Evaluating Disinfectants Potential for Inactivation of Microbial Species in Laboratory Prototype Distribution Network

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List of Abbreviations

ATCC	American Type Culture Collection
CCD	Central composite design
CFU	Colony-forming units
Cl_2	Chlorine
ClO_2	Chlorine dioxide
DBPs	Disinfection by-products
DPD	*N*,*N*-Diethyl-*p*-Phenylenediamine
DWDS	Drinking water distribution system
DWTPs	Drinking water treatment plants
GWI	Global water initiative
mg/L	Milligram per litre
$Na_2S_2O_3$	Sodium thiosulphate
NaOCl	Sodium hypochlorite
NH_2Cl	Chloramine
NH_4OH	Ammonium hydroxide
UNESCO	United Nations Educational, Scientific and Cultural Org
UNICEF	United Nations International Children's Emergency Fund
WEF	World Economic Forum
WHO	World Health Organization

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ABSTRACT

Microbial population in the water may be inactivated by the process of disinfection. Disinfection of the drinking water mainly performed by chlorination technique which can kill bacteria by destroying their metabolism and protein synthesis processes but failed to completely eradicate the microbial population from the drinking water. Drinking water supplies agencies has the responsibility to completely prevent the drinking water supplies from the microbial population. The present study compared the microbial inactivation efficiency of the two main disinfectants chlorine and chloramine to completely inactivate the gram-negative bacterial strains *(Salmonella enterica and Shigella dysenteriae).* These bacterial strains are the causative agent of Salmonellosis and Shigellosis. The study was conducted in two phases. In first phase, bench scale experiments were conducted to optimize the disinfectant dose and contact time for microbial inactivation. The contact time of 1 min, 10 mins and 30 mins were selected against 0.5, 1.0 and 2.0 mg/L disinfectant dosages for bench scale study. In second phase, the experiments were performed in a laboratory prototype distribution network to simulate real time conditions. Active microbial inoculum was inoculated at 10^6 CFU/ml in 100 L de-chlorinated tap water in prototype network. The physicochemical quality of tap water assessed before experimentation. Chloramine shows greater inactivation efficiency than chlorine in both bench scale study and the prototype study. The study concludes that chloramine is more effective and efficient disinfectant than chlorine in the prototype distribution network as gram-negative microbial strains were completely inactivated within 30 mins of the contact time when optimal dose of 2 mg/L was applied

Chapter 1

Introduction

Water is essential for human life and health. The basic need for human health and support human is drinking water (UNESCO, 2021). The water catastrophe is the fifth global hazard in terms of impact on society (WEF, 2021). Globally, around 785 million human beings are deprived of access to fundamental water resources.

According to recent estimates 2300 million people around the globe are confined to water-burdened countries, out of which nearly 733 million are forced to dwell in excessive and severely water-burdened countries (UN-Water, 2021). Similar estimates by UNICEF report that around 1420 million people and nearly 450 million children dwell in areas with excessive vulnerability in access to water resources (UNICEF, 2021).

Nearly two-thirds of the world's population or four billion people are plagued by extreme water shortages (Mekonnen and Hoekstra, 2016). It was estimated that if these disparities in water resources are not equalized, globally around 700 million people would be displaced because of extreme water scarcity by 2030 (GWI, 2013). Only 20% of the population of Pakistan has the ability to acquire safe drinking water, whereas the remaining 80% is forced to utilize insecure drinking water resources due to extreme shortage of safe drinking water sources (Daud et al., 2017).). Brian Glazer, an oceanographer said that where there is the presence of water, there must be the existence of microbial community that live there (Ghose, 2015). 1.72 to 2 million

people die off from to the ingestion of unsafe drinking water and its various subsequent diseases such as diarrhea (Clayton et al., 2017).

Inappropriate treatment and poor management of drinking water resources may result in the outbreak of severe water borne illnesses (WHO, 2017). In drinking water distribution networks, such water borne diseases are connected to the cross-connection, contamination throughout the storage and intermittent water supply. Microbial regrowth with inside the ingesting water distribution systems (DWDS) noticeably relies upon at the treatment implemented to the supply water however additionally at the supply water quality (Favere et al., 2021).

The Primary aims of the drinking water distribution system is supplying aesthetically acceptable and healthy water and maintaining the quality of the water until it reaches the consumer end (Nescerecka et al., 2014).

Disinfection of drinking water occurs by two main methods physical disinfection and chemical disinfection. Chemically disinfected water supplies have a large societal hazard in addition to their benefits. When the disinfectants interact with organic material in the source water, they form hazardous chemical substances known as disinfection by-products (DBPs). The production of these substances and their potential adverse health impacts have raised serious concerns over this chemical disinfection process (Komaki et al., 2014).

Karl W. Scheele in 1774 discovered chlorine. And Humphrey Davy in 1810 identified chlorine as an element (IARC). In 1897, chlorination was used for the first time for the purpose of disinfection of drinking water. In Maindstone (Kent, UK), following an

outbreak of typhoid a disinfection solution of chlorine mixed with bleach was prepared. In the twentieth century, chlorination became a regular practice for disinfecting drinking water.

A major reason for water treatment was and has been shielding consumers from contaminations that might be hostile or harmful to human health. The second main reason for water treatment is to remove the impurities which are not directly harmful to human health but can cause discoloration and corrosion. These impurities may be removed from the water by setting up obstructions like filtration and coagulation which can cause precipitation and capturing of the particle. Disinfection is the final barrier.

Chlorination is ineffective in achieving the reduction below the permissible limits against various waterborne pathogens, such as Legionella, Cryptosporidium, Giardia Noroviruses and Hepatitis A Virus (HAV) due to their resistant nature and ability to persist in water (WHO, 2017). According to Razzolini et al. (2010), resistant microbial strains that produce toxins and colonize biofilms such as Aeromonas sp are harder to inactivate by chlorination. Each type of microbial strain has a specific residual chlorine concentration to which it inactivates completely (Martínez-Hernández et al., 2013). Chlorine-based disinfectants also poses a risk coupled with the handling and storage of chemical disinfectants (Ghebremichael et al., 2011). These limitations have instigated the search of an alternative disinfection method.

Chloramine, mainly monochloramine (NH_2Cl), as a disinfectant has been prioritized recently over chlorine, owing to its greater residence time and stability in drinking water distribution networks and hence better overall disinfection (Leopold and Freese, 2009). Chloramine decays slowly as compared to chlorine, primarily because of its

lower reactivity with organic materials naturally present in the raw water. This benefits in reducing the formation of regulated DBPs, typically 80–97% less Haloacetic acids (HAAs) and Trihalomenthanes (THMs) when compared to chlorination. The amount of chlorine added for the disinfection of drinking water after the treatment mainly depends upon the treatment technique, but generally the chlorine added was sufficient enough to provide the desired residual chlorine inside the range of 0.5-1 mg/L.

1.1 Problem Statement:

Chlorination is applied as the only disinfection method in Pakistan. But there is a substantial lack of the studies about responses of microbial populations to chlorination process. In Pakistan, only little work has been reported regarding the bacteriological quality of water. In addition, no proper conditions have ever been testified at any treatment plant for effective chlorination system to meet the drinking water standards. DBPs production potential, inefficient against resistant species, irritant odor, low stability, ecological risk, toxic residues, and specific residual concentration for each microbe demands a substitute disinfectant to ensure the microbially safe drinking water at user end point (Diao et al., 2004).

1.2 Objectives:

The present study focuses on the following:

i. Chlorine and chloramine dose optimization for inactivation of microbial species in batch setup

ii. Comparison of Inactivation efficiency of chlorine and chloramine to inactivate microbial species

Chapter 2

Literature Review

The application of chemicals for disinfection is widespread, agents such as chlorine (Cl), ozone (O_3), chlorine dioxide (ClO_2) and chloramine (NH_2Cl) are most commonly used for the inactivation of pathogens in water. However, these disinfectants can and often react with natural organics (TOC & OM) in the water and produce threatening disinfection by-products. In 1974, trichloromethane was identified as first DBP, since then about 800 DBP compounds have been detected. Trihalomethanes (THMs) have been enormously reported in natural water treated by chlorination.

2.1 Bacterial action of disinfectants:

Chlorine and chloramine are known to exhibit speedy biocidal effect in aqueous solution. At first, chlorine upon its reaction with H_2O, forms hypochlorous acid.

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

Conditional to the pH, hypochlorous acid may partly convert into hypochlorite ions:

$$2H_2O + Cl_2 + \rightarrow H_3O + Cl^- \; HOCl + H_2O + HOCL \rightarrow H_3O^+ + OCl^-$$

The meticulous mechanism by which hypochlorous acid obliterates microorganisms has never been demonstrated experimentally, however it has been theorized that hypochlorous acid permits oxygen to emerge, which in turn purportedly combines with components of cell protoplasm, destroying the organism. This detaches chlorine and oxygen atoms as:

$$OCl^- \rightarrow Cl^- + [O]$$

Buse et al., (2019) studied the inactivation of *Legionella pneumophilia* colonizing polyvinyl chloride and copper drinking water biofilms by chlorination and chloramination and from the results found that free chlorine was more significantly affected in inactivation than monochloramine on PVC biofilms whereas monochloramines show greater inactivation the free chlorine on Cu biofilms.

Khan et al., (2020) compared chlorination and chloramination inactivation efficiency on microbial strains through a scaled-up water distribution network with a central composite design (CCD). He concluded that the ideal equilibrium needs to be accomplished in water distribution networks through multifactorial streamlining. For both disinfectants, approximately 3 mg/L of the disinfectant dose was retained whereas the contact time was 62 and 155 minutes respectively.

Grunert et al., (2018) experimented on a new-found method for assessing the efficiency of drinking water disinfectants. From his results he confirmed the high efficiency of chlorine and chlorine dioxide, justifying their use as standard disinfectants for evaluating the efficacy of new disinfectants. These findings show that a test rig is an effective tool for evaluating new disinfectants and disinfection techniques.

Qureshi et al., (2020) studied the inactivation dynamics of gram -ve and gram +ve microbes contained in drinking water. This study compared the biocidal competence of chlorine and monochloramine to inactive the gram -ve *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and the gram +ve *Staphylococcus aureus*. A lab scale testing batch was setup in carefully monitored conditions to understand the response of these microbial species to the applied disinfectant dosages 1 and 2 mg/l. The bench-scale experiments shown that chlorine and monochloramine are very effective in damaging

the gram-negative cultures in monocultures. They concluded that for prolonged exposures, monochloramine is advisable owing to its immediate microbial inactivation upon contact as well as enhanced stability in drinking water supplies. These results fill a major gap in the water sector of microbial inactivation DWDs.

Krishna et al., (2013) evaluated the impacts of Nitrification and Chloramination metabolites on microbial strains population in Chloraminated system. 5 lab scale reactors were operated to stimulate Chloraminated distribution system. Cloning and qPCR techniques were established to help characterize and enumerate, mixed microbial communities in the reactors maintained at a residual range of 2.18–0.03 mg/L. Bacterial classes *Nitrospira, Solibacteres, Betaproteobacteria* and *Sphingobacteria* were predominant at low concentrations of chloramine residuals, while *Gammaproteobacteria* & *Actinobacteria* became dominant at higher concentrations. *Methylobacterium Pseudomonas* and *Sphingomonas* were shown to be prevalent before to the commencement of nitrification, and *Sphingomonas* increased with the commencement of nitrification. When the chloramine residuals reduced to below 0.65 mg/L, *Oligotropha, Nitrosomonas urea* was discovered along with 2 new ammonia-oxidizing bacteria. Furthermore, nitrification alone was unable to account for the chloramine degradation rates reported in this study. The conclusions of this study are anticipated to shift attention away from nitrifiers and toward heterotrophic bacteria, which may be the key to devising a control method for better chloramine residue management.

Potgieter et al., (2018) studied seasonal and geographic dynamics of microbial communities present in large-scale drinking water distribution systems in South Africa utilizing 3 successive disinfection procedures (i.e., Chloramination, hypochlorination & Chlorination). From the treatment plants outflow and 17 selective sites throughout the distribution system (a length of around 150-km), bulk samples were taken monthly. The bacterial community composition was determined using Illumina MiSeq sequencing of the 16S rRNA gene's V4 hypervariable region. The drinking water was dominated by bacterial populations of *Alpha* and *Betaproteobacteria*, as in earlier investigations *Betaproteobacteria* population grew during post Chloramination. According to this study, the diversity, richness and evenness of observed bacterial communities were greater in the months of winter as compared to summer, contrary to earlier studies. Fluctuations in average duration of water in the distribution system, as well as matching variations in disinfectant residual concentration, were likely influenced by seasonal fluctuations and temperature changes. The bacterial communities' spatial dynamics revealed distance decay, it was found that the diversity of bacterial communities grew increasingly different as the distance between sampling locations increased. When evaluating the overall distribution system, these spatial impacts were the major factors in diminished temporal variations in the bulk water community. However, chronological variations were generally stronger than spatial changes at specific sampling sites, indicating the impact of seasonality. This study highlights the importance of long-term research in order to fully comprehend temporal patterns that would otherwise be ignored in short-term studies. In addition, rigorous enduring investigations are essential for measuring the impacts of changes in source

water quality. And ambient changes in the composition of the microbial communities of the drinking water distribution system are influenced by environmental conditions and process operations.

