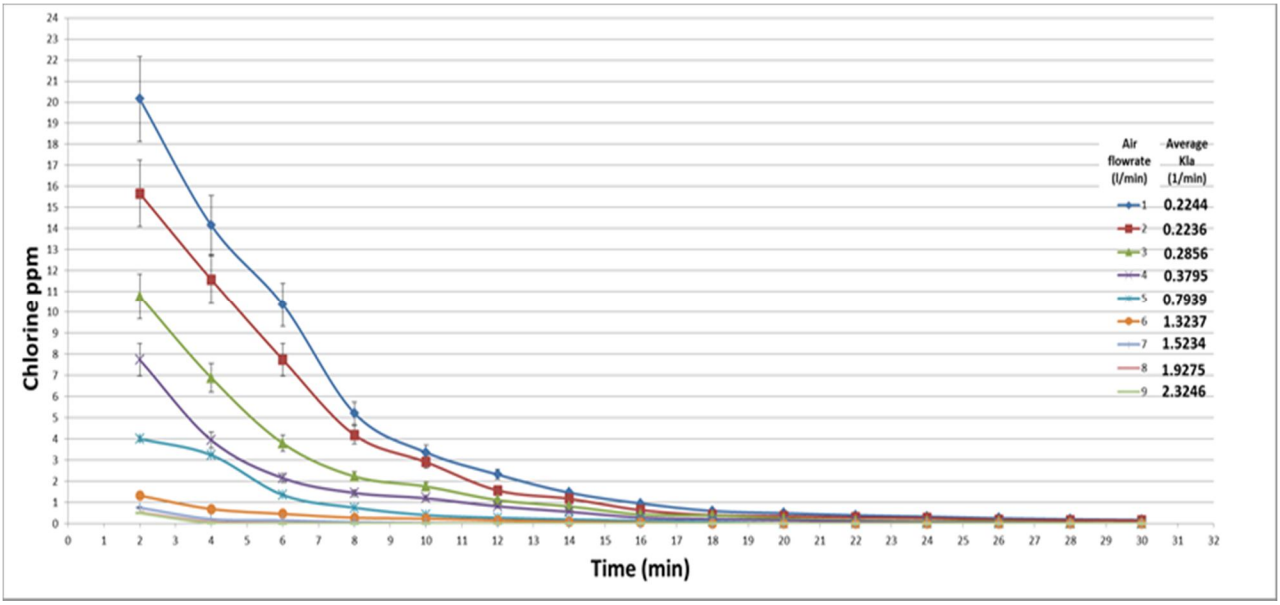

Figure 7.11: Effect of low range air flowrate on chlorine decay when using chlorine gas as chlorine source

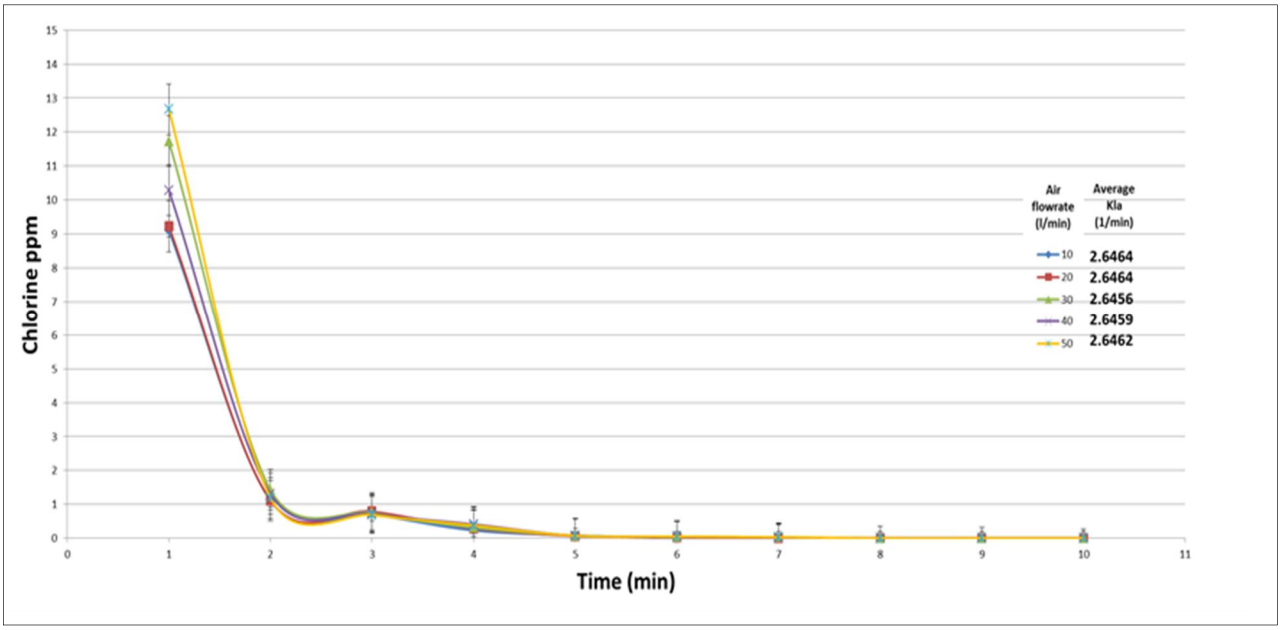

Figure 7.12: Effect of high range air flowrate on chlorine decay when using chlorine gas as chlorine source

7.4.5 Effect of air flow rate on mass transfer coefficient ($K_l a$)

The average values of *Kla* are plotted against air flow rate. Figures 7.13 and 7.14 summarize the results.

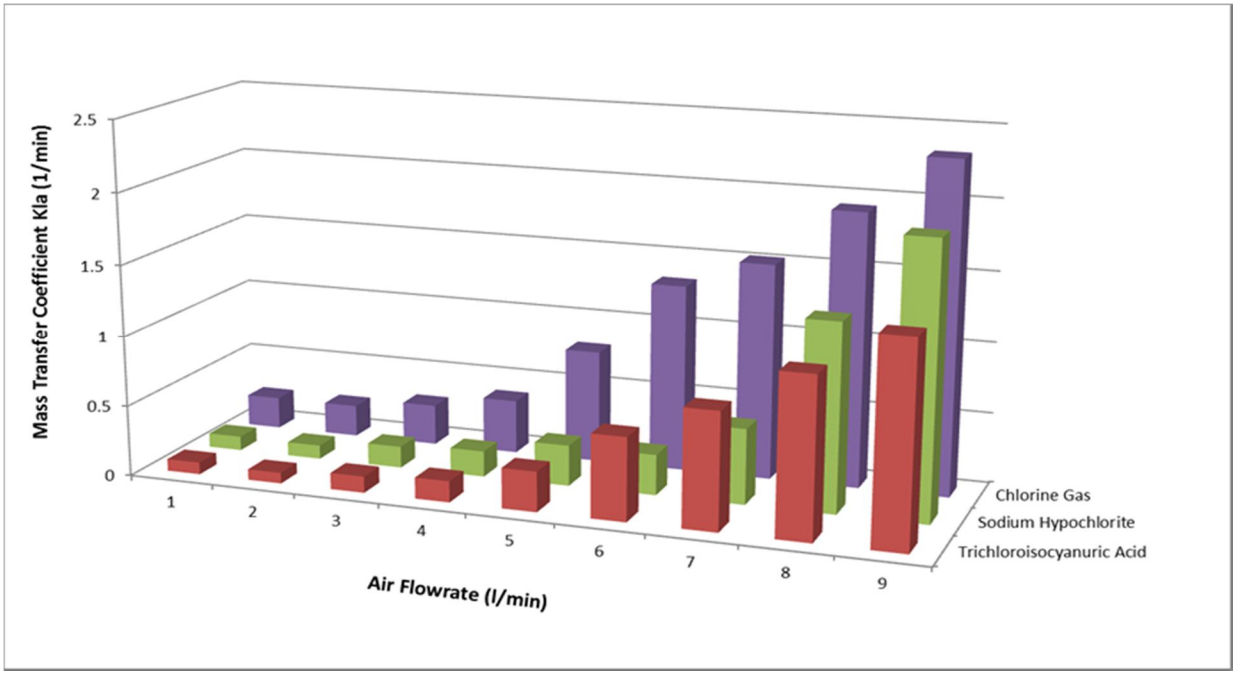

Figure (7.13): Effect of air flowrate on mass transfer coefficient $K_l a$ (Low range flowrate)

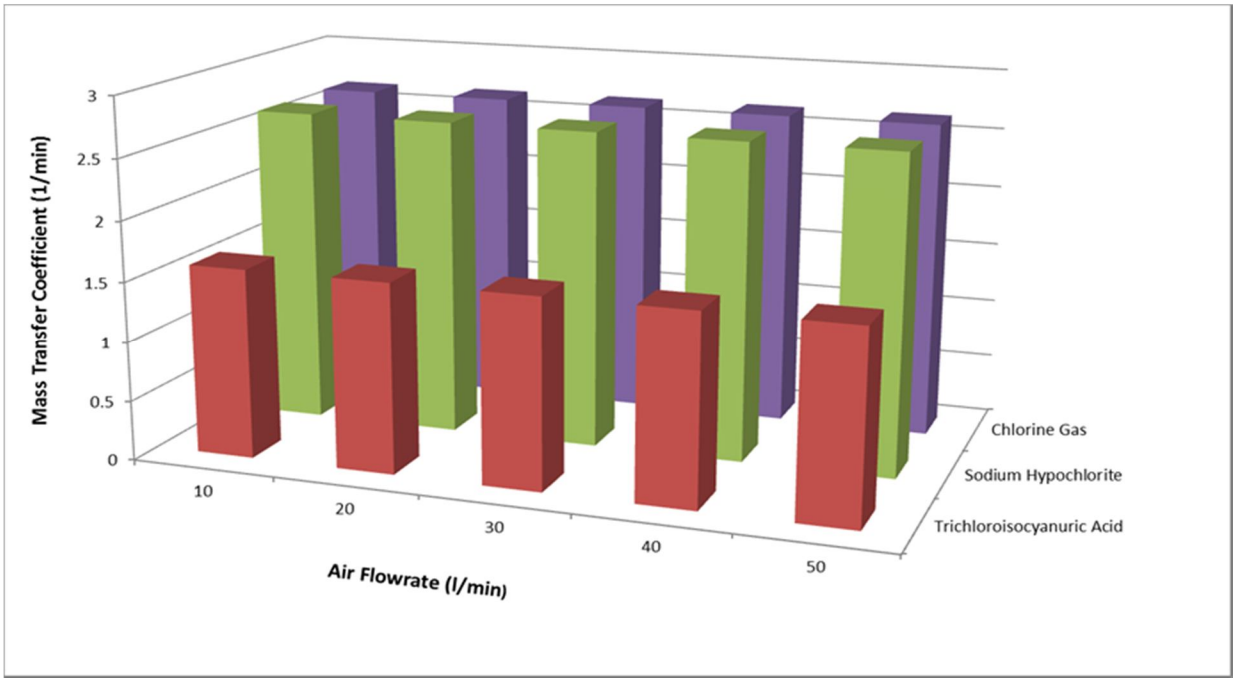

Figure (7.14): Effect of air flowrate on mass transfer coefficient $K_l a$ (High range flowrate)

7.4.6 Effect of chlorination on water pH

In all the experiments of the three chlorine sources, pH was monitored to give the results in figure 7.15. As chlorine concentration decreased as pH went close to 7.