Liu et al., (2019) evaluated the impact of chlorine on opportunistic pathogens (OPs) which causes waterborne illness in drinking water distribution systems (DWDS). Chlorine (Cl_2) and ultraviolet/chlorine (UV/Cl_2) were used to treat two models DWDS injected with groundwater to investigate the impacts of chloring on these OPs divided in four distinct phases of the DWDS (bulk water, loose deposits, biofilms & corrosion products). Researchers used 16S ribosomal RNA gene sequencing & qPCR for profiling the microbial population and quantifying target genes. With the same residual chlorine content, single Cl_2 by itself was less efficient as compared to UV/Cl_2 in controlling the regrowth of Opportunistic Pathogens in the water. The OPs found in biofilms, corrosion products, and loose deposits, on the other hand, appeared resistant to Cl_2 & UV/Cl_2, indicating the resistance of the OPs during this stage to the disinfection processes. Spearman correlative analysis revealed significant microbiological correlations between the Opportunistic Pathogens and *Acanthamoeba* ($p<0.05$), indicating that ecological interactions may exist in the DWDS. The structure of the microbial community under UV/Cl_2 and Cl_2 once observed through 16S ribosomal RNA gene sequencing of samples was observed to be significantly different. This research could have ramifications for managing OPs in DWDS that have been disinfected with UV/Cl_2.

Li et al., (2020) explored the community composition and function of juvenile biofilm under the influences of diverse substrate materials (high-density polyethylene, stainless

steel, cast iron, copper and polyvinyl chloride) and disinfectants (chlorine and chloramine), measured using 16S rDNA sequencing. In chlorine-disinfection samples, the dominant classes were *Actinobacteria* (5.90%–40.03%) and *Alphaproteobacteria* (39.14%–80.87%), while in a chloraminated group, the dominant classes were *Betaproteobacteria* (3.79%–68.50%) and *Alphaproteobacteria* (17.46%–74.18%). In the chlorinated samples, the rarely discussed genus *Phreatobacter* became prominent, however was later suppressed by the action of chloramine and copper ions. According to the Adonis test otherwise known as Permutational multivariate analysis of variance and Principle Coordination Analysis (PCoA), different disinfectants were the main drivers of community composition, and the communities of bacteria varied significantly over time. According to Bray-Curtis dissimilarity, biofilm communities developed on cast iron were very different from those developed on other materials, possessing a single dominant genus "*Dechloromonas"*. Metagenomics based estimates using 16S rDNA have been utilized to find antibiotic biosynthesis and beta-lactam resistance operational routes, these routes demonstrated that pathways varied considerably for chlorinated & chloraminated groups.

Jia et al., (2020) evaluated the effect of chlorine-resistant bacteria on drinking water distribution systems and found that they endangers the purity of the water. By analyzing the 16S rDNA gene, a bacterium (identified as *Pseudomonas peli)* was identified from an urban water supply network in northern China. The chlorine tolerance of this strain was very high, with The CT value (a term used to denote the product of disinfectant concentration and contact time) required to inactivate this *P. peli* isolate to 3 lg unit (i.e., 99.9%) was between 51.3 mg min/L to 90.4 mg min/L,

which was indirectly associated with free chloride concentration. Flow cytometry had revealed that chlorine dioxide can inactivate bacteria quicker and more effectively than free chlorine. Free chlorine & chlorine dioxide inactivated *P. peli* through compromising the permeability of the cell membrane. According to thiazole orange plus propidium iodide staining, the *P. peli* was also receptive to ultraviolet radiation; a UV exposure of 40 mJ/cm^2 resulted in inactivation of 4 lg unit (99.99 %). When assessing the disinfection kinetics of *P. peli,* the Hom Model fared better than the Chick Chick-Watson models.

Wang et al., (2020) examined both Antibiotic Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARGs) in the effluent of two hospitals and two wastewater treatment plants (WWTPs). Network analysis revealed eight genra of microbes, including *Bacteroides, Myroides, Mycobacterium, Morganella, Enterococcus, Ferruginibacter, Thermomonas,* and *Romboutsia*, are the most likely hosts of ARGs. In WWTPs, chlorine or ultraviolet (UV) disinfection was used to remove ARGs and their putative bacterial hosts in a synchronous and consistent manner. UV, chlorine, and synergistic UV/chlorine disinfection showed the processes of ARB and ARG elimination, as well as conjugation transfer of RP4 plasmids. ARB inactivation was improved by 1.4 log when compared to UV alone; photoreactivation was efficiently resisted by the combination of UV/chlorine (UV 8 mJ/cm^2, chlorine 2 mg/L). Degradation of ARGs, on the other hand, proved more difficult than inactivation of ARBs. ARGs were reported to have achieved log elimination (0.58–1.60) until the dose of UV was increased to 320 mJ/cm^2. Whereas, during 2 mg/L chlorine coupled with UV, the elimination of ARGs increased from 1 to 1.5 log. Increasing low-dose chlorine (1–2

mg/L) under Ultra Violet radiation had a synergistic effect that significantly increased ARB and ARG elimination at the same time. The horizontal gene transfer had the same synergistic impact as the vertical gene transfer (HGT). The conjugation transfer frequency was increased by a dose of non-lethal chlorine (0.5 mg/L), confirming that the mRNA expression levels of type IV secretion system (T4SS) proteins vir4D, vir5B, and vir10B were dramatically elevated. UV/chlorine greatly lowered the threat of RP4 plasmid conjugation transfer. These findings could have important consequences for assessing and limiting the threat of ARGs spreading and transferring.

Han et al., (2021) studied the combined effects of (UV) ultraviolet chloramine disinfection in a drinking water supply system using metagenomics to assess the distribution, community structure, diversity and hosts. The combined method decreased number of viral species (6%) and gene abundance (52%) but failed to eradicate the viruses from the water in their entirety, according to the findings. Based on culturing methods, the United States Environmental Protection Agency has claimed that viral elimination efficiency from water can reach from 99-99.99%. Only 93.46% of all viruses were removed, according to metagenomic research. As a result, the culturing method for detecting viruses in water cannot consistently detect viruses in drinking water. Lentivirus may infect both humans and vertebrates and is resistant to UV and chloramine treatment. In the water supply system under investigation, bacteria were the primary virus hosts (61.50%). Most of the viruses were parasite in synechococcus. Both effluent water and pipe network water samples showed Pseudomonas as the most common viral host. There was increase of 342.6% of *Pseudomonas aeruginosa* host in pipe which requires higher attention. They concluded

that combined UV chloramine disinfection was more effective as compared to single UV disinfection (51.9% from 0.79%) for the removal of virus.

Huo et al., (2021) investigated the influence of disinfectants on the presence of bacteria, including heterotrophic plate count (HPC), total coliforms and various opportunistic pathogens (OPs), and amoeba masses in DWDSs from 5 different drinking water treatment plants. The results of the study revealed residual chlorine higher than 0.05 mg/L and total chlorine higher than 0.35mg/L, when HPCs fell lower than 500 CFU/mL. There was a good relationship between HPC and OPs. The growth of OPs in DWDSs can be controlled in DWDSs by limiting HPC. Meanwhile, *Pseudomonas aeruginosa*, *Legionella spp*., and *Hartmannella vermiformis* were detected positively (100%) and *Legionella pneumophila* was detected in more than 90% of all the samples risking human health. The growth of OPs in DWDSs can be effectively controlled by keeping the free chlorine residual between 0.15–0.20 mg L1 and the total chlorine residual at 0.35–0.50 mg/L. This study suggested that growth of *Mycobacterium avium*, *Acanthamoeba spp* and *Legionella pneumophila* the particle number should be kept below 300. The turbidity of the water should be kept between 0.25 and 0.35 NTU. There has been a reported trade-off between the selection of disinfectant residuals and/or particles for controlling the growth of OPs in DWDs.

Li et al., (2014) investigated the impact of chlorine and chloramine on microbial biomass & community structure in DWDS with AR reactors. When the biomass contained in the biofilms achieved a pseudo steady state, the chlorine alone performed as a better disinfectant than chloramine and the other disinfectants, with HPC reduced by 3-log and 1-log, respectively. No significant variation in the relative abundance of

the core populations of the biofilms on the coupon with respect to the growth within the two-month timeframe. According to 16S rRNA-based T-RFLP analysis. The most dominant T-RFs in biofilm and buck water were both 88bp, with 67bp, 493bp, & 497bp as subdominant T-RFs. Compared to the control group, pyrosequencing data demonstrated that the relative abundance of prominent bacteria changed on a consistent basis at the class level, indicating that bacteria that are less sensitive to disinfectants needed more attention.

Donohue et al., (2019) investigated the disinfection efficacy of the chloramine on five pathogens which were selected for the study. The disinfected water samples were collected from public water utilities all cross United States. Probe quantitative methods were selected for the bacterial quantification. The results revealed that the chloramine is effective in controlling the gram-negative Legionella pneumophilia.

Rose et al., (2007) conduct an experiment to test the susceptibility of seven selected bacterial strains against monochloramine. The selected bacterial strains include *Bacillus anthracis, Brucella suis, Brucella melitensis , Francisella tularensis , Burkholderia pseudomallei, Yersinia pestis and Burkholderia mallei.* The experiment was performed at three different temperatures 5˚,10˚and 15˚. The monochloramine was routinely maintained on potable water which produces a 2-log reduction in the six bacterial species within 4.2 h.

Li et al., 2021 investigated the resistance of *Staphylococcus aureus and Escherichia coli* against chlorine and chloramine. The no-disinfectants particles in drinking water were selected to build particle-associated bacterial systems. The results shows that the

particles cam have protective effects on bacteria in half of chlorine experiments and 90% of chloramination.

Lee et al., 2011 examined the penetration of free chlorine and monochloramine biofilms into an undefined mixed-culture nitrifying biofilm. Microelectrodes were used to examine the subsequent influence on biofilm activity. Viability assessed buy the use of dissolved oxygen electrode whereas confocal laser scanning microscopy with live/dead backlight. Monochloramine entered biofilms 170 times quicker than free chlorine, while free chlorine penetration was inhibited following monochloramine administration. DO profiles providing the evidence that the biofilms were inactivated with the monochloramines penetration.

Chiao et al., (2014) employing culture dependent and independent methodologies, to investigate the influence of monochloramine disinfection on the complex bacterial community structure in DWS. The study's findings show that bacterial populations in drinking water have varying levels of resistance to chloramine, and that the procedure favors the disinfection of resistant bacterial strains.

Chapter 3

Materials and Methods

The study mainly comprises of two phases. The first phase is the batch study which comprises of bench scale experiments for the optimization of disinfection dosages of chlorine and chloramine to inactivate the microbial strain. The second phase of the study involve the prototype study in which the experiments were carried out in carefully monitored conditions in the laboratory prototype distribution network model present in the Environmental Toxicology Lab, IESE, NUST, Islamabad, Pakistan. Two-gram negative bacterial strains *Salmonella enterica* and *Shigella dysenteriae* were selected. Freeze dried culture of *S. enterica and S. dysenteriae* was obtained and revived according to ATCC recommendations (ATCC, 2004).

3.1 Preparation of Precursors

3.1.1 Bacterial Innoculum

After reviving the microbial strains, *Salmonella enterica* and *Shigella dysenteriae* colonies were taken from nutrient agar plates and inoculated to the nutrient broth test tubes. These tubes were incubated at 37°C for 24 hours. The bacterial culture was twice rinsed with a phosphate buffer with the pH of 7 to avoid the accumulation of non-cellular components in the system. Afterwards 10 minutes of centrifugation at 4000 rpm was conducted till a pellet formed at the base. The optical density of solution was measured using spectrophotometer to approximate bacterial count to 10^6 CFU/ml (Amiri *et al.,* 2010). Approximately 1.0 ml of this suspension was added to 500 ml flasks each containing autoclaved distilled water. Before disinfection, serial dilutions

were prepared for spread plate count (SPC) after culture inoculation. The result of this process was an actual number of around 10^6 CFU/ml bacteria in the sample prior to chlorine disinfection experiment.

3.1.2 Chlorine and Monochloramine Solutions

5% sodium hypochlorite stock solution was prepared for free chlorine in distilled water to get chlorine solutions. Final free chlorine concentrations of 0.5, 1.0 and 2.0 mg/L were achieved by preparing further dilutions. The concentrations of free chlorine were verified through *N,N*-Diethyl-*p*-Phenylenediamine (DPD) ferrous titrimetric method according to standard method (APHA, 2017).

Monochloramine (NH_2Cl) stock solutions were prepared through the dissolution of Ammonium hydroxide (1M) solution in a solution of Sodium hypochlorite (1M) which was later cooled to 0°C. NaOCl solution was added gradually with stirring and cooling to NH_4OH solution. NH_4:NaOCl mixing ratio was set at 3:1. To minimize the disproportionation of NH_2Cl to dichloramine ($NHCl_2$) pH was adjusted at 8.5, as $NHCl_2$ forms at pH < 8 (U.S. Environmental Protection Agency, 1999). The Final dose of chloramine was measured through *N,N*-Diethyl-*p*-Phenylenediamine (DPD) ferrous titrimetric method according to standard method.

3.2 Phase 1: Batch Study

3.2.1 Experimental setup

For bench-scale experiments, 500 ml capped flasks were used. All the glassware was washed and autoclaved prior to use. The flasks were kept at room temperature (around 20°C) and covered with aluminum foil to provide dark conditions in order to avoid

photolysis. For chlorination, 5% NaOCl solution was prepared and added in one set of flasks (experimental set). The control was left without any disinfectant. Same inputs and precursors were used for chloramine setup with the exception of ammonium chloride to prepare monochloramines, leaving control disinfectant-free. All experiments were conducted in replicates.

3.2.2 Sampling and Analysis

After the addition of bacterial inoculum, chlorine and chloramine dose, samples were periodically collected at 1,10 and 30 minutes in designated sterile glassware with appropriate preservation for further analysis.

3.2.3 Sample Collection and Preservation

Microbial samples (5 ml) were obtained in sterile test tubes, at selected time intervals. 0.1 mL sodium thiosulfate was added to quench any residual disinfectant. After sampling the samples were stored in the dark at low temperatures (4°C) for further microbial analysis. The addition of $Na_2S_2O_3$ fixes excess chlorine and inhibits its actions on microorganisms to avoid interference with the exact SPC. Afterwards samples (50 mL) were collected and underwent pre-chlorination, physicochemical measurements were conducted following selected time intervals and for samples of chlorine or monochloramine residual, pH and temperature analysis was conducted immediately after sampling.

3.2.4 Standard Plate Count (SPC)

Agar plates for SPC were made by pouring roughly 20 mL of molten agar into petri plates, evenly distributing it, and incubating it upside down at 37 C for 24 hours. Serial dilutions were used to obtain an accurate and countable range of microbial colonies,

i.e., 30-300 colonies. Pipetting 0.1 mL of serial dilution onto a sterile petri plate containing agar and gently spreading it with a spreader coated in 70% alcohol and flamed was used to plate each dilution.. Once the sample was spread uniformly on the plate, it was placed in incubator at 37°C for 24 hrs. Before and after treatment samples were taken to measure bacterial count in order to check bacterial inactivation of both disinfectants. Viable cell count was made after 24 hours using Colony Counter. Viable ells represent bacteria that are able to form colonies via reproduction.

3.2.5 Physicochemical Analysis

Samples were taken before and after the addition of disinfectant to measure residual chlorine and monochloramine, pH, temperature, Electrical Conductivity, TDS, turbidity, Hardness, Alkalinity, DO. Testing instruments and methods used are tabulated in

Parameters	Technique/ Instruments	References
pH	pH meter	APHA, 2017
Temp (°C)	Thermometer	
EC (µS/cm)	Conductivity meter	
Turbidity (NTU)	Turbidimeter	
TDS	Gravimetric method	
Residual Chlorine (mg/L)	DPD Titrimetric method	
Microbial analysis (CFU/mL)	Spread Plate Count	
Alkalinity	Titration Method	
Hardness	Titration Method	
Do	Do meter	

3.2.6 Residual chlorine and chloramine measurement

Chlorine and monochloramine residuals were determined by *N*,*N*-Diethyl-*p*-Phenylenediamine (DPD) ferrous titrimetric method according to standard method (APHA, 2017).

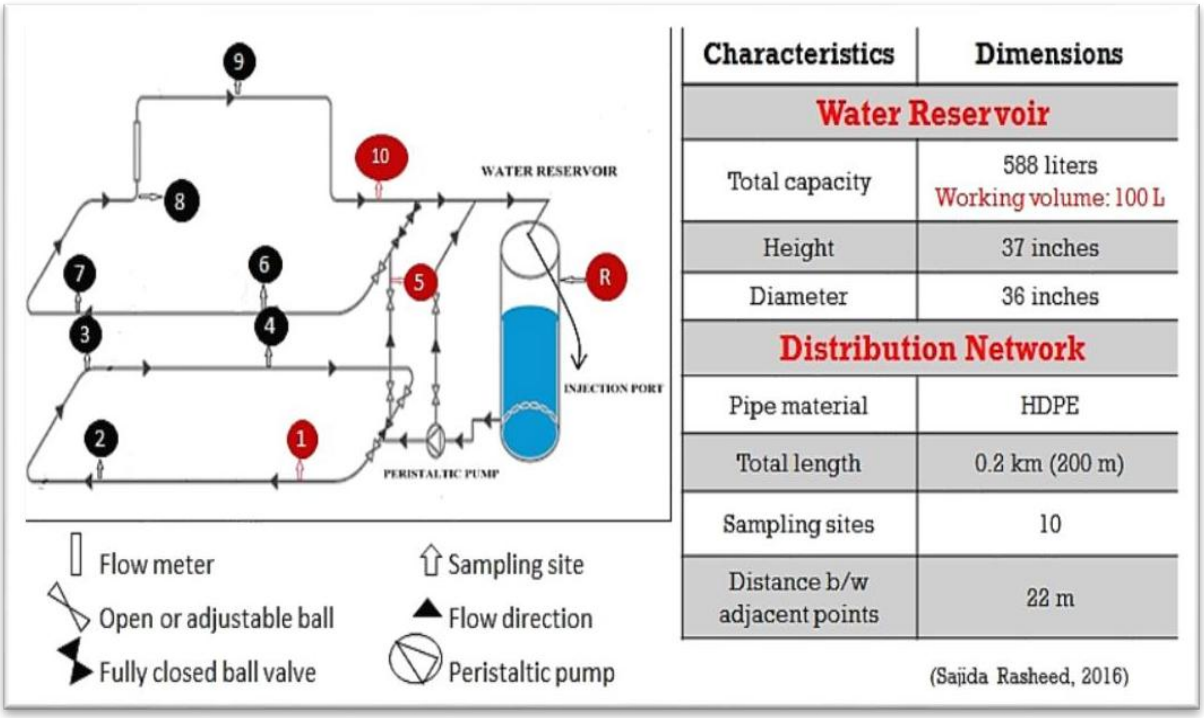
3.3 Phase 2: Prototype Study:

2 mg/L disinfection dosage of chlorine and chloramine optimized from the bench scale experiments was applied in the laboratory prototype distribution network to study the inactivation of gram-negative bacterial strains at 1, 30 and 60 minutes of the contact time. The water used for the study was de-chlorinated tap water wherein all precursors and bacterial culture was added maintaining sterile conditions. 24 hours freshly cultured inoculum of *Salmonella enterica and Shigella dysenteriae* in nutrient broth was centrifuged at 4000 rpm, suspended twice in phosphate buffer and set at 10^6 CFU/ml. Sampling was performed using autoclaved sampling bottles at specific contact times. Bacterial count was determined by Spread Plate Count. Same procedure was carried out for chloramine study.

3.3.1 Specification of Laboratory Prototype DN Model

To investigate the influence of drinking water factors on chlorine degradation and bacterial regrowth, a laboratory scale distribution network system (Prototype) was established at IESE, SCEE, NUST with water reservoir capacity of 588 liters, working volume was kept 100 liters in the study. The model of the distribution network was built

of 1-inch PVC pipe, fittings, and valves. The conceptual diagram depicts a tap water reservoir (1) that operates a water pump (2) by gravity flow. Septum ports labelled (3), (4), and (5) will be utilized to inject contaminants into the system. The travel distance between the contaminant injection septum (3) and the free chlorine sensor sampling location (6) is approx. 20 feet (6.1 m). Union fittings will be used to add and remove pipe sections as necessary.



Characteristics	Dimensions
Water Reservoir	
Total capacity	588 liters Working volume: 100 L
Height	37 inches
Diameter	36 inches
Distribution Network	
Pipe material	HDPE
Total length	0.2 km (200 m)
Sampling sites	10
Distance b/w adjacent points	22 m

(Sajida Rasheed, 2016)

Figure 3.1: Specifications of prototype distribution network

Chapter 4

Results and Discussion

In this section, the results attained from the experiments conducted in two phases are discussed. The first phase contains results attained from the bench scale experiments whereas second phase include results attained from the prototype study. The present study aimed to determine the optimum dosages of chlorine and chloramine for maximum inactivation of the *Salmonella enterica* and *Shigella dysenteriae.*

4.1 Phase:1: BENCH SCALE DISINFECTION STUDIES

4.1.1 Chlorination:

Chlorination was performed for maximum inactivation of the microbial strains and to determine an optimum dose. The medium used for the inactivation study was dechlorinated tap water and the physicochemical parameters were measured at each sampling interval. Ct value was obtained by multiplying the chlorine dose with time. The initial inoculum count was set to 10^6 CFU/ml. The chlorine dosages applied were 0.5, 1.0 mg/L and 2.0 mg/L respectively. The contact time for maximum microbial inactivation was 1 minute, 10 minutes and 30 minutes respectively for the bench scale experiments. Ct values (mg.min/liter) to estimate the residual chlorine and time required for desired bacterial inactivation, also useful in comparing disinfectants' efficiencies. (Amiri et al., 2010)

4.1.1.1 *Salmonella enterica* Inactivation Study

0.5 mg/L dose of chlorine was applied and inactivation of *Salmonella enterica* was observed at 1,10 and 30 minutes of the contact time. At 1 min of the contact time, the initial *Salmonella enterica* inoculum of 2.20×10^{6} CFU/ml was reduced to 1-Log. At 10 minutes of the contact time, further 2-Log reduction was observed. 3-Log reduction was observed at 30 minutes of the contact time as shown in the Figure 4.1

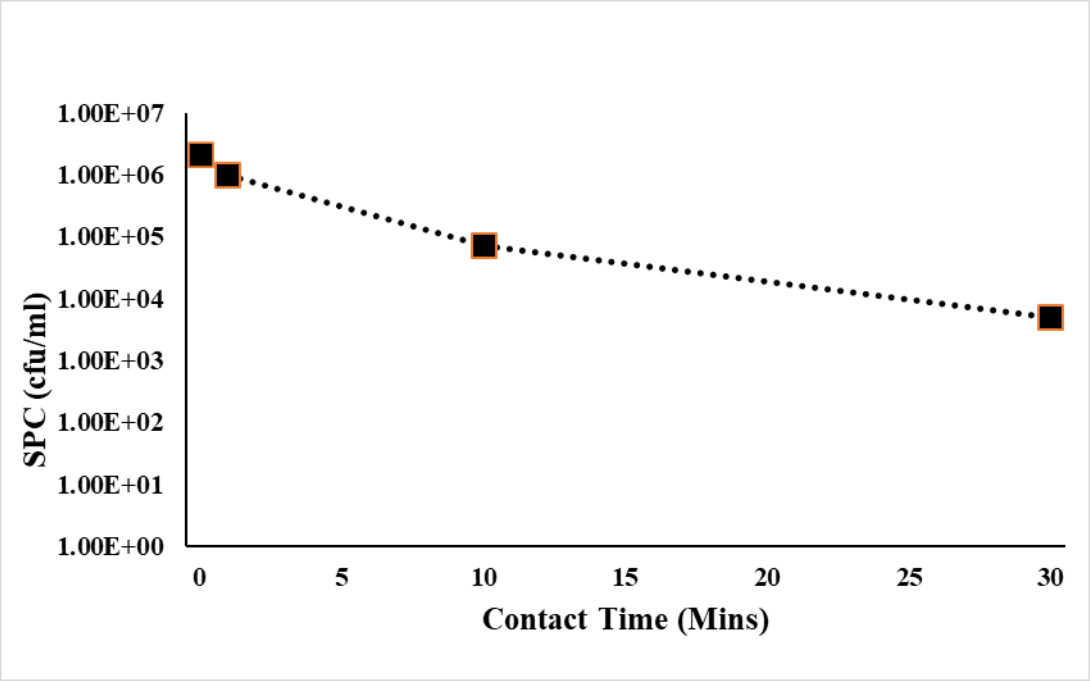


Figure 4.1: *Salmonella enterica* inactivation at 0.5 mg/L Chlorine dose

Residual chlorine measured was 0.4 mg/L, 0.3 mg/L and 0.1 mg/L at 0.5, 5 and 15 Ct value as shown in Table 4.1

Ct value (mg.min/L)	Residual free chlorine (mg/L)	Log_{10}-Removal (CFU/ml)
0.5 mg/L chlorine dose		
0.5	0.4	9.8×10^5
5	0.3	7.1×10^4
15	0.1	5.0×10^3

Table:4.1 Log inactivation of *Salmonella enterica* at various CT values

When a dose of 1.0 mg/L chlorine was applied, the microbial count was reduced to 2-Log after 1 minute of the contact time. A 3-Log reduction was observed at 10 minutes of the contact time whereas after 30 minutes of the contact time, no evident change was observed in microbial count and similar 3-Log inactivation was achieved after 30 minutes of the contact time as shown in Figure 4.2.