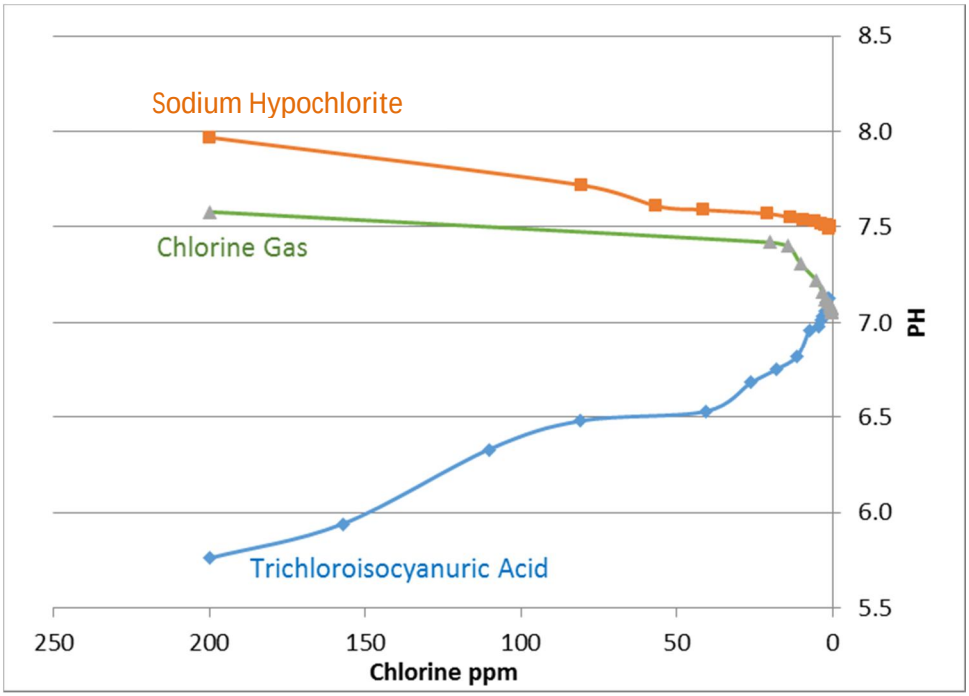

Figure 7.15: pH variation in shock chlorination for the three chlorine sources

7.4.7 Shock chlorination rate

Results are expressed as ppm reduction for dechlorination and ppm increase for chlorination during the same time intervals. Every bar is the net difference between one reading and the next one.

To make a fair comparison, the chlorination values near to zero ppm are compared with the dechlorination values near 200 ppm because at these two values the driving forces are at their maxima. Figure 7.16 shows the results. It is shown that the chlorination is faster than dechlorination rate by an average of 25%.

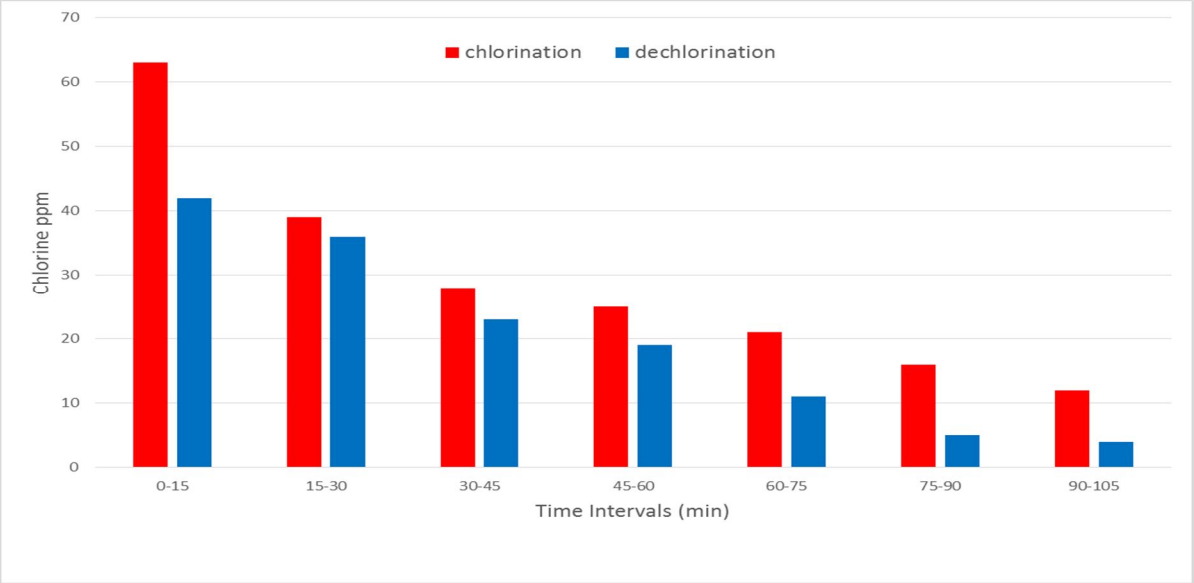

Figure 7.16: Comparison between chlorination and dechlorination rate

7.4.8 Effect of initial cfu ml^{-1} on chlorine demand

The results are expressed according to two outputs. If the sample shows bacterial growth and looks turbid to the naked eye, it is marked as "failed". If the sample shows no-bacterial growth and looks clear to the naked eye, the sample is "Successful". Table 7.2 illustrates the results.

Table 7.2: The obtained results, green = no growth = successful, red = growth = fail

		Chlorine ppm									
		200	100	50	25	12	6	3	1.5	0.75	0.37
Initial cfu/ml	50000										
	25000										
	12500										
	6250										
	3125										
	1562										
	781										
	390										
	195										
	97										

7.5 Discussion

7.5.1 The dechlorination rate

The aim of this study was to investigate increasing the release of dissolved Chlorine from water by increasing the mass transfer area, concentration gradient and turbulence using aeration. This hypothesis is supported and data for such a process is given.

Chlorine is widely used in water industry. Changing away from the use of chlorine would be expensive (Powell, 2010; Fawell, 2014). Therefore, it is better to keep using disinfection by chlorination and trying to avoid the disadvantages.

The perfect drinking water system should be free of biological contaminants and free of chemicals, especially biocides. The results show that shock chlorination and shock dechlorination by aeration can produce such water. The proposed purification process aims to kill the entire microorganism load with a high dose of chlorine then removing this chlorine by aeration.

The findings of this study pave the road for using shock chlorination followed by shock dechlorination for producing high quality drinking water. Dissolved chlorine can easily be removed from water by forced aeration as it provides three advantages over stagnant aeration. First is the significant increase in mass transfer area as shown in Figure 7.2. Second is the increase in mass transfer boundary layer turbulence between air bubble and bulk water. The third is the increase in mass transfer concentration gradient along the water-bubble interface.

In this study, three sources of chlorine were examined for their response to dechlorination by aeration. The first was trichloroisocyanuric acid which is used frequently in swimming pools due to its reaction with water to produce cyanuric acid which is considered as a chlorine stabilizer that prevents chlorine from volatizing that may result from high surface area with respect to water mass in swimming pools (Harnvajanawong et al., 2004). The second is sodium hypochlorite (bleach). It is the most used chlorine source in all applications as a result of its abundance, cheapness and ease of use. The third was chlorine gas.

The results from all three chlorine sources show the general trend is relatively fast chlorine removal from water but they do differ slightly in removal rate, the final steady state

concentration and how fast they reach this steady concentration. For trichloroisocyanuric acid the chlorine removal was optimum at 9 L min^{-1} of air and needed 6 minutes to reach the minimum steady concentration. For the low flowrate range the results obeyed the hypothesis that assumes more air pumping leads to faster chlorine removal. For the high range over 10 L min^{-1} the removal rate did not increase as a response to an increase in air flowrate. This because of "the column effect"; that is, in a column, at high air flow rates and when the froth starts to form there will be a bubble "traffic jam" along the water column and bubbles increase in size as they rise. These two factors drive bubbles to be adjacent to each other reducing the total surface area for mass transfer as well as losing the turbulence along the bubble-water interface. Therefore, at higher air flowrates further increasing air flowrate had two counteractive effects; first, is increasing the three driving forces of aeration "increasing surface area, increasing turbulence, and increasing concentration gradient" while the opposite is "the column effect". The more the air flowrate, the more the domination of column effect and the less chlorine transfer.

In chapter four, it was concluded that, in an air-water bubbling system, the column effect should not start effectively before 50 L min^{-1} but in this work it started after 10 L min^{-1}. This is because the relatively high concentrations of trichloroisocyanuric acid and sodium hypochlorite seem to work as frothers which stimulate the column effect at lower air flow.

It was also noticed that in aerating trichloroisocyanuric acid chlorine removal is lower when compared with sodium hypochlorite and chlorine gas. Furthermore, the chlorine concentration did not reach zero even with a long aeration time and stuck on 0.02 ppm. This is because of the stabilizing effect of cyanuric acid that is produced from the reaction of trichloroisocyanuric acid and water (Harnvajanawong *et al.*, 2004).

Using trichloroisocyanuric acid in shock chlorination would give many problems and limitations. Concentration of cyanuric acid, formed when trichloroisocyanuric acid is added to water, above 30 ppm leads to a phenomenon called "chlorine lock" in which cyanuric acid locks the ability of chlorine to kill bacteria. Hypochlorous acid is another product from trichloroisocyanuric acid and water which decreases the pH to a level unacceptable for drinking water (Harnvajanawong *et al.*, 2004).

For the sodium hypochlorite solution the dechlorination was faster than trichloroisocyanuric acid. Sodium hypochlorite has also the ability for reaching zero chlorine level. It seems that the acidic pH; not the presence of cyanuric acid alone hinders the dechlorination.

Shock chlorination using trichloroisocyanuric acid and sodium hypochlorite is known in the treatment of swimming pools and domestic wells. Using either of them is not a recommended practice in drinking water production for many reasons. First, is because of corrosion, especially when the construction material is cast iron. Second, is the difficulty of lowering chlorine level across the distribution system naturally. Third, there will be reaction residuals lead to a significant unacceptable effect on water pH away from the neutral value of 7. Therefore, using chlorine gas is the recommended method for shock chlorination and shock dechlorination because there will be no residual chemicals after the dechlorination.