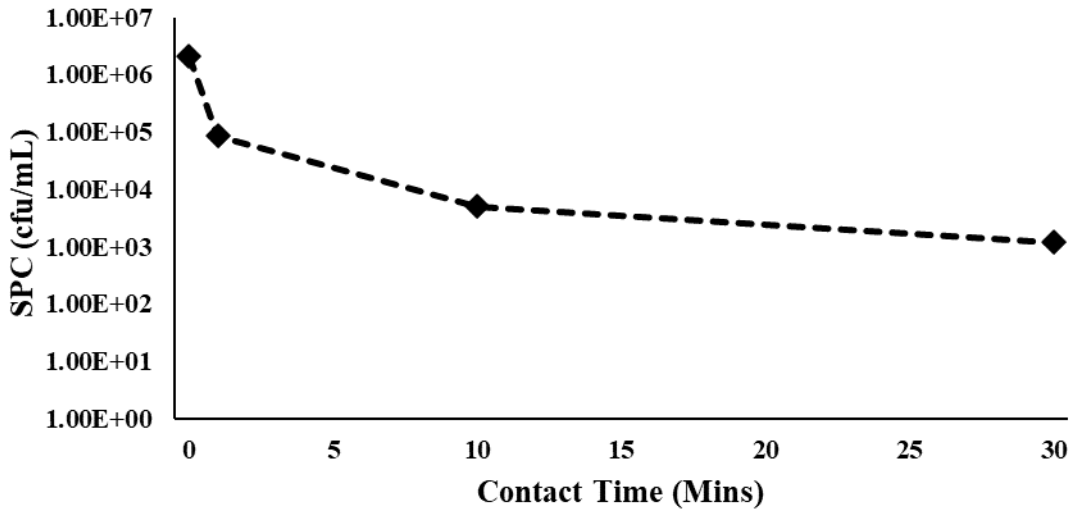


Figure4.2: *Salmonella enterica* inactivation at 1.0 mg/L Chlorine dose

The residual chlorine was measured at 1, 10 and 30 minutes of the contact time was 0.8, 0.6 and 0.4 mg/L as shown in Table 4.2

Ct value (mg.min/L)	**Residual free chlorine (mg/L)**	**Log_{10}-Removal (CFU/mL)**
1.0 mg/L chlorine dose		
1	0.8	8.5×10^{4}
10	0.6	5.0×10^{3}
30	0.4	3.0×10^{2}

Table4.2: *Salmonella enterica* log inactivation at various Ct values

At 2.0 mg/L chlorine dose, 2-Log inactivation was observed at 1 minute of the contact time. After 10 minutes of the contact time, 4-Log reduction of *Salmonella enterica* was observed whereas complete inactivation of *Salmonella enterica* was achieved after 30 minutes of the contact time at 2.0 mg/L chlorine dose as shown in Figure 4.3

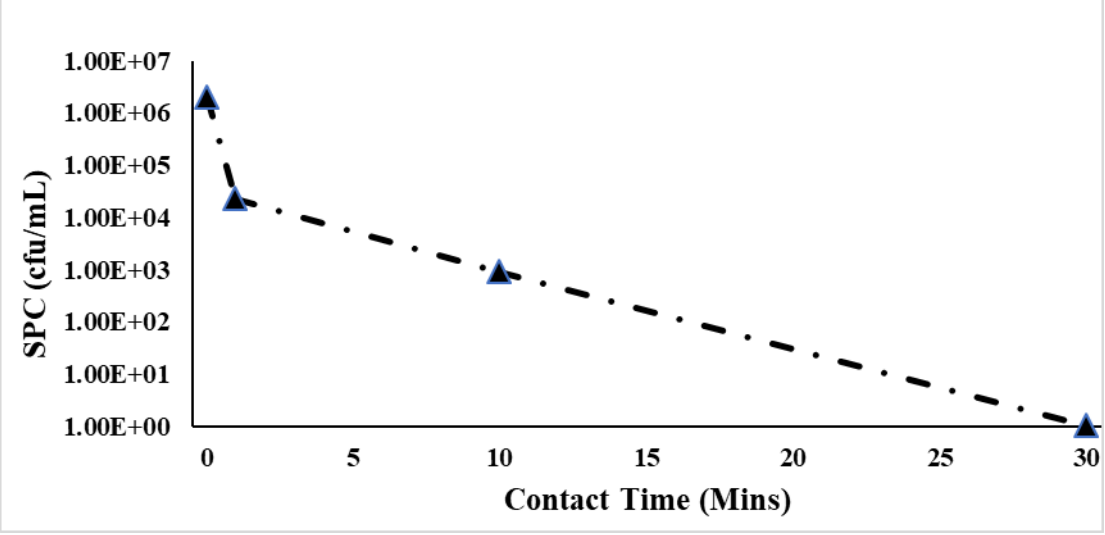


Figure:4.3 *Salmonella enterica* inactivation at 2.0 mg/L Chlorine dose

The results are contrary to study conducted by LeChevallier in 1988 on factors promoting bacterial survival in chlorinated water supplies and the results in shows that 99 percent of viable bacterial counts decreased on exposure to 0.8 mg/L of hypochlorous acid at pH 7 for 1 min.

The residual chlorine measured at 2, 20 and 60 mg.min/L of the ct values was 1.4, 0.8, 0.4 mg/L respectively as shown in Table 4.3

Ct value (mg.min/L)	**Residual free chlorine (mg/L)**	**Log_{10}-Removal (CFU/mL)**
2.0 mg/L chlorine dose		
2	1.4	1.00×10^{4}
20	0.8	9×10^{2}
60	0.4	1.0×10^{0}

Table 4.3: *Salmonella enterica* inactivation at various CT values

4.1.1.2 *Shigella Dysenteriae* Inactivation Study

0.5 mg/L chlorine dose was applied and inactivation of *Shigella dysenteriae* was observed at 1, 10 and 30 minutes of the contact time. At 1 minute of the contact time, no evident log reduction was observed in the initial *Shigella dysenteriae* inoculum of 2.20×10^{6} CFU/ml. At 10 minutes of the contact time, further a lower 1-Log reduction was observed. No greater reduction in log value was achieved after 30 minutes of the contact time as shown in the Figure 4.4

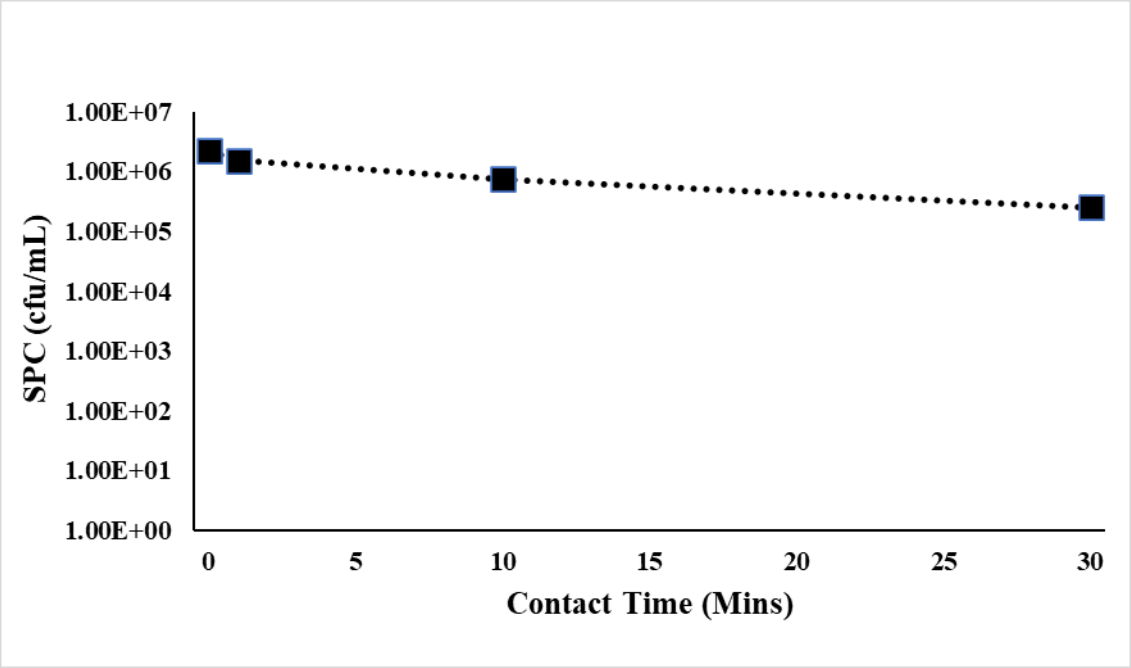


Figure4.4*: Shigella dysenteriae* inactivation at 0.5 mg/L Chlorine dose

The residual chlorine measured at 0.5, 5 and 15 mg.min/L of the ct values was 0.3, 0.2, 0.1 mg/L respectively as shown in Table 4.4

Ct value (mg.min/L)	Residual free chlorine (mg/L)	Log_{10}-Removal (CFU/mL)
0.5 mg/L chlorine dose		
0.5	0.3	1.55×10^{6}
5	0.2	7.60×10^{5}
15	0.1	2.55×10^{5}

Table:4.4 *Shigella dysenteriae* inactivation at various Ct values

When 1.0 mg/L chlorine dose was applied, the microbial count was reduced to 1-Log after 1 minute of the contact time. A 2-Log reduction was observed at 10 minutes of the contact time whereas after 30 minutes of the contact time, no evident change was

observed in microbial count and similar 2-Log inactivation was achieved after 30 minutes of the contact time as shown in Figure 4.5

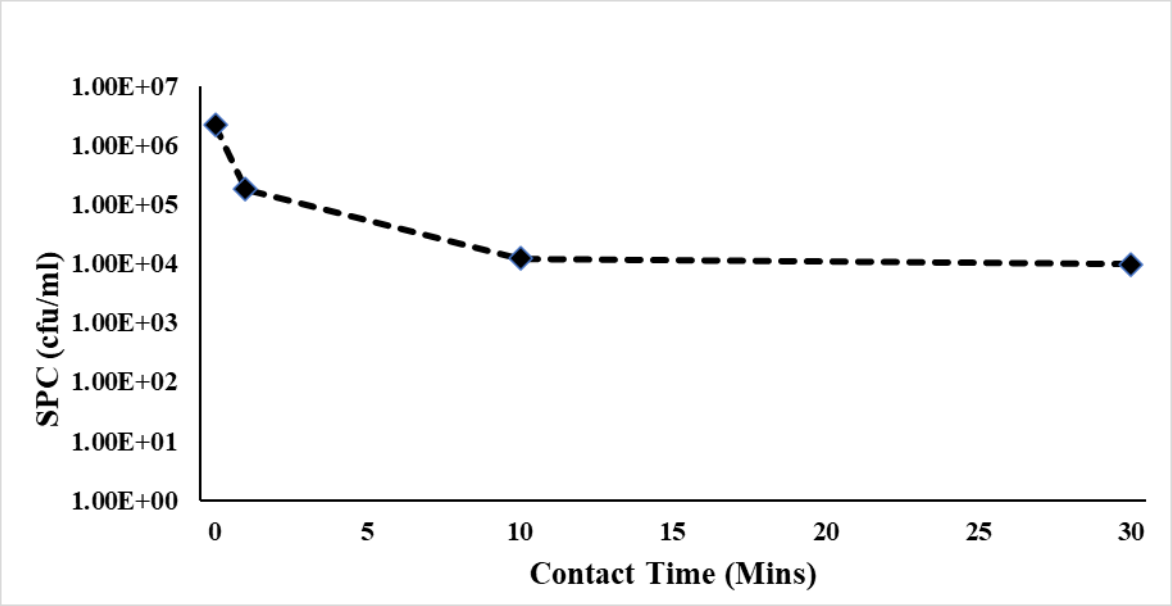


Figure 4.5: *Shigella dysenteriae* inactivation at 1.0 mg/L Chlorine dose

These results are contrary to the study conductedby Sidra Tul Muntaha in 2014 and LeChavelliar in 1985 that higher chlorine dose is required to completely inactivate the *shigella spp*.

The residual chlorine was measured at 1,10 and 30 minutes of the contact time was 0.6, 0.4 and 0.2 mg/L as shown in Table 4.5

Ct value (mg.min/L)	Residual free chlorine (mg/L)	Log_{10}-Removal (CFU/mL)
1.0 mg/L chlorine dose		
1	0.6	1.78×10^{5}
10	0.4	1.21×10^{4}
30	0.2	9.8×10^{3}

Table 4.5: *Shigella dysenteriae* inactivation at various Ct values

At 2.0 mg/L chlorine dose, 2-Log inactivation was observed at 1 minute of the contact time. After 10 minutes of the contact time, 3-Log reduction of *Shigella dysenteriae* was observed whereas after 30 minutes of the contact time at 2.0 mg/L chlorine dose, 5-Log reduction of *Shigella dysenteriae* was observed as shown in Figure 4.6

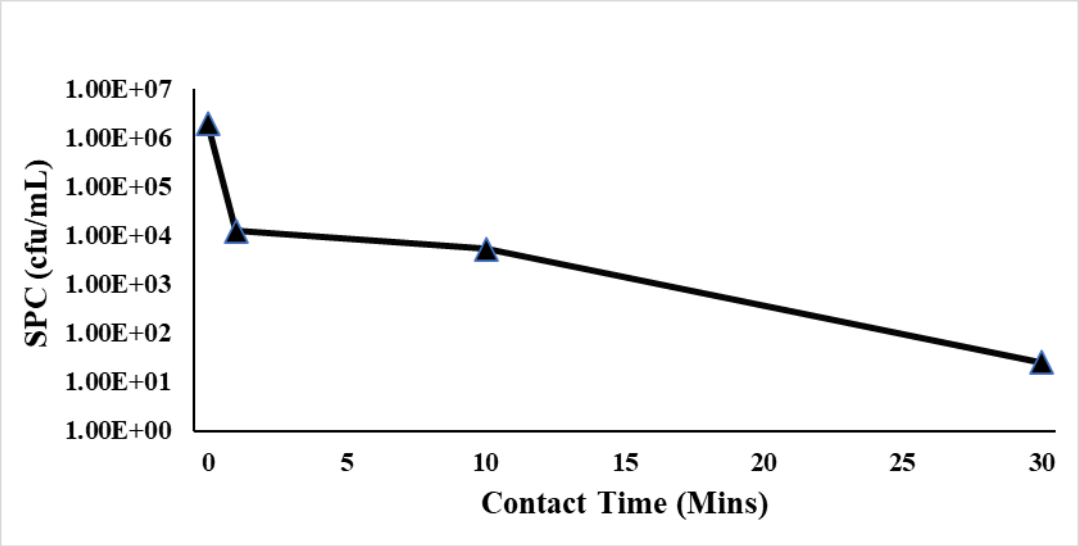


Figure 4.6: *Shigella dysenteriae* inactivation at 2.0 mg/L Chlorine dose

Similar result was reported by LeChevallier et al., (1985) that higher chlorine doses (1.5 mg/L) were necessary to produce injured *Shigella spp.* and *Yersinia enterocolitica* than to produce injured *Escherichia coli* or *coliform* bacteria (0.25 to 0.5 mg/L).

The residual chlorine measured at 2, 20 and 60 mg.min/L of the Ct values was 0.9, 0.6, 0.3 mg/L respectively as shown in Table 4.6

Ct value (mg.min/L)	Residual free chlorine (mg/L)	Log_{10}-Removal (CFU/mL)
2.0 mg/L chlorine dose		
2	0.9	1.25×10^{4}
20	0.6	5.4×10^{3}
60	0.3	2.50×10^{1}

Table 4.6: *Shigella dysenteriae* inactivation at various Ct values

4.1.2 Chloramination:

Chloramination was performed for maximum inactivation of the microbial strains and to determine an optimum dose in comparison to chlorination. The medium used for the inactivation study was dechlorinated tap water and the physicochemical parameters were measured at each sampling interval. Ct value was obtained by multiplying the chlorine dose with time. The initial inoculum count was set to 10^6 CFU/ml. The chlorine dosages applied were 0.5 mg/L, 1.0 mg/L and 2.0 mg/L respectively. The contact time for maximum microbial inactivation was 1,10 and 30 minutes respectively for the bench scale experiments.

4.1.2.1 *Salmonella enterica* Inactivation Study

0.5 mg/L chloramine dose was applied and inactivation of *Salmonella enterica* was observed at 1,10 and 30 minutes of the contact time. At 1 minute of the contact time, the initial *Salmonella enterica* inoculum of 2.20×10^6 CFU/ml was reduced to 3-Log. At 10 minutes of the contact time, further 4-Log reduction was observed. 5-Log reduction was observed at 30 minutes of the contact time as shown in the Figure 4.7

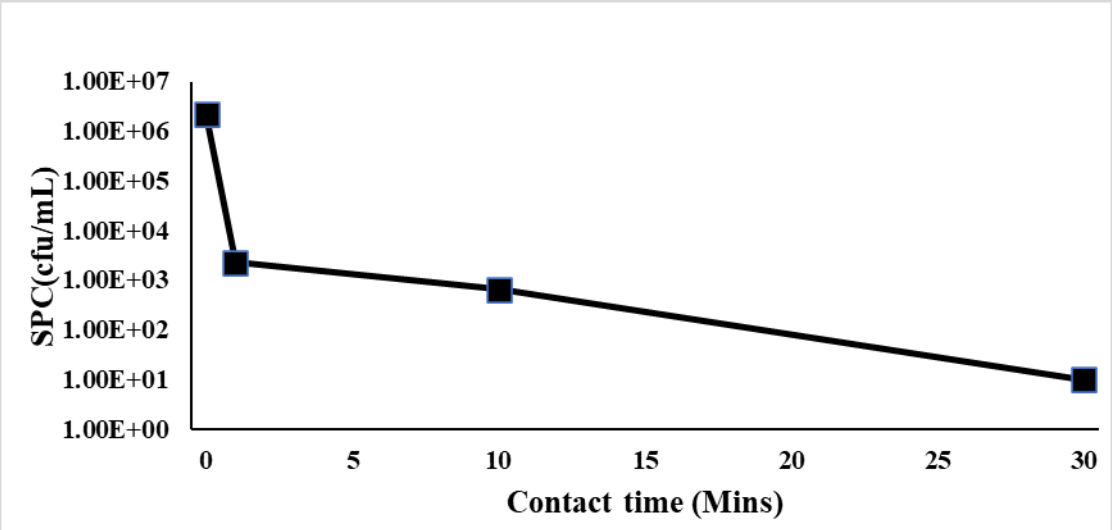


Figure 4.7: *Salmonella enterica* inactivation at 0.5 mg/L Chloramine dose

Residual chlorine measured was 0.3, 0.2 and 0.1 mg/L at 0.5, 5 and 15 Ct value as shown in Table 4.7

Ct value (mg.min/L)	**Residual monochloramine (mg/L)**	**Log_{10}-Removal (CFU/mL)**
0.5 mg/L chloramine dose		
0.5	0.3	2.25×10^{3}
5	0.2	6.50×10^{2}
15	0.1	1.00×10^{1}

Table 4.7: *Salmonella enterica* inactivation at various Ct values

When 1.0 mg/L chloramine dose was applied, the microbial count was reduced to 4-Log after 1 minute of the contact time. A 5-Log reduction was observed at 10 minutes of the contact time whereas complete inactivation was achieved after 30 minutes of the contact time as shown in Figure 4.8

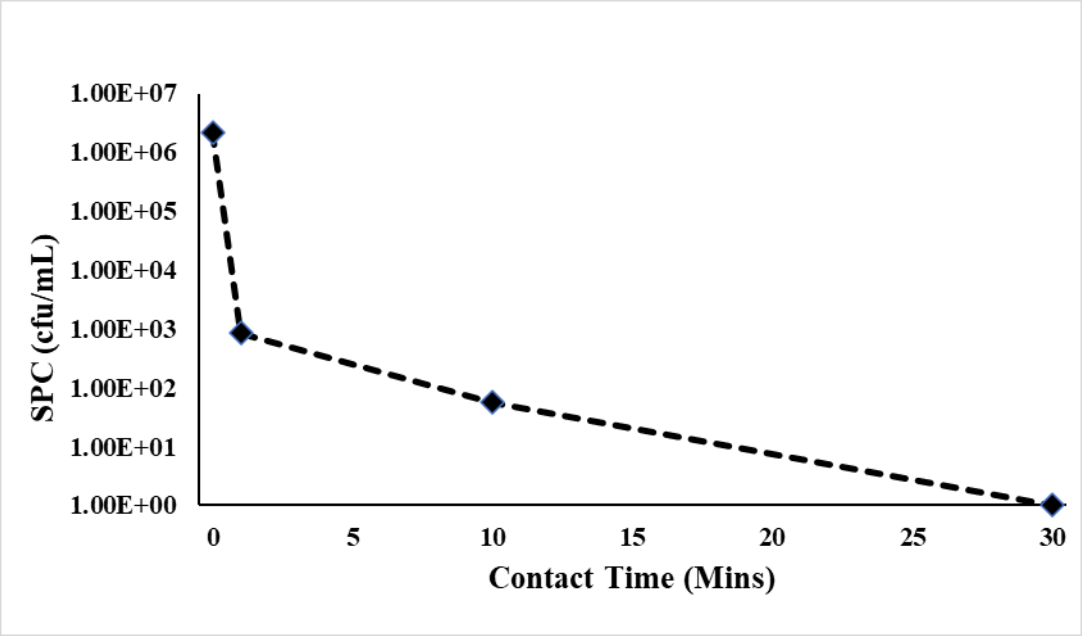


Figure 4.8: *Salmonella enterica* inactivation at 1.0mg/L Chloramine dose

The residual chlorine was measured at 1,10 and 30 minutes of the contact time was 0.6, 0.4 and 0.4 mg/L as shown in Table 4.8

Ct value (mg.min/L)	Residual monochloramine (mg/L)	Log_{10}-Removal (CFU/mL)
1.0 mg/L chloramine dose		
1	0.6	8.50×10^{2}
10	0.4	5.60×10^{1}
30	0.4	1.0×10^{0}

Table 4.8: *Salmonella enterica* inactivation at various Ct values

At 2.0 mg/L chloramine dose, 5-Log inactivation was observed at 1 minute of the contact time. After 10 minutes of the contact time, 6-Log reduction of *Salmonella enterica* was observed whereas complete inactivation of *Salmonella enterica* was

achieved after 30 minutes of the contact time at 2.0 mg/L chloramine dose as shown in Figure 4.9

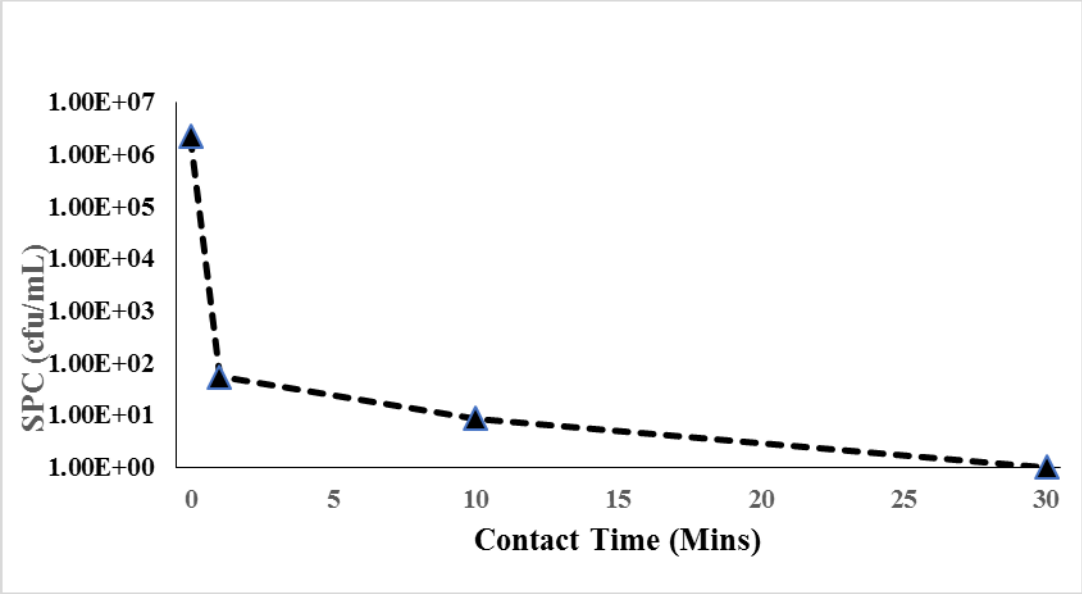


Figure 4.9: *Salmonella enterica* inactivation at 2.0 mg/L Chloramine dose

Similar results were reported in the study conducted by Qureshi et al., in 2020 on the comparison of chlorine and chloramine inactivation efficacy on various gram-negative and gram-positive microbial strains and the result shows that this sudden inactivation in microbial viable count predicts monochloramines are greatly effective in reducing bacterial population in water.