Chlorination by chlorine gas is usually implemented by injecting it through water pipes. For shock chlorination this is not enough even though chlorination with chlorine gas is known to be fast. Also, releasing high doses of chlorine to the air through the shock dechlorination process implies a high health risk on the people that may live near water treatment facilities. Therefore, a new design for recycling chlorine in shock chlorination equipment is needed. Figure 7.5 gives a preliminary idea for such design. In chapter eight there is a proposed design for such equipment.

Chlorine is a very corrosive substance; so it should be fully controlled to prevent the decay of the distribution system. On the other hand, acceptable Chlorine levels in treatment and distribution facilities should be kept acceptable. Mostly, water distribution systems are made of three well-known construction materials; cast iron, concrete, and plastic. For cast iron chlorine is not recommended. Concrete and plastic are less affected by corrosion (Al-Jasser, 2007). Therefore, minimizing the chlorine level in water distribution system is another advantage for dechlorination.

Using bubble aeration has the advantage over stagnant aeration due to three physical phenomena. First is higher mass transfer area. The second is higher Reynolds number through the mass transfer interface, the third is a higher mass transfer concentration gradient; that is, the bubble is rising fast through the water column which leads to shorter time to mass transfer then there is a higher concentration gradient. Smaller bubbles have larger mass transfer area; less Reynolds

number and less concentration gradient as they climb up slowly through the water column which enables them to collect more chlorine inside hence there is less concentration difference between the bubble and the bulk water. However, the comparison between small or large bubbles is a trade off and needs further investigation.

pH was monitored against chlorine removal for each chlorine source. For trichloroisosyanuric acid at 200 ppm chlorine the pH was acidic at 5.76. As chlorine was withdrawn by aeration the pH became less acid till reaching 7.13 at 1.32 ppm chlorine. For sodium hypochlorite at 200 ppm the pH was 7.97 then went to 7.5 at final chlorine concentration of 0.68 ppm. For chlorine gas at 200 ppm, pH was 7.58 then dropped to 7.05 when the chlorine concentration became 0.17. The reason behind these trends is that the continuous withdrawal of chlorine from the system leads to continuous withdrawal of hypochlorous acid and chlorine oxide; hence, decreasing the acidity or basicity of the water depending on which one is dominating. For the trichloroisosyanuric acid, degrading of the produced syanuric acid to ammonia will increase the basicity as well as the previous reason.

The pH results give important limitations that should be considered when selecting a shock chlorinator. Using trichloroisosyanuric acid will produce high residual of cyanuric acid which raises water acidity to unacceptable levels. The same is for sodium hypochlorite but is less marked and in basic direction. Sodium is also a side product of water dechlorination by sodium hypochlorite but its health effect is not that dangerous as stoichiometrically the produced sodium when using 200 ppm sodium hydroxide is approximately 131 ppm. Sodium is an essential component in mineral water, so this sodium concentration may still acceptable. Chlorine gas is the best for use in such application as its chlorination and dechlorination is nearly completely reversible.

Practically, when low chlorine concentration is needed for shock chlorination; say less than 30 ppm, the preferred chlorinator is sodium hypochlorite because of its affordable effect on water pH. For higher chlorine levels chlorine gas would be recommended but with some modifications in chlorination facilities.

Mass transfer coefficient is a direct reflection to the chlorine depletion rate in water. The faster the decrease of chlorine ppm the greater the mass transfer coefficient is. When plotting the term

$ln\frac{(C^*-C)}{(C^*-C_\circ)}$ against the time, the slope represents the value of K_la. The sign of K_la was negative in all points which indicate that the process represent dissolved gas depletion from water to gas bubble.

K_la values are necessary for design purposes. Therefore they should approach the reality not the values predicted by mathematics. That is, the first few points have fast chlorine decrease hence more vertical slopes while the many others have slow decrease hence more horizontal slopes. When taking the average of these slopes, the average will show horizontality which is much away from design reality and will cause an unfair or magnified equipment. To deal with that, the average slope for the range from 200 ppm to nearly 1 ppm was taken into account as this range represents the most reliable chlorine depletion rate. Within this range equipment will designed well and economically.

It is clearly noticed that K_la values are very high at higher air flowrates. This is a direct result of the increase of mass transfer area that resulting from higher number of bubbles per certain time period and water volume.

K_la values for low range air flowrates (1-9 l/min) shows an increase with respect to air flowrate for all the three chlorine sources. This is due to the parallel increase in mass transfer area that resulting from the increase of bubbles per unit volume and time. After that, when reaching 10 l/min bubbles come to be crowded inside the column and; in majority of cases, attached to each other. This attachment reducing the overall mass transfer area as bubbles will not be longer exposed to water. Over the range of 10 to 50 l/min K_la shows nearly constant values because there will be a counteractive effect between increasing the air flowrate and decreasing the mass transfer area resulting from bubble crowd.

The variation of K_la ($K_l \times a$) or (mass transfer coefficient multiplied by the mass transfer area), is a direct result of varying the mass transfer area alone. K_l is nearly constant at the same other variables such as pressure and temperature.

Every value in figures (7.13) and (7.14) is the mean of many K_la values with respect to chlorine concentration. K_la increases as the starting chlorine concentration increases. This because the term$(C^* - C)$; the transferred moles, is increasing also.

7.5.2 The chlorination rate

This set of experiments was designed to compare chlorination by chlorine gas and dechlorination by forced aeration rates.

It is clear that chlorination is faster than dechlorination by an average of 25 %. The explanation behind this can be illustrated by the comparison between chlorine and air bubbles rising in water. When a chlorine bubble rises in water column, the gas that transfers across the bubble-water interface is only the chlorine. On the other hand, when an air bubble is rising along the water column, the transferred gasses across the interface are chlorine and water vapor.

The solubility of a gas in liquid and the counter action of the release of a gas from the liquid under the same conditions is governed by mass transfer rate. This depends mainly on the concentration gradient. In the chlorination process the concentration gradient continues to be the maximum level from the bottom to the top of the column while in dechlorination it start at the maxima and decreases as it rise up. However, in chlorination the lower the concentration of chlorine in water the higher the chlorine transfer rate as a result of higher concentration gradient while for dechlorination process the higher the concentration in the water the higher the transfer rate for the same reason.

The starting point of the dechlorination unit design in Section 8.2 is the “air/water demand per ppm of chlorine reduction”. The findings of this chapter assumes that as the chlorination is faster than dechlorination by a factor of 25%, hence, the “air/water demand per ppm of chlorine reduction” should be reduced by the same percentage.

After chlorination the chlorinated water should reside in the column for a short time to kill the bacteria. Approximate calculations indicate that, at a chlorine level of 200 ppm, the contact time should be about 11 seconds (Wagnet *et al.*, 2005). These calculations are proposed to work under moderate or low bacterial load and chlorine concentrations. Generalizing these figures for high chlorine levels is not fully recommended but can give a preliminary indication. Furthermore, such calculations are not for a complete kill of all the microorganisms but for a reduction to certain cfu ml-1 according to industrial bio-purification demands. Therefore, in designing a chlorination unit both chlorination and contact time have to be addressed.

For the low chlorine concentrations that are conventionally used in treatment processes, the dissolution time is not important as it takes little time to rise from zero to 4 ppm. However, for shock chlorination, chlorine has to rise from zero to 200 ppm. This time should be kept in mind in design calculations.

All in all, the chlorination unit dimensions can be assumed to be the same as dechlorination. This assumption depends on the fact that if dechlorination takes 2 minutes, the chlorination should take one minute and 30 seconds (dechlorination takes 25% more the time of chlorination) this gives 30 seconds to kill all the bacteria. If more time is needed to do so, it should be taken into account

7.5.3 Effect of initial cfu ml^{-1} on chlorine demand

The experiments were designed to show whether low initial bacterial concentration affects (lowers) the chlorine demand in drinking water. Also it is aimed to answer the question about the ability of chlorine to completely kill all the bacteria in water.

The first question arises because most previous published research deals with bacterial concentrations of 10^5 cfu ml^{-1} or more of; for example, *E-coli*. Such bacterial concentration is described as "the detection limit of the cell quantification assay" (Helbling and Vanbriesen, 2007). Therefore, to investigate the effect of lower initial bacterial concentration on chlorine demand a new technique was needed.

Mathematically, the formula describing the relation between the initial concentration and the contact time with a biocide is given by Chicks law (Hoff, 1986)

$$log\frac{N}{N_o} = -kt \quad \text{..... 7.16}$$

Where:

N_o: is the original number of microorganisms.

N: is the number of microorganisms remaining at time t.

t: is the contact time.

k: is proportionality constant.

This equation shows that: First, the biocidal efficacy is independent of the biocide concentration; i.e., even with low biocidal concentrations, the desired final bacterial concentration can be reached if it is given enough time. Unfortunately, this case cannot be implemented practically because the concentration of chlorine and other biocides is depleted quickly; hence, does not remain effective. Second, is that the zero bacterial concentration cannot be reached. If N is replaced by zero; which is the case of killing all microorganisms, this will give a time of infinity.

Practically, a concentration-time relation is used in all water treatment designs. This relationship is predicted empirically for each microorganism or group of microorganisms. Hence, the final bacterial concentration is biocide concentration dependent and zero cfu ml^{-1} can be reached if enough time and biocidal concentration is available. The practical difficulty behind the total killing if microorganisms in water are that the drinking water distribution system has not enough time and concentration to reach that extent. In other words, the water net is designed to work with a low chlorine concentration of 4 ppm maximally. This concentration is an optimization between two conditions, first, while more chlorine leads to more biocide efficacy but second is that the more chlorine that is introduced at the treatment plant produces more chlorine at the consumer tab. Therefore, studying the ability to increase the chlorine concentration to reach zero bacterial concentration under these two conditions is meaningless. Using a shock chlorination followed by shock dechlorination for drinking water paves the road for using high chlorine doses to reach zero bacterial concentration.

Returning back to the first question, this set of experiments answered it clearly by yes. The initial bacterial concentration is directly proportional to the chlorine needed to complete destruction even with low bacterial cfu ml^{-1} levels.

Theoretically and logically, the biocidal level enough to kill a single bacterium is the same to kill billions of them but in high bacterial content and low biocidal level the dominant phenomenon is the bacterial consumption of biocides that may explain the direct proportionality (Castro and Neves, 2003). On the contrary, at low bacterial content and high biocides levels this hypothesis does not seem to be acceptable because of the limited ability of small amount of bacteria to consume large amount of chlorine. The explanation behind continuing direct proportionality at low bacterial content is quorum sensing. This bacterial property gives the bacteria resistive properties against the severe environment. That is, the bacteria sense each other. When sensing

that they are becoming low in population, they possess higher resistive abilities. Hence, to kill these few bacteria, relatively more biocidal concentration is required. Therefore, single bacterium at the beginning of the treatment process is less resistant than a bacterium in the later stages. The later bacteria need relative more biocide to be killed.