The residual chlorine measured at 2, 20 and 60 mg.min/L of the Ct values was 1.2, 0.8, 0.8 mg/L respectively as shown in Table 4.3

Ct value (mg.min/L)	Residual free chlorine (mg/L)	Log_{10}-Removal (CFU/mL)
2.0 mg/L chloramine dose		
2	1.2	5.40×10^{1}
20	0.8	8.67×10^{0}
60	0.8	1.0×10^{0}

Table 4.9: *Salmonella enterica* inactivation at various Ct values

4.1.2.2 *Shigella dysenteriae* Inactivation Study

0.5 mg/L chloramine dose was applied and inactivation of *Shigella dysenteriae* was observed at 1,10 and 30 minutes of the contact time. At 1 minute of the contact time, 1-log reduction was observed in the initial *Shigella dysenteriae* inoculum of 2.20×10^{6} CFU/ml. At 10 minutes of the contact time, further a lower 3-Log reduction was observed. After 30 minutes of the contact time, 4-Log reduction was achieeved as shown in the Figure 4.10

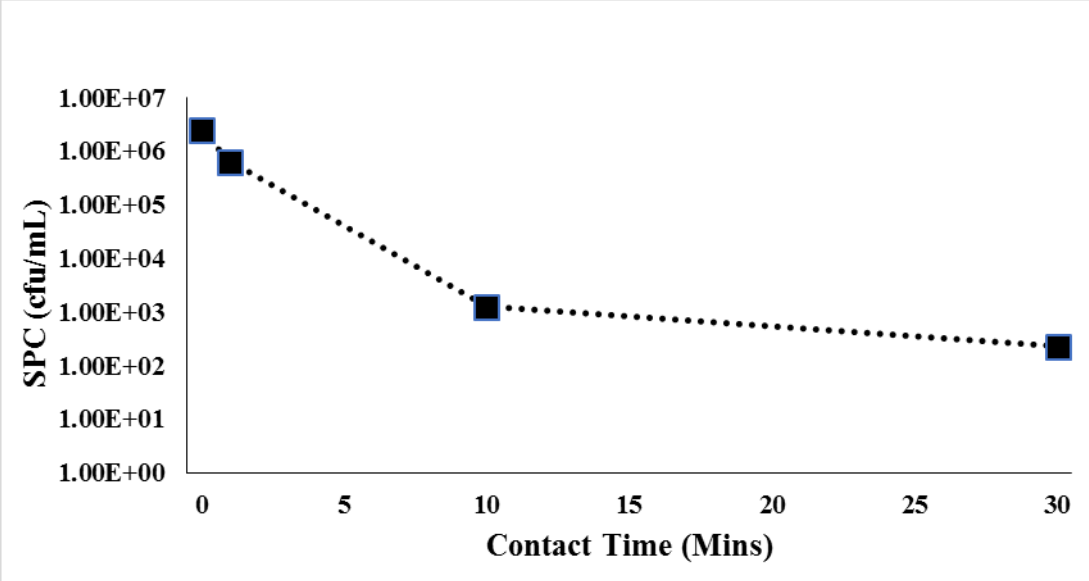


Figure 4.10: *Shigella dysenteriae* inactivation at 0.5 mg/L Chloramine dose

Residual chlorine measured was 0.3, 0.2 and 0.1 mg/L at 0.5, 5 and 15 Ct value as shown in Table 4.10

Ct value (mg.min/L)	Residual free chlorine (mg/L)	Log_{10}-Removal (CFU/mL)
0.5 mg/L chloramine dose		
0.5	0.4	6.40×10^{5}
5	0.3	1.25×10^{3}
15	0.1	2.25×10^{2}

Table 4.10: *Shigella dysenteriae* inactivation at various Ct values

When 1.0 mg/L chloramine dose was applied, the microbial count was reduced to 2-Log after 1 minute of the contact time. A 4-Log reduction was observed at 10 minutes of the contact time whereas complete inactivation was achieved after 30 minutes of the contact time as shown in Figure 4.11

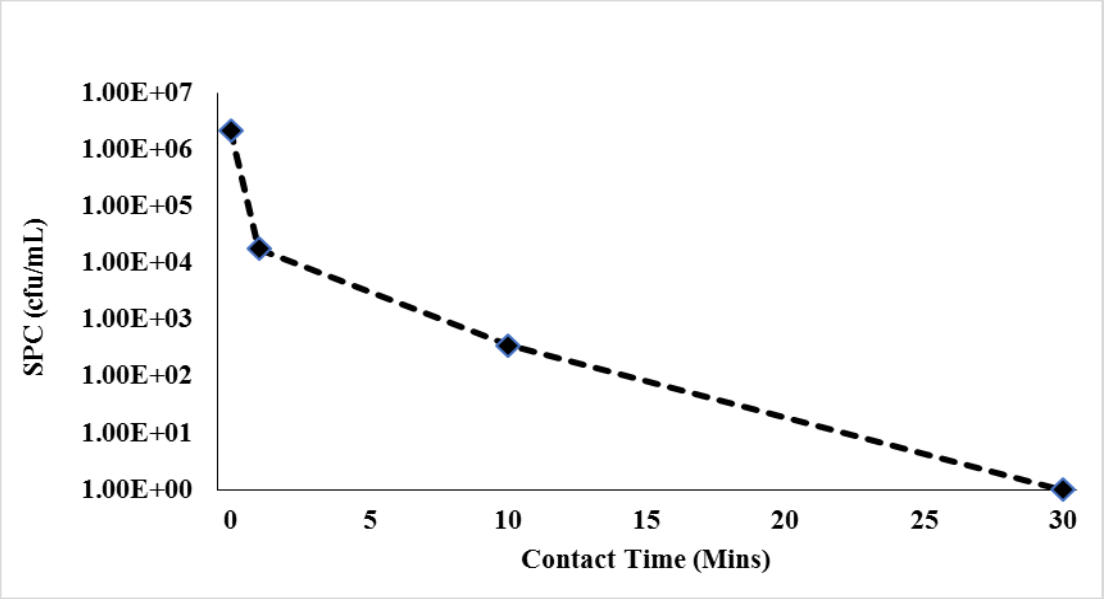


Figure 4.11: *Shigella dysenteriae* inactivation at 1.0 mg/L Chloramine dose

These results contradict the finding of the study conducted by Chiao et al., 2014 on differential resistance of drinking water bacterial population to monochloramine and the results demonstrate that bacterial populations in drinking water exhibit differential resistance to chloramine and the process selects for the disinfection of resistant bacterial strains

The residual chlorine was measured at 1,10 and 30 minutes of the contact time was 0.7, 0.5 and 0.3 mg/L as shown in Table 4.11

Ct value (mg.min/L)	**Residual free chlorine (mg/L)**	**Log_{10}-Removal (CFU/mL)**
1.0 mg/L chloramine dose		
1	0.7	1.75×10^{4}
10	0.5	3.5×10^{2}
30	0.3	1.0×10^{0}

Table 4.11: *Shigella dysenteriae* inactivation at carious Ct values

At 2.0 mg/L chloramine dose, 4-Log inactivation was observed at 1 minute of the contact time. After 10 minutes of the contact time, 6-Log reduction of *Salmonella enterica* was observed whereas complete inactivation of *Shigella dysenteriae* was achieved after 30 minutes of the contact time at 2.0 mg/L chloramine dose as shown in Figure 4.12

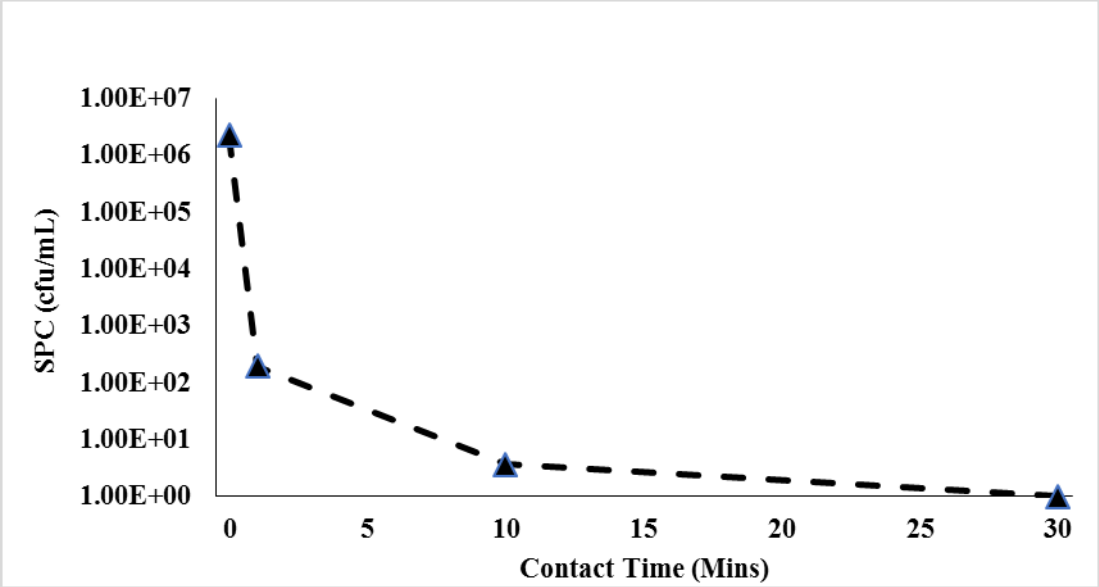


Figure 4.12: *Shigella dysenteriae* inactivation at 2.0 mg/L Chloramine dose

These results are contrary to the results of the study conducted by Donohue et al., 2019 on gram negative bacterial strain and observed that chloramine was effective at *controlling L. pneumophila*. Gram-negative more sensitive to monochloramines

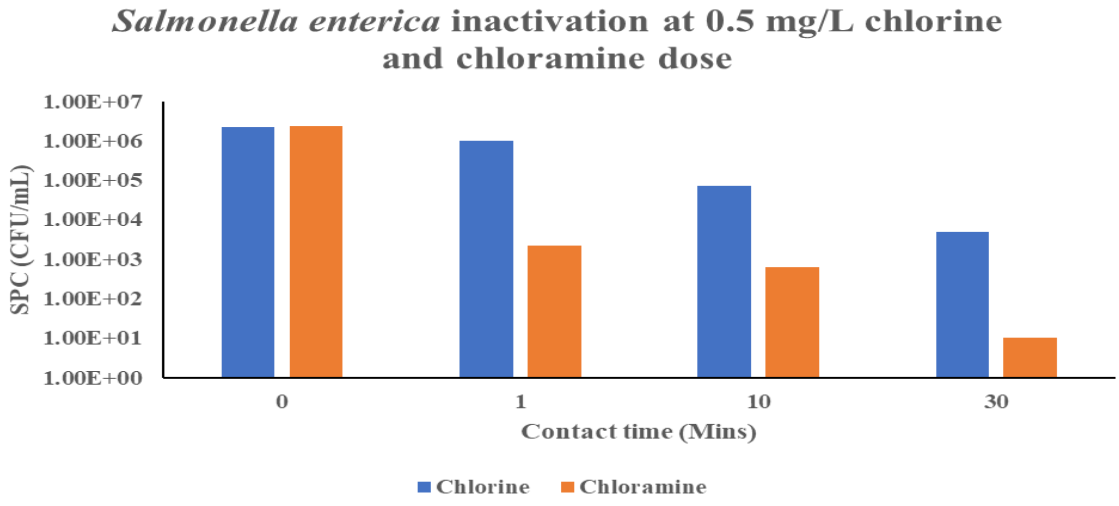
The residual chlorine measured at 2, 20 and 60 mg.min/L of the ct values was 1.2, 0.8, 0.8 mg/L respectively as shown in Table 4.12

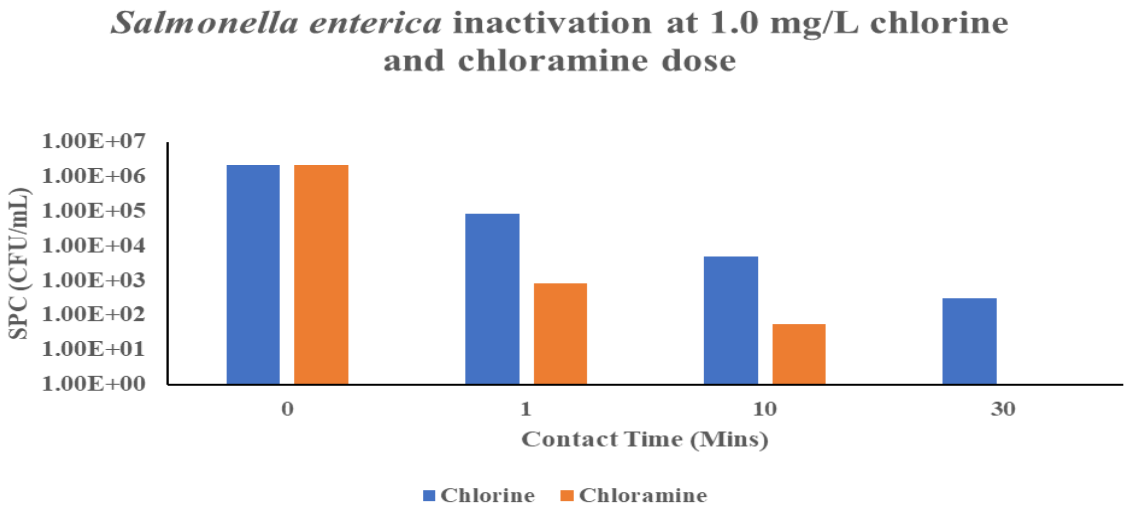
Ct value (mg.min/L)	Residual free chlorine (mg/L)	Log_{10}-Removal (CFU/mL)
2.0 mg/L chloramine dose		
2	1.0	1.98×10^{2}
20	0.7	3.67×10^{0}
60	0.6	1.0×10^{0}

Table 4.12: *Shigella dysenteriae* inactivation at various Ct values

4.1.3 Chlorine and Chloramine Disinfection Comparison

Figure 4.13 shows the comparison of chlorine and monochloramine disinfection dosages effectiveness on the inactivation of gram-negative *Salmonella enterica* in the drinking water. It is clear from the figure that monochloramine shows a higher inactivation rate than chlorine. The inactivation of monochloramine is rapid and it takes less time to inactivate gram-negative *Salmonella enterica*. 1.0 mg/L dose of monochloramine completely inactivates the *Salmonella enterica* at 30 minutes of the contact time whereas 2.0 mg/L dose chlorine completely inactivates the *Salmonella enterica* which confirms the higher inactivation efficiency of monochloramine than chlorine.


Salmonella enterica inactivation at 0.5 mg/L chlorine and chloramine dose
SPC (CFU/mL)
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
1
10
30
Contact time (Mins)
Chlorine
Chloramine


Salmonella enterica inactivation at 1.0 mg/L chlorine and chloramine dose
SPC (CFU/mL)
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
1
10
30
Contact Time (Mins)
Chlorine
Chloramine

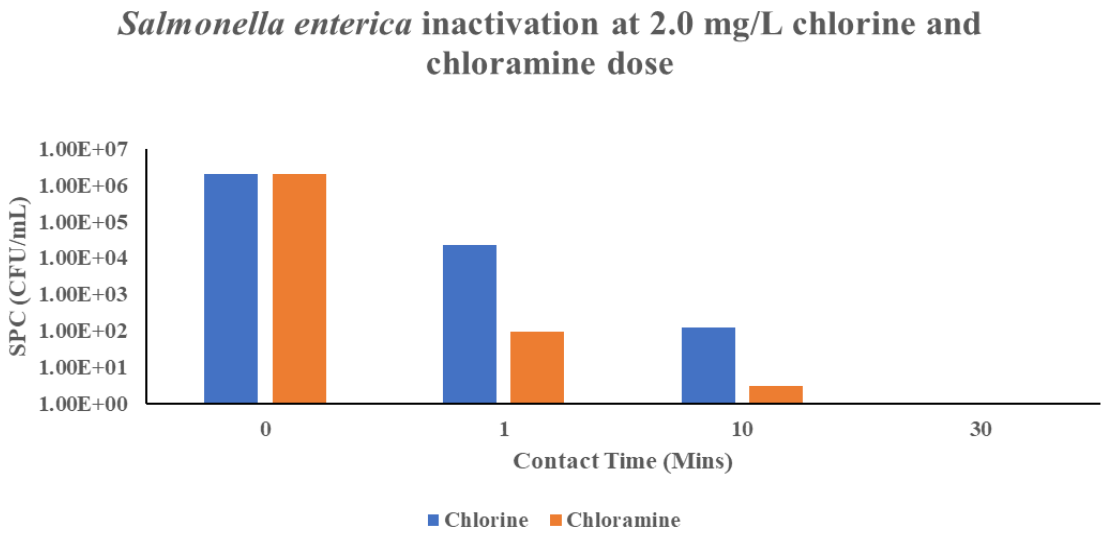
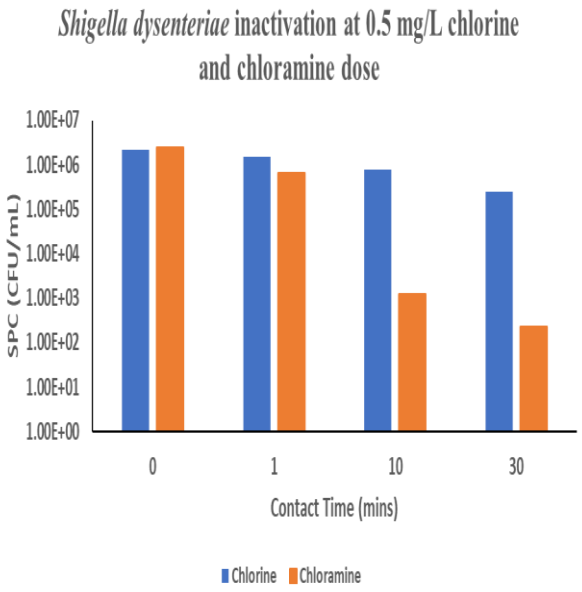
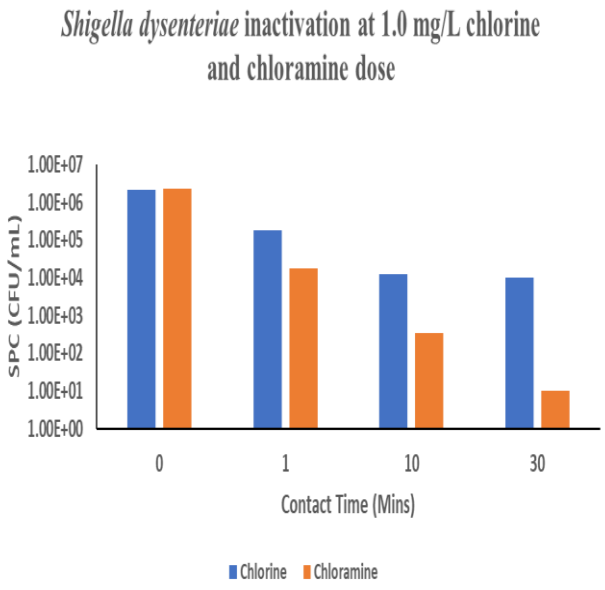
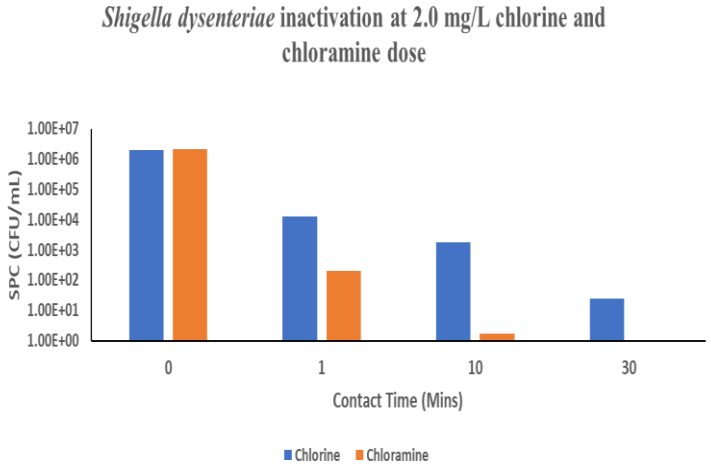

Salmonella enterica inactivation at 2.0 mg/L chlorine and chloramine dose
SPC (CFU/mL)
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
1
10
30
Contact Time (Mins)
Chlorine
Chloramine

Figure 4.14 shows the comparison of chlorine and monochloramine disinfection dosages effectiveness on the inactivation of gram-negative *Shigella dysenteriae* in the drinking water. It is clear from the figure that monochloramine shows a higher inactivation rate than chlorine. Chlorine fails to completely inactivate the *Shigella dysenteriae* after 30 minutes of the contact time whereas monochloramine completely inactivates the *Shigella dysenteriae* after 30 minutes of the contact time.







Doubling the dose from 1 to 2 mg/L at pH 8.5 and temperature 23°C, an additional 2-3 log-removal was observed as also reported by Gagnon et al., 2004 that increasing the amount of disinfectant leads to a further 1-2.5 log-inactivation of HPC bacteria in water.

Parameters	0.5	1.0	2.0	WHO
pH	7.7	7.7	7.7	6.5-8.5
EC	997	995	994	1000
Teperature	30	30	29.5	15-35
TDS	517	517	516	<1000
Hardness	308	308	311	<500
Alkalainity	327	329	340	<500
Turbidity	0.5	0.5	0.6	<1
DO	6.8	6.8	6.8	5-9.5

Table 4.13: Physicochemical parameters analysis of bench scale study

4.2 Phase-2: Prototype Study Results

The optimized dose of 2 mg/L of both chlorine and chloramine disinfectant from the bench scale experiments were further applied in the laboratory prototype distribution network to compare the inactivation efficiencies of chlorine and monochloramine. Separate experiments of chlorine and monochloramine were performed on the laboratory scale in the phase-2 of the study.

4.2.1 *Salmonella enterica* Inactivation at 2.0 mg/L Chlorine Dose

2.0 mg/L of chlorine dose was employed on the 2.20×10^6 CFU/mL of the *Salmonella enterica* within the distribution network. After 1 minute of the contact time, there was 1-log reduction observed at all the sampling points. 3-Log reduction was observed after 30 minutes of the contact time at sampling point 1 whereas 4-log reduction in the viable count of *Salmonella enterica* was observed after 30 minutes contact time at sampling

point 2 and sampling point 3. After 60 minutes of the contact time, *Salmonella enterica* was completely inactivated at sampling point 2 and sampling point 3. As shown in the Figure 4.15

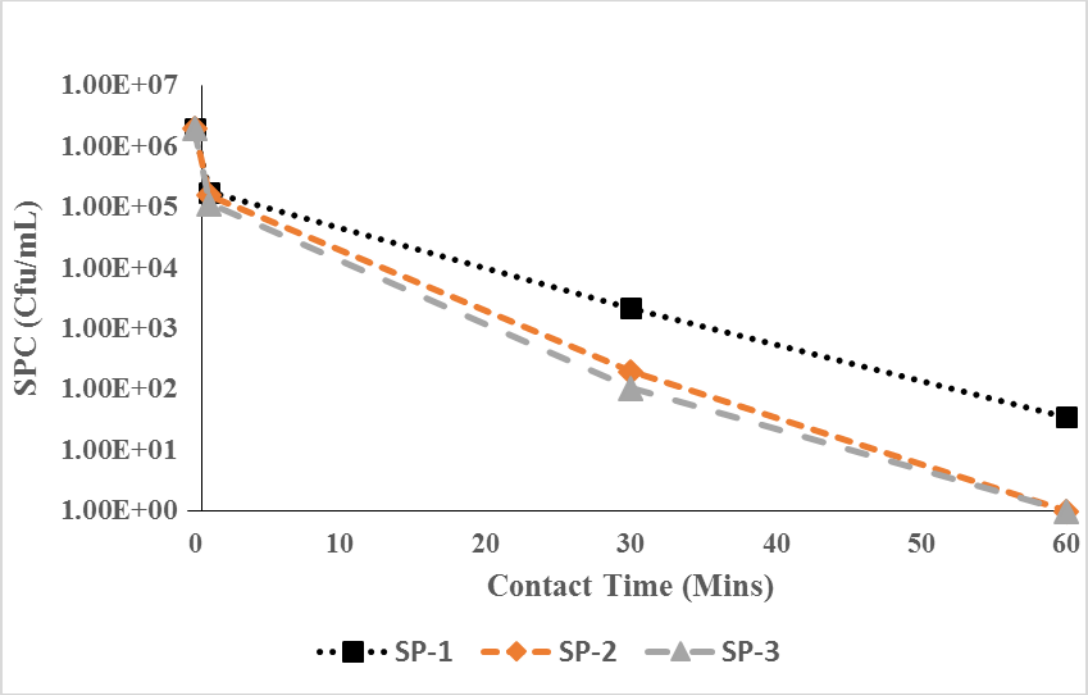


Figure 4.15: *Salmonella enterica* inactivation at 2.0 mg/L Chlorine dose

4.2.2 *Shigella dysenteriae* Inactivation 2.0 mg/L Chlorine Dose

2.0 mg/L of the chlorine dose was applied to the 2.20×10^{6} CFU/ml of the *Shigella dysenteriae* within the distribution network. After 1 minute of the contact time, there was 1-log reduction observed at all the sampling points. 3-Log reduction was observed in viable count of *Shigella dysenteriae* after 30 minutes of the contact time at all the sampling points. After 60 minutes of the contact time, *Shigella dysenteriae* was completely inactivated at sampling point 2 and sampling point 3. As shown in the Figure 4.16