7.6 Conclusions

7.6.1 The dechlorination rate

Forced aeration is a promising technique that can be used for removing chlorine in a fast and safe way in drinking water industry. This would enable shock chlorination followed by shock dechlorination to produce microorganism and biocide free drinking water. Chlorine gas was found to be the fastest and cleanest chlorine source to response to dechlorination by aeration followed by sodium hypochlorite and trichloroisosyanuric acid respectively.

7.6.2 The chlorination rate

On average, chlorination is faster than dechlorination by 25%. This gives a good tolerance and enough idea about the dimensions of a chlorination unit. The design of a chlorination unit is not that different from a dechlorination unit. As a rule of thumb, they can be the same (see Chapter 8)

7.6.3 Effect of initial cfu ml^{-1} on chlorine demand

At low bacterial levels, initial bacterial concentration is directly proportional to chlorine demand. The behavior of low bacterial population is the same as high bacterial populations, halving the bacterial concentration will half the chlorine demand. Also, enough chlorine doses can kill all the microorganisms in water.

CHAPTER EIGHT

DESIGN CONCEPT

Chapter Eight

Design Concept

The core results obtained in this thesis can be translated to modifications in the currently used water treatment processes. The following sections give ideas for recommended and assumed design cases.

8.1 Proposed shock chlorination and shock dechlorination unit location

The location of a chlorination-dechlorination unit can be in one of three places. First, is where a standard chlorination step is currently put; i.e. at the end of the water treatment process. The second is at the beginning of the treatment process, and the third is splitting the unit into chlorination part which may be put at the beginning of the process while the dechlorination part is just before discharging the water to the distribution net. The prevention of the accumulation of disinfectant by-products in the discharged water is the dominant consideration in deciding the unit location.

If used at the beginning of the process, the chemical and biological contaminants will be at their maxima. Applying shock chlorination will thus tend to produce the maximum amount of disinfectant by-products. After chlorination the water stream should pass through gravel, sand or activated carbon filters which may adsorb most of these products. The absorptivity of the disinfection by-products is not well studied.

If it is decided to put the unit just before discharging to the distribution net, the precursors of the disinfectant by-products will be at their minima as a result of the previous treatment and thus applying shock chlorination and shock dechlorination should produce lower levels of by-products.

Splitting the unit into two parts of shock chlorination and shock dechlorination has the same disadvantages of putting both units at the beginning but has the advantage of longer contact time for the chlorine; hence lower chlorine concentrations will be required and hence lower subsequent aeration capital and running costs. On the other hand, the performance of activated carbon will be affected by the high dose of chlorine and will need for more frequent reactivation.

It is recommended to put the shock chlorination and dechlorination units at the end of the treatment process due to the minimum production of disinfectants by-products. Furthermore, the conventional soft pre-chlorination; i.e. low dose of chlorine at the beginning of the treatment process, is recommended to kill most of the microorganisms without interfering with the activated carbon filters.

8.2 Shock dechlorination unit design

The starting point for designing the shock dechlorination column or basin is the data from chapter six. That is, the optimum chlorine removal was found to be at an air flowrate of 9 l/min through a 20 cm diameter column containing 50 litres of 200 ppm chlorinated water within two minutes.

Put as a single expression; excluding the two minutes, we get:

$$L_{air,\,opt} = 9\times10^{-4}\ \frac{la}{lw\,.\,ppm}\ \text{ or }\ \frac{\frac{m^3}{hr}}{\frac{m^3}{hr}\,.\,ppm}\ \text{ or }\ ppm^{-1} \qquad \text{.....8.1}$$

Where: *la* and *lw* are the air and water flowrates respectively (unit volume).

This number can be used as a basis of any shock dechlorination basin with an even sparger layout.

The two minutes period is neglected because the time cannot be embedded into the above constant to be for example ($4.5\times10^{-4}\ \frac{la}{lw\,.\,ppm\,.min}$). This may be correct if the relation between chlorine depletion and time is linear, i.e. within the first minute the chlorine drops from 200 to 100 ppm and drops from 100 to nearly zero during the second minute. In reality it drops from 200 to 3.94 at the first minute and to 0.02 during the second minute. Therefore, adding a variable number to a constant is not acceptable. To deal with such problem it is better to complete the design with the constant ($9\times10^{-4}\ \frac{la}{lw\,.\,ppm}$) then multiply the resulting design dimensions by 2 “minutes” as the residence time.

Both of these methods give the same design. The difference between them is the physical and logical acceptance.

8.2.1 Design case 1: Dechlorination channel

To design a shock dechlorination basin with a water flowrate of 10000 m^3 day^{-1} with 150 ppm chlorine to be reduced to 0.02 ppm, the design steps will be:

Water flowrate = $\frac{10000\ m^3/day}{24\ \frac{hr}{day} \times 60\ \frac{min}{hr}} = 6.94\ m^3\ min^{-1}$

Air flowrate = (6.94 m^3/min) × (149.98 ppm) × ($9\times10^{-4}\ \frac{la}{lw\ .\ ppm}$) = 0.937 $m^3\ min^{-1}$

If it is desired to make the basin as a channel with an assumed width of 5m and height of 3 meters. This will give water velocity of:

Water velocity inside the channel = $\frac{6.94\ m^3/min}{5\times3\ m^2}$ = 0.464 m min^{-1}

Since the residence time is 2 minutes, hence the length of the channel in which the spargers should be spread will be:

0.464 m min^{-1} × 2 min = 0.928 m

8.2.2 Design case 2: Dechlorination column

If it is desired to design a dechlorination column to treat 2000 m^3 day^{-1} from 150 to 0.02 ppm, the design steps will be:

Water flowrate = $\frac{2000\ m^3/day}{24\ \frac{hr}{day} \times 60\ \frac{min}{hr}} = 1.39\ m^3\ min^{-1}$

Air flowrate = (1.39 m^3 min^{-1}) × (149.98 ppm) × ($9\times10^{-4}\ \frac{la}{lw\ .\ ppm}$) = 0.187 m^3/min

Assuming there is an available standard column with 1.25 m diameter, this will give water velocity of:

Water velocity inside the column = $\frac{1.39\ m^3/min}{\frac{\pi}{4}(1.25^2)\ m^2}$ = 1.13 m/min

Since the residence time is 2 minutes, hence the column length will be:

1.13 m/min × 2 min = 2.26 m

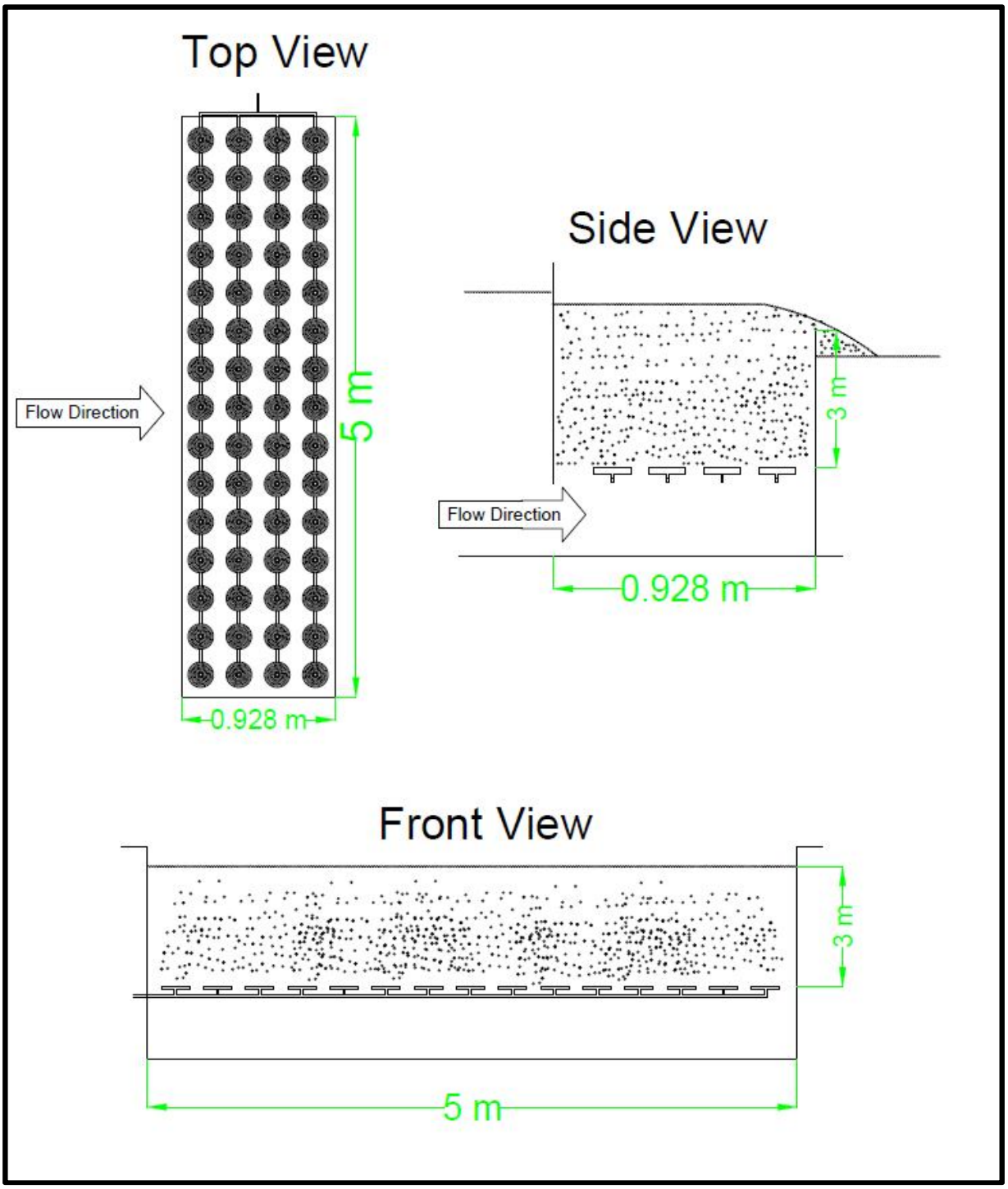

Figure 8.1: The proposed design of the channel dechlorinator in 8.2.1

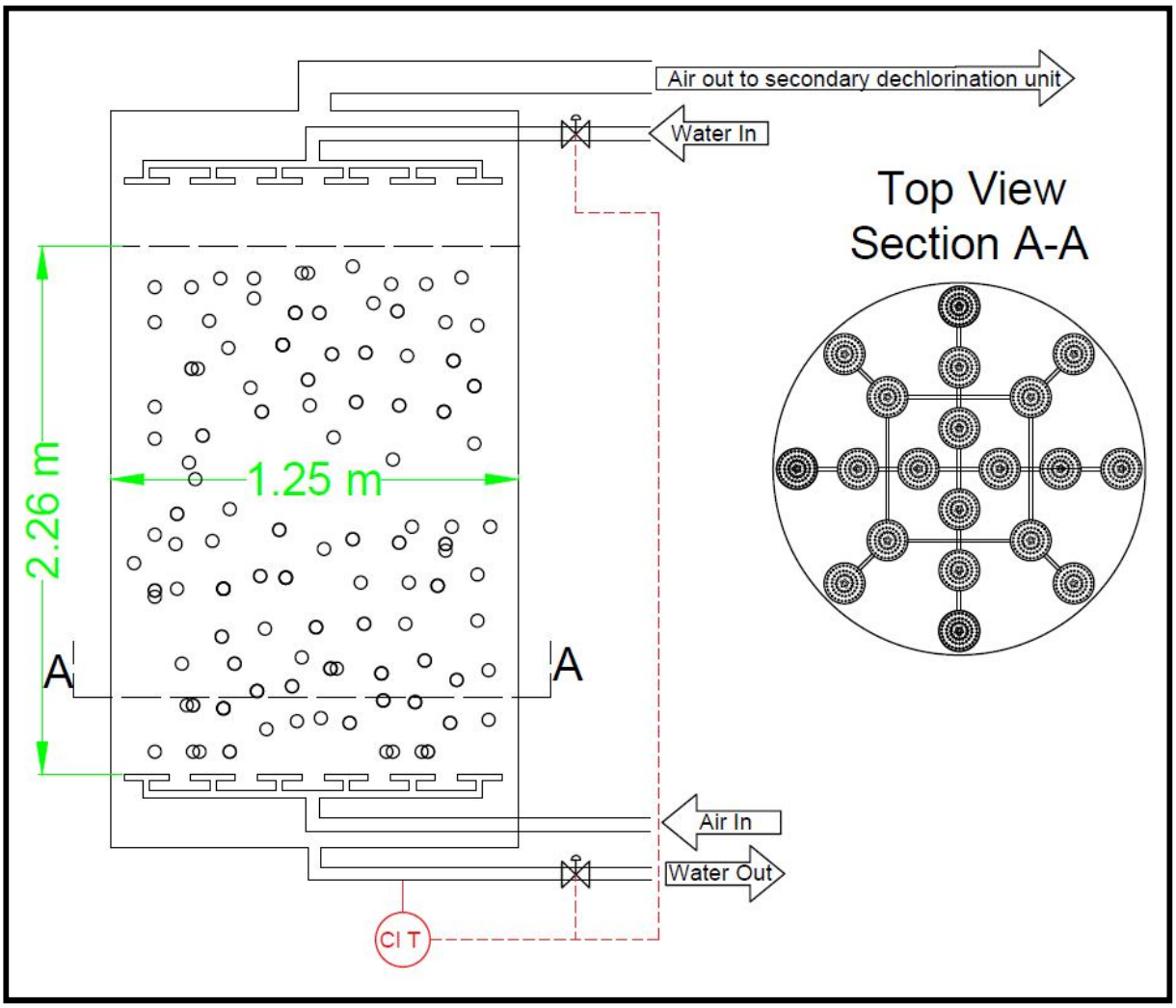

Figure 8.2: The proposed design of the column dechlorinator in 8.2.2

From the above calculations and assumptions, it can be concluded that the channel is suitable for in-situ, stationary large scale designs while the column is recommended for mobile medium and small scales designs. In other words, a channel dechlorinator may be used in water treatment plants while a column dechlorinator is preferred in trucks equipped with chlorination and dechlorination units for emergency treatment.

8.3 Shock chlorination by chlorine gas unit design

The conventional shock chlorination is implemented mainly using liquid chlorine sources not by chlorine gas. Therefore, there is no known shock chlorination equipment designed for use with such gas. In this section, a design is proposed in light of the results obtained in chapter seven for both column and channel shock chlorinator.

8.3.1 Shock chlorination column design

The shock chlorination column for use with chlorine gas is assumed to have the same dimensions as the shock dechlorination by aeration column described in 8.2.2 according to the conclusion that the chlorination rate is faster than the dechlorination by a factor of 0.25. The main differences between the chlorination and dechlorination column designs are, chlorine gas replaces air, the outlet gas is recycled, and a control system is added to compensate the lack of data of chlorine efficacy and dissolution rate in water. Figure 8.3 illustrates the design.

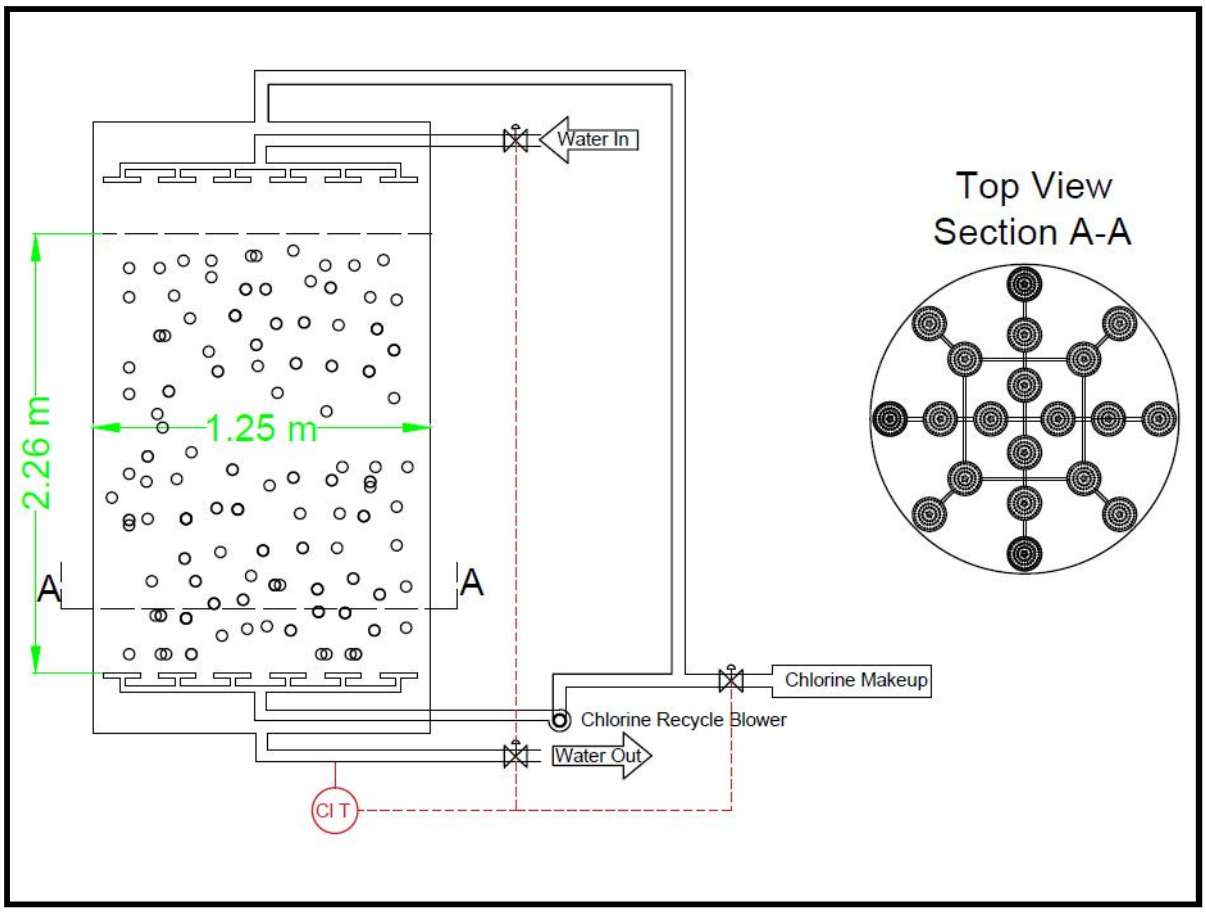

Figure 8.3: Shock chlorination column design

8.3.2 Shock chlorination Channel design

The chlorination channel is assumed to have the same design as the dechlorination channel. The differences are, again, chlorine gas replaces air, the outlet gas is recycled, and a feedback control system is added. The control system controls a gate or a series of valves fixed on the discharge holes to keep the water level above these holes. It also controls the chlorine makeup.

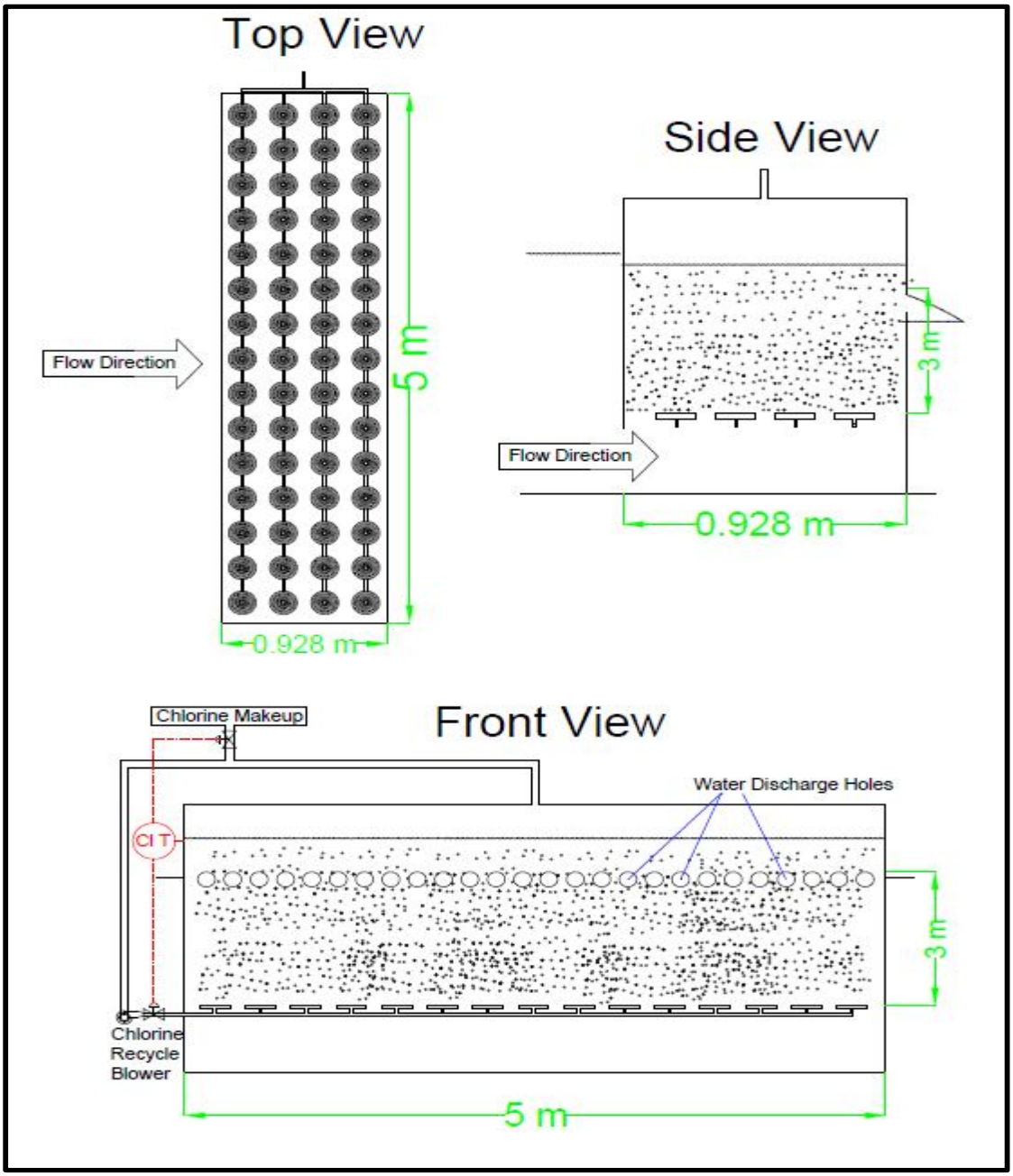

Figure 8.4: Shock chlorination channel design

8.4 Upgrading the aeration unit to froth flotation unit

A conventional aeration system consists of a medium swimming pool size basin equipped with a mesh of spargers as in figure (2.5). This design is relatively straight forward to upgrade to a froth flotation system. However, to make such an upgrade the air demand for aeration should be close in value to be used in froth flotation. The froth flotation has flexible pumping range while most

of aeration basins work with a wider range of air demand. Therefore the air supply should not be a big challenge.

The conventional aeration basin design consists of a net of spargers at the bottom of a basin. The water flows continuously over these spargers. The spargers in most cases are circular and equidistant within the basin. Therefore, it is likely that the design will be restricted to this originally existing facility and the other design variables should be adjusted accordingly.

The findings of this thesis that can be considered as a starting point to such upgrade are the air/water ratio, air flow rate, and the significance of column length on purification efficiency. The design criteria of the modification should identify the column lengths and number, the ratio of high/low bacterial streams, and the air flow rate. Column number is limited to the spargers number while their length will be flexible up to the maximum basin depth. The ratio of high/low bacterial streams should be kept as high as the control system can offer due in order to avoid water wasting. For the air flow rate, the calculation should depend on air velocity and take into consideration the column diameter. In Chapter 5 the optimum air flow rate was 50 L min^{-1} in a 20 cm diameter column. The air velocity will be about 1.59 m min^{-1}. This number can be used as a basis for estimating air flow rate.

8.5 Estimating CT value for design duties

Most of literatures and design protocols are aimed to estimate CT values for reducing *Giardia Lamblia* three-log (99.9%) and four-log of viruses inactivation (99.99%) (EPA, 2009). Therefore, new tests should be designed for every water source to identify the new CT value to kill all the microorganisms.

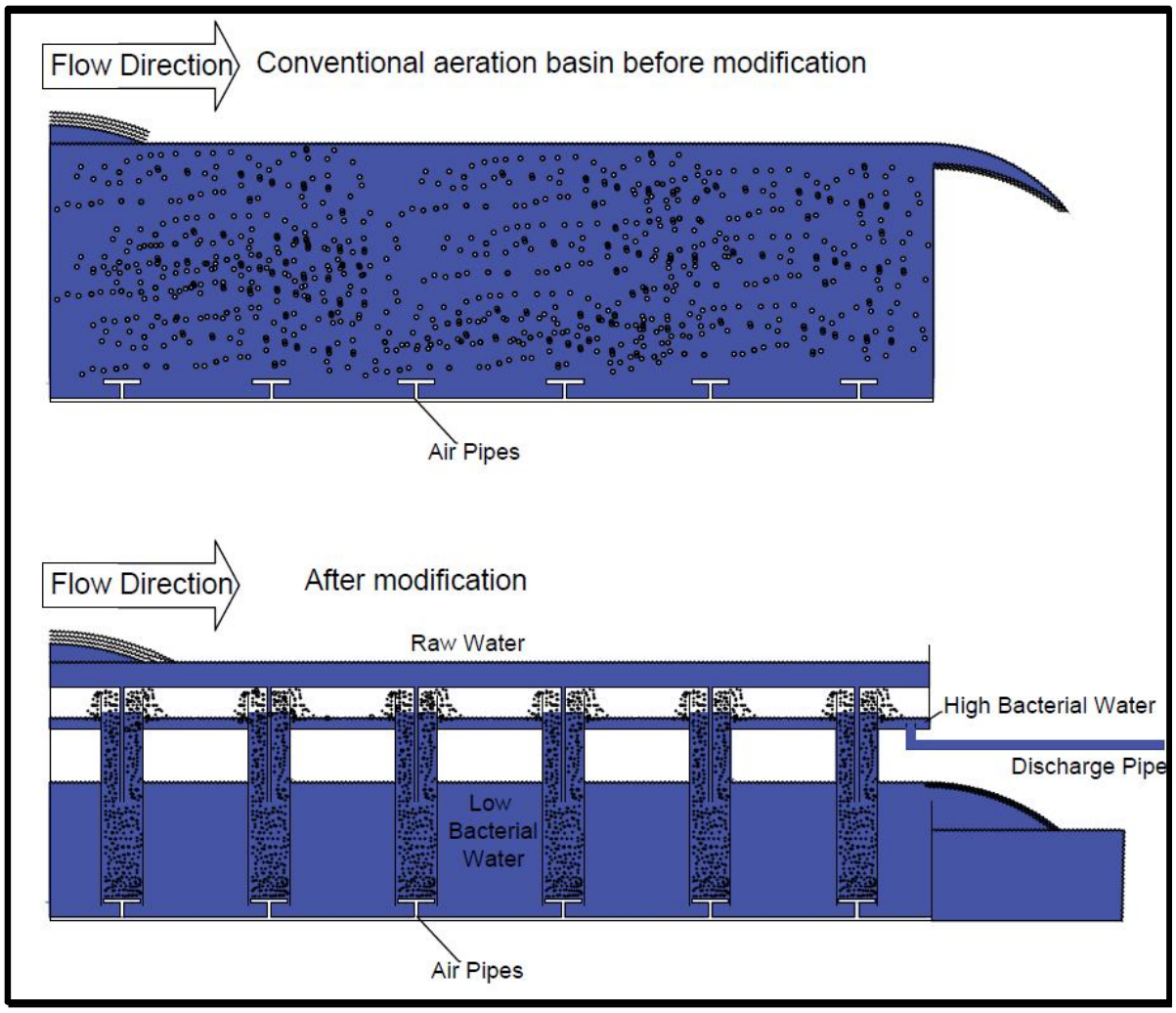

Figure 8.5: Aeration basin before and after modification

Tests should be implemented as follows:

1- Prepare 20 samples of 500 ml of raw water.

2- Prepare 20 samples of distilled water chlorinated with 10 ppm, 20 ppm, and so on till 400 ppm. Samples are 500 ml each.

3- Prepare samples of autoclaved nutrient broth according to test time range. For example, prepare 200 samples if it is needed to cover a time range of 10 minutes with a test frequency of one minute.

4- Add every single sample in step 2 to a single sample in step 1.

5- Every minute, take 1 ml of every mixture prepared in step 4 and add it to a single sample prepared in step 3 to make 10 new samples.

6- Collect all the samples produced from step 5 and send to incubation for 24 hours and 37 C^{o}.

7- After these 24 hours, every sample has microorganisms growth is fail and every sample still clear with no growth is successful.

8- The least CT sample is the required one for design needs.

8.6 Further Development

This section summarizes some potential further developments:

1- Shock chlorination can be implemented in a treatment plant. The discharge water in the distribution net is then kept very highly chlorinated. At the last possible point before consumer's tap, a dechlorination unit is added. This "possible point" may be attached to the last re-pumping station or at the start of a final part of the distribution. The advantage of this process is ensuring that the water is kept free of microorganisms and the pipe lines kept free of biofilms.

   This procedure can be used also to treat the distribution nets that already suffer from biofilms. The advantage of this method is to treat biofilm without shutdown.

2- A shock chlorination and dechlorination unit can be loaded on a truck to be temporarily used in any pipe line that suffers from high contamination due to accidents, process failure or when urgent purification is needed such as refugee camps during disasters. The design problem described in 9.3 gives justification to such idea. In this case an emergency outlet and inlet valves should be added to the pipeline during the design process or may be added later. The working procedure of these valves is illustrated in figure 9.6. When there is an emergency, the main valve in the pipeline is closed and the water diverted to the treatment unit then returned back to the pipeline.

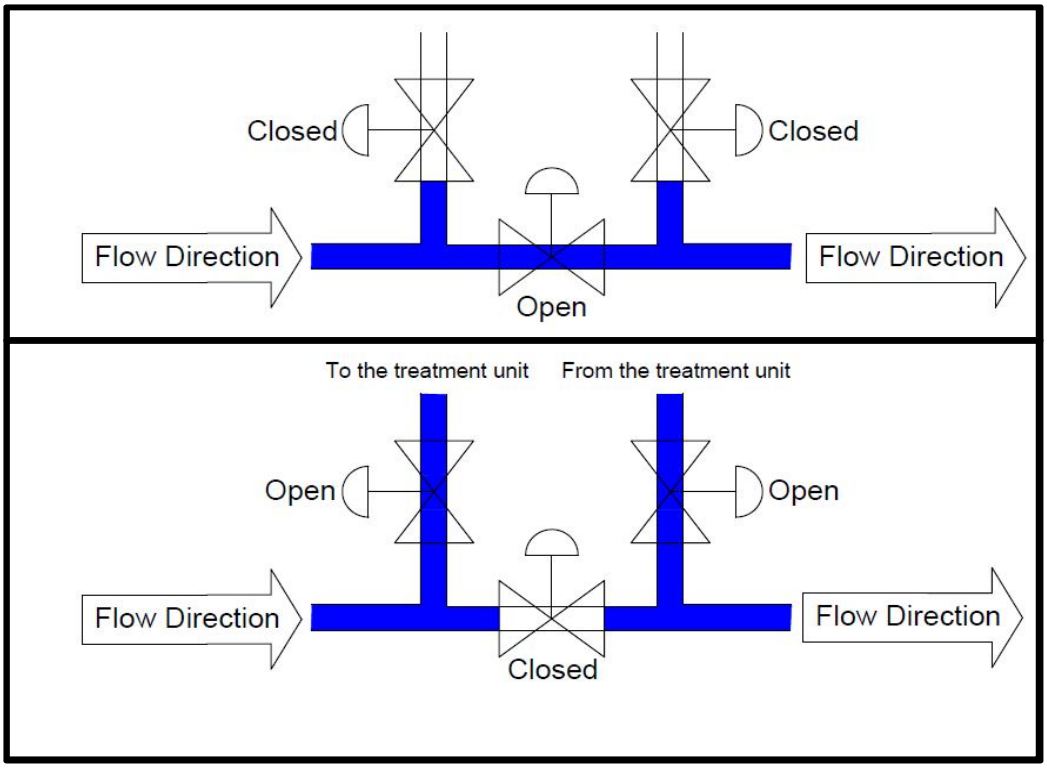

Figure 8.6: modification of pipe line for emergency mobile treatment. Up is the conventional operation. Down is emergency operation

CHAPTER NINE

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Chapter Nine

Conclusions and Recommendations for Future Work

1- Froth flotation can be developed to reach an acceptable bacterial separation level. This development can be done by discovering new frothers and collectors that may not affect drinking water health, taste or odor. For instance, trichloroisocyanuric acid was found to be a good frother but it affects the drinking water pH when used as a shock chlorinator. Therefore, its use is currently limited to swimming pools.

2- Froth flotation can be used as an alternative to filtration and sedimentation pretreatment processes. The nature and response of fine and suspended particles in raw water to froth flotation will need more investigation. This step; if successful can be easily combined with the froth flotation modification that described in Section 8.4.

3- Forced high air bubbling is a promising technique for gas-liquid and liquid-liquid separation. It has three advantages; greater surface area, more liquid-gas interface turbulence, and a larger concentration gradient. Therefore, such processes should be assessed technically and economically.

4- Packed bed and tray towers can be used to enhance the performance of both chlorinators and dechlorinators. These towers have the advantage of higher contact time but the disadvantage of low concentration gradients. These counter effects should be studied practically to give the best compromise result.

5- Air exiting the dechlorination column may contain a high chlorine content breaching regulations, particularly close to urban areas. This will require a secondary air dechlorination unit. A column filled with water saturated with soluble chemicals such as sodium thiosulfate, sodium bisulfite, sodium sulfite, or sulfur dioxide can be suitable such a process. A research path can be developed to investigate which of these substances solutions is better for dechlorinating an air saturated stream.

6- Chlorine decay through the distribution net is attributed to many reasons such as the reaction with pipe materials and consumption by microorganisms, organic material and natural aeration. One of the hidden and unknown reasons is the pressure drop along the distribution system. This reason should be investigated and compared with the other reasons for its significance.

7- *E-coli k-12* was taken as guidance bacteria through this study. The effect of the new developed froth flotation technique on various bacterial strains may be investigated as a new research path in this field.

8- Disinfectant CT value that is available for some microorganisms is somewhat vague on two points. First, there is no CT value to completely kill the entire microorganism. Instead, the data available are for 2, 3 or 4 log-reduction. Second, the CT data are for certain microorganisms. Even this is too close to the scientific methodology but it is too far from the engineering point of view. Designers are dealing with rivers and reservoirs water. Availability of CT values for the water body as a whole with a variety of bacterial load will help in engineering designs and decisions more than individual values. Therefore, new studies should be implemented to gather such data for rivers or reservoirs

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APPENDEX

ERROR MARGINS FOR FIGURES 5.3 – 5.8

A

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.2	0.9	1.2	0.4	0.3	0.2	0.2
10	0.2	0.2	0.2	1.0	1.3	0.5	0.4	0.2	0.2
15	0.2	0.3	0.3	1.0	1.4	0.5	0.4	0.2	0.2
20	0.2	0.3	0.3	1.0	1.4	0.5	0.4	0.2	0.2
25	0.2	0.3	0.3	1.0	1.5	0.5	0.4	0.2	0.2
30	0.2	0.3	0.3	1.0	1.5	0.5	0.4	0.2	0.2

B

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.3	0.7	1.0	0.7	0.6	0.2	0.2
10	0.2	0.3	0.3	0.8	1.2	0.8	0.7	0.2	0.2
15	0.2	0.3	0.4	0.9	1.3	0.9	0.8	0.2	0.3
20	0.2	0.3	0.4	1.0	1.4	1.0	0.8	0.3	0.3
25	0.2	0.4	0.4	1.0	1.4	1.0	0.8	0.3	0.3
30	0.2	0.4	0.4	1.0	1.4	1.0	0.8	0.3	0.3

C

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.3	0.5	0.7	0.7	0.6	0.2	0.2
10	0.2	0.3	0.4	0.5	0.8	0.9	0.7	0.2	0.2
15	0.2	0.4	0.5	0.6	0.9	1.0	0.8	0.2	0.2
20	0.2	0.5	0.5	0.7	1.0	1.1	1.0	0.3	0.2
25	0.2	0.5	0.6	0.8	1.1	1.2	1.0	0.2	0.2
30	0.2	0.5	0.6	0.8	1.1	1.2	1.0	0.3	0.2

D

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.3	0.7	0.8	0.6	0.7	0.3	0.2
10	0.2	0.3	0.5	0.8	0.9	0.8	0.9	0.3	0.3
15	0.2	0.5	0.6	0.9	1.0	0.9	1.0	0.3	0.3
20	0.2	0.6	0.7	1.0	1.1	1.0	1.2	0.3	0.3
25	0.2	0.6	0.7	1.1	1.2	1.1	1.2	0.3	0.3
30	0.2	0.6	0.7	1.1	1.2	1.1	1.2	0.3	0.3

E

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.4	0.6	0.9	0.9	0.8	0.3	0.3
10	0.2	0.3	0.5	0.7	1.1	1.1	0.9	0.3	0.3
15	0.2	0.5	0.6	0.8	1.2	1.3	1.1	0.3	0.3
20	0.2	0.6	0.7	0.9	1.3	1.4	1.3	0.3	0.3
25	0.2	0.6	0.7	1.0	1.4	1.5	1.3	0.3	0.3
30	0.2	0.6	0.7	1.0	1.4	1.5	1.3	0.3	0.3

Error margins for figure 5.3 A, B, C, D and E (± cm)

A

	10	30	50	70	90	110	130	150	170
5	0.1	0.2	0.2	0.8	1.0	0.4	0.3	0.2	0.2
10	0.1	0.2	0.2	0.8	1.1	0.4	0.3	0.2	0.2
15	0.1	0.2	0.2	0.9	1.2	0.4	0.4	0.2	0.2
20	0.1	0.2	0.2	0.9	1.2	0.4	0.4	0.2	0.2
25	0.1	0.2	0.2	0.9	1.3	0.4	0.4	0.2	0.2
30	0.1	0.2	0.2	0.9	1.3	0.4	0.4	0.2	0.2

B

	10	30	50	70	90	110	130	150	170
5	0.1	0.2	0.2	0.6	0.8	0.6	0.5	0.2	0.2
10	0.1	0.2	0.3	0.7	1.0	0.7	0.6	0.2	0.2
15	0.1	0.3	0.3	0.8	1.1	0.8	0.6	0.2	0.2
20	0.1	0.3	0.4	0.8	1.2	0.9	0.7	0.2	0.2
25	0.1	0.3	0.4	0.8	1.2	0.9	0.7	0.2	0.2
30	0.2	0.3	0.4	0.8	1.2	0.9	0.7	0.2	0.2

C

	10	30	50	70	90	110	130	150	170
5	0.1	0.1	0.2	0.4	0.6	0.6	0.5	0.2	0.2
10	0.1	0.2	0.3	0.5	0.7	0.7	0.6	0.2	0.2
15	0.1	0.3	0.4	0.5	0.8	0.8	0.7	0.2	0.2
20	0.2	0.4	0.5	0.6	0.9	0.9	0.8	0.2	0.2
25	0.2	0.4	0.5	0.7	0.9	1.0	0.9	0.2	0.2
30	0.2	0.4	0.5	0.7	0.9	1.0	0.9	0.2	0.2

D

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.3	0.6	0.7	0.5	0.6	0.2	0.2
10	0.2	0.3	0.4	0.7	0.8	0.7	0.7	0.2	0.2
15	0.2	0.4	0.5	0.8	0.9	0.8	0.8	0.2	0.3
20	0.2	0.5	0.6	0.9	1.0	0.9	1.0	0.3	0.2
25	0.2	0.5	0.6	1.0	1.0	0.9	1.0	0.2	0.2
30	0.2	0.5	0.6	1.0	1.0	0.9	1.0	0.3	0.3

E

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.3	0.5	0.8	0.7	0.7	0.3	0.2
10	0.2	0.3	0.4	0.6	0.9	0.9	0.8	0.3	0.2
15	0.2	0.4	0.5	0.7	1.0	1.1	0.9	0.3	0.3
20	0.2	0.5	0.6	0.8	1.1	1.2	1.1	0.3	0.2
25	0.2	0.5	0.6	0.9	1.2	1.3	1.1	0.3	0.3
30	0.2	0.5	0.6	0.9	1.2	1.3	1.1	0.3	0.3

Error margins for figure 5.4 A, B, C, D and E (± cm)

A

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.2	0.9	1.2	0.4	0.3	0.2	0.2
10	0.2	0.2	0.2	1.0	1.3	0.5	0.4	0.2	0.2
15	0.2	0.3	0.3	1.0	1.4	0.5	0.4	0.2	0.2
20	0.2	0.3	0.3	1.0	1.4	0.5	0.4	0.2	0.2
25	0.2	0.3	0.3	1.0	1.5	0.5	0.4	0.2	0.2
30	0.2	0.3	0.3	1.0	1.5	0.5	0.4	0.2	0.2

B

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.3	0.7	1.0	0.7	0.6	0.2	0.2
10	0.2	0.3	0.3	0.8	1.2	0.8	0.7	0.2	0.2
15	0.2	0.3	0.4	0.9	1.3	0.9	0.8	0.2	0.3
20	0.2	0.3	0.4	1.0	1.4	1.0	0.8	0.3	0.3
25	0.2	0.4	0.4	1.0	1.4	1.0	0.8	0.3	0.3
30	0.2	0.4	0.4	1.0	1.4	1.0	0.8	0.3	0.3

C

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.3	0.5	0.7	0.7	0.6	0.2	0.2
10	0.2	0.3	0.4	0.5	0.8	0.9	0.7	0.2	0.2
15	0.2	0.4	0.5	0.6	0.9	1.0	0.8	0.2	0.2
20	0.2	0.5	0.5	0.7	1.0	1.1	1.0	0.3	0.2
25	0.2	0.5	0.6	0.8	1.1	1.2	1.0	0.2	0.2
30	0.2	0.5	0.6	0.8	1.1	1.2	1.0	0.3	0.2

D

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.3	0.7	0.8	0.6	0.7	0.3	0.2
10	0.2	0.3	0.5	0.8	0.9	0.8	0.9	0.3	0.3
15	0.2	0.5	0.6	0.9	1.0	0.9	1.0	0.3	0.3
20	0.2	0.6	0.7	1.0	1.1	1.0	1.2	0.3	0.3
25	0.2	0.6	0.7	1.1	1.2	1.1	1.2	0.3	0.3
30	0.2	0.6	0.7	1.1	1.2	1.1	1.2	0.3	0.3

E

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.4	0.6	0.9	0.9	0.8	0.3	0.3
10	0.2	0.3	0.5	0.7	1.1	1.1	0.9	0.3	0.3
15	0.2	0.5	0.6	0.8	1.2	1.3	1.1	0.3	0.3
20	0.2	0.6	0.7	0.9	1.3	1.4	1.3	0.3	0.3
25	0.2	0.6	0.7	1.0	1.4	1.5	1.3	0.3	0.3
30	0.2	0.6	0.7	1.0	1.4	1.5	1.3	0.3	0.3

Error margins for figure 5.5 A, B, C, D and E (± cm)

A

	10	30	50	70	90	110	130	150	170
5	0.2	0.3	0.3	1.2	1.5	0.6	0.4	0.3	0.3
10	0.2	0.3	0.3	1.2	1.6	0.6	0.5	0.3	0.3
15	0.2	0.3	0.3	1.3	1.8	0.6	0.5	0.3	0.3
20	0.2	0.3	0.3	1.3	1.9	0.6	0.5	0.3	0.3
25	0.2	0.3	0.3	1.3	1.9	0.7	0.5	0.3	0.3
30	0.2	0.3	0.4	1.3	1.9	0.7	0.5	0.3	0.3

B

	10	30	50	70	90	110	130	150	170
5	0.2	0.3	0.4	0.9	1.3	0.9	0.8	0.3	0.3
10	0.2	0.3	0.4	1.0	1.6	1.1	0.8	0.3	0.3
15	0.2	0.4	0.5	1.2	1.7	1.2	1.0	0.3	0.3
20	0.2	0.4	0.5	1.3	1.8	1.3	1.1	0.3	0.3
25	0.2	0.5	0.5	1.3	1.8	1.3	1.1	0.3	0.3
30	0.2	0.5	0.5	1.3	1.8	1.3	1.1	0.3	0.3

C

	10	30	50	70	90	110	130	150	170
5	0.2	0.2	0.4	0.6	0.9	0.9	0.8	0.3	0.3
10	0.2	0.3	0.5	0.7	1.1	1.1	0.9	0.3	0.3
15	0.2	0.5	0.6	0.8	1.2	1.2	1.1	0.3	0.3
20	0.2	0.6	0.7	0.9	1.3	1.4	1.3	0.3	0.3
25	0.2	0.6	0.7	1.0	1.4	1.5	1.3	0.3	0.3
30	0.2	0.6	0.7	1.0	1.4	1.5	1.3	0.3	0.3

D

	10	30	50	70	90	110	130	150	170
5	0.3	0.3	0.4	0.9	1.0	0.8	1.0	0.4	0.3
10	0.3	0.4	0.6	1.0	1.2	1.0	1.1	0.4	0.3
15	0.3	0.6	0.8	1.1	1.3	1.1	1.3	0.4	0.4
20	0.3	0.7	0.8	1.3	1.5	1.3	1.5	0.4	0.3
25	0.3	0.7	0.9	1.5	1.5	1.4	1.6	0.4	0.4
30	0.3	0.8	0.9	1.5	1.5	1.4	1.6	0.4	0.4

E

	10	30	50	70	90	110	130	150	170
5	0.3	0.3	0.5	0.8	1.1	1.1	1.0	0.4	0.3
10	0.3	0.4	0.6	0.9	1.4	1.4	1.2	0.4	0.4
15	0.3	0.6	0.8	1.0	1.5	1.6	1.4	0.4	0.4
20	0.3	0.8	0.9	1.2	1.7	1.8	1.6	0.4	0.4
25	0.3	0.8	1.0	1.3	1.8	1.9	1.7	0.4	0.4
30	0.3	0.8	1.0	1.3	1.8	1.9	1.7	0.4	0.4

Error margins for figure 5.6 A, B, C, D and E (± cm)

A

	10	30	50	70	90	110	130	150	170
5	0.3	0.3	0.4	1.4	1.8	0.7	0.5	0.3	0.4
10	0.3	0.4	0.4	1.5	2.0	0.7	0.6	0.4	0.4
15	0.3	0.4	0.4	1.6	2.2	0.8	0.6	0.4	0.4
20	0.3	0.4	0.4	1.6	2.3	0.8	0.7	0.4	0.4
25	0.3	0.4	0.4	1.6	2.3	0.8	0.6	0.4	0.4
30	0.3	0.4	0.4	1.6	2.3	0.8	0.7	0.4	0.4

B

	10	30	50	70	90	110	130	150	170
5	0.3	0.3	0.4	1.0	1.6	1.1	0.9	0.3	0.4
10	0.3	0.4	0.5	1.3	1.9	1.3	1.0	0.4	0.4
15	0.3	0.5	0.6	1.5	2.1	1.4	1.2	0.4	0.4
20	0.3	0.5	0.6	1.5	2.2	1.6	1.3	0.4	0.4
25	0.3	0.6	0.7	1.5	2.2	1.6	1.3	0.4	0.4
30	0.3	0.6	0.7	1.5	2.2	1.6	1.3	0.4	0.4

C

	10	30	50	70	90	110	130	150	170
5	0.3	0.3	0.4	0.7	1.1	1.1	1.0	0.4	0.3
10	0.3	0.4	0.6	0.9	1.3	1.3	1.1	0.4	0.4
15	0.3	0.6	0.8	1.0	1.4	1.5	1.3	0.4	0.4
20	0.3	0.7	0.9	1.1	1.6	1.7	1.5	0.4	0.3
25	0.3	0.8	0.9	1.2	1.7	1.8	1.6	0.4	0.4
30	0.3	0.8	0.9	1.2	1.7	1.8	1.6	0.4	0.4

D

	10	30	50	70	90	110	130	150	170
5	0.3	0.3	0.5	1.0	1.2	1.0	1.2	0.4	0.4
10	0.3	0.5	0.7	1.2	1.5	1.2	1.4	0.4	0.4
15	0.3	0.7	0.9	1.4	1.6	1.4	1.5	0.5	0.5
20	0.3	0.9	1.0	1.6	1.8	1.6	1.8	0.5	0.4
25	0.3	0.9	1.1	1.8	1.9	1.7	1.9	0.5	0.4
30	0.3	0.9	1.1	1.8	1.9	1.7	1.9	0.5	0.5

E

	10	30	50	70	90	110	130	150	170
5	0.3	0.3	0.6	0.9	1.4	1.4	1.3	0.5	0.4
10	0.4	0.5	0.8	1.1	1.7	1.7	1.5	0.5	0.5
15	0.4	0.8	1.0	1.3	1.9	2.0	1.7	0.5	0.5
20	0.4	0.9	1.1	1.5	2.1	2.3	2.0	0.5	0.4
25	0.4	1.0	1.2	1.6	2.2	2.4	2.1	0.5	0.5
30	0.4	1.0	1.2	1.6	2.2	2.4	2.1	0.5	0.5

Error margins for figure 5.7 A, B, C, D and E (± cm)

A

5	0.3	0.4	0.4	1.7	2.2	0.8	0.6	0.4	0.4
10	0.3	0.4	0.4	1.8	2.3	0.9	0.7	0.4	0.4
15	0.3	0.5	0.5	1.9	2.6	0.9	0.8	0.4	0.4
20	0.3	0.5	0.5	1.9	2.7	0.9	0.8	0.4	0.5
25	0.3	0.5	0.5	1.9	2.7	0.9	0.8	0.4	0.4
30	0.3	0.5	0.5	1.9	2.7	0.9	0.8	0.4	0.4

B

	10	30	50	70	90	110	130	150	170
5	0.3	0.4	0.5	1.2	1.8	1.3	1.1	0.4	0.4
10	0.3	0.5	0.6	1.5	2.2	1.5	1.2	0.4	0.4
15	0.3	0.6	0.8	1.8	2.5	1.7	1.4	0.5	0.5
20	0.3	0.6	0.8	1.8	2.6	1.9	1.6	0.5	0.5
25	0.3	0.7	0.8	1.8	2.6	1.9	1.6	0.5	0.5
30	0.3	0.7	0.8	1.8	2.6	1.9	1.6	0.5	0.5

C

	10	30	50	70	90	110	130	150	170
5	0.3	0.3	0.5	0.9	1.3	1.2	1.2	0.4	0.4
10	0.3	0.5	0.7	1.0	1.6	1.6	1.3	0.4	0.4
15	0.3	0.7	0.9	1.2	1.7	1.8	1.5	0.4	0.5
20	0.3	0.9	1.0	1.3	1.9	2.1	1.8	0.5	0.4
25	0.3	0.9	1.1	1.5	2.0	2.2	1.9	0.4	0.4
30	0.3	0.9	1.1	1.5	2.0	2.2	1.9	0.5	0.5

D

	10	30	50	70	90	110	130	150	170
5	0.4	0.4	0.6	1.2	1.4	1.2	1.4	0.5	0.5
10	0.4	0.6	0.9	1.5	1.7	1.5	1.6	0.5	0.5
15	0.4	0.9	1.1	1.7	1.9	1.7	1.8	0.5	0.5
20	0.4	1.0	1.2	1.9	2.1	1.9	2.2	0.6	0.5
25	0.4	1.1	1.3	2.1	2.2	2.0	2.3	0.5	0.5
30	0.4	1.1	1.3	2.1	2.2	2.0	2.3	0.6	0.5

E

	10	30	50	70	90	110	130	150	170
5	0.4	0.4	0.7	1.1	1.7	1.6	1.5	0.5	0.5
10	0.4	0.6	0.9	1.3	2.0	2.1	1.7	0.6	0.5
15	0.4	0.9	1.2	1.5	2.2	2.3	2.0	0.6	0.6
20	0.4	1.1	1.3	1.8	2.5	2.7	2.4	0.6	0.5
25	0.4	1.2	1.4	1.9	2.6	2.8	2.4	0.6	0.6
30	0.4	1.2	1.4	1.9	2.6	2.8	2.4	0.6	0.6

Error margins for figure 5.8 A, B, C, D and E (± cm)