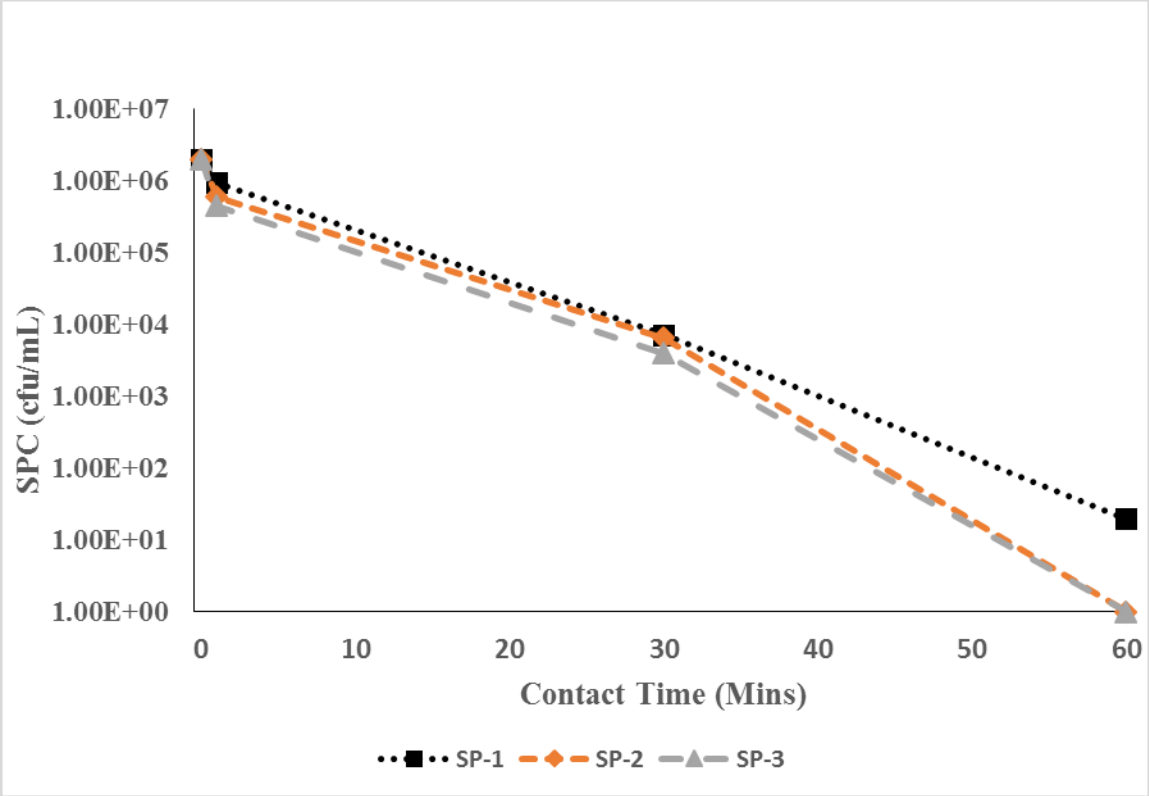


Figure 4.16: *Shigella dysenteriae* inactivation at 2.0 mg/L Chlorine dose

4.2.3 *Salmonella enterica* Inactivation at 2.0 mg/L Chloramine Dose

2.0 mg/L of the chloramine dose was applied to the 2.20×10^6 CFU/mL of the *Salmonella enterica* within the distribution network. After 1 minute of the contact time, there was 4-log reduction observed at all the sampling points. 5-Log reduction was observed after 30 minutes of the contact time at sampling point 1 whereas complete reduction in the viable count of *Salmonella enterica* was observed after 30 minutes of the contact time at sampling point 2 and sampling point 3. After 60 minutes of the contact time, *Salmonella enterica* was completely inactivated at all the sampling points as shown in the Figure 4.17

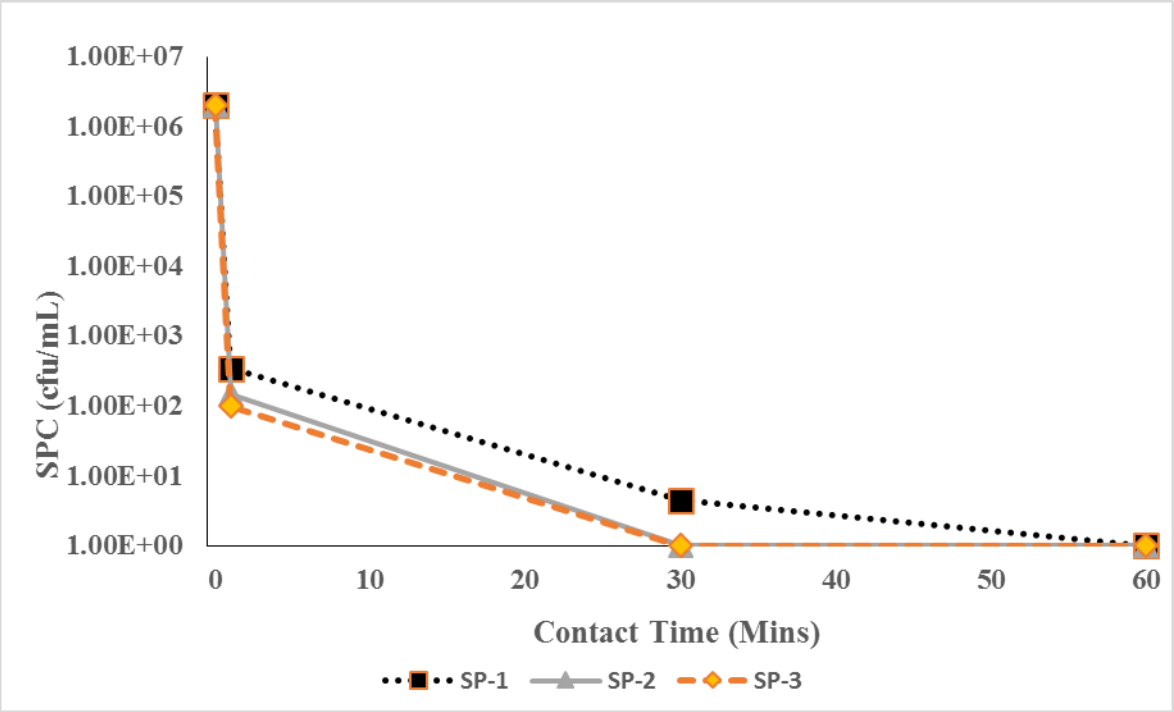


Figure 4.17: *Salmonella enterica* inactivation at 2.0 mg/L chloramine dose

The results contradict the results of the study conducted by Buse et al., (2019) and he observed that monochloramine is more effective on biofilm growth and thus suppresses the survival of *Legionella pneumophila* in the drinking water distribution networks.

4.2.4 *Shigella dysenteriae* Inactivation 2.0 mg/L Chloramine Dose

2.0 mg/L of the chloramine dose was applied to the 2.20×10^{6} CFU/ml of the *Shigella dysenteriae* within the distribution network. After 1 minute of the contact time, there was 3-log reduction was observed at the sampling point 1 whereas 4-log reduction was observed at sampling points 2 and 3. 5-Log reduction was observed in viable count of *Shigella dysenteriae* after 30 minutes of the contact time at sampling point 1 and complete inactivation was observed at sampling point 2 and 3. After 60 minutes of the

contact time, *Shigella dysenteriae* was completely inactivated at all the sampling points as shown in the Figure 4.18

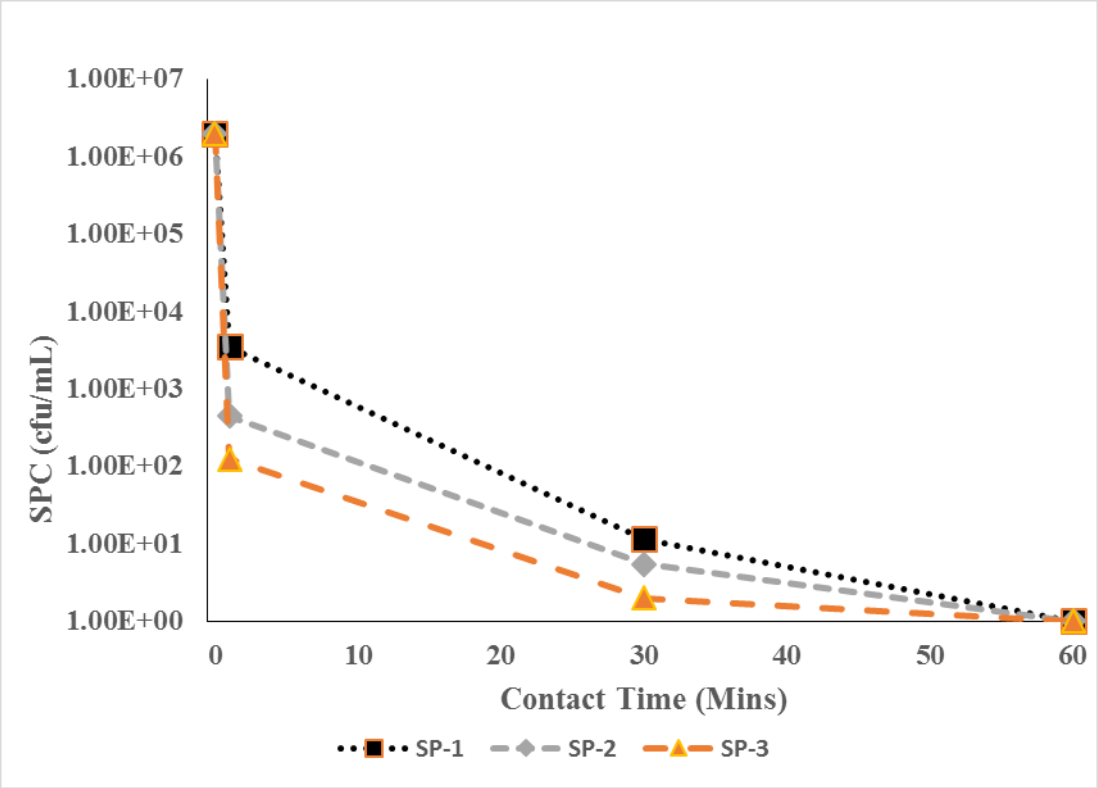


Figure 4.18: *Shigella dysenteriae* inactivation at 2.0 mg/L Chloramine dose

The results show similar trend of the study conducted by Qureshi et al., (2020), the results shows that greater inactivation rates of monochloramine as compared to free chlorine on gram-negative bacteria.

4.2.5 Chlorine and Chloramine Inactivation Comparison

Figure 4.19 shows the comparison of chlorine and monochloramine 2.0 mg/L disinfection dosage effectiveness on the inactivation of gram-negative *Salmonella enterica* within the laboratory prototype distribution network. It is clear from the figure that monochloramine shows a higher inactivation rate than chlorine. The inactivation

of monochloramine is rapid and it takes less time to inactivate gram-negative *Salmonella enterica*. Monochloramine completely inactivates the *Salmonella enterica* at 30 minutes of the contact time whereas chlorine completely inactivates the *Salmonella enterica* after 60 minutes of the contact time which confirms the higher inactivation efficiency of monochloramine than chlorine.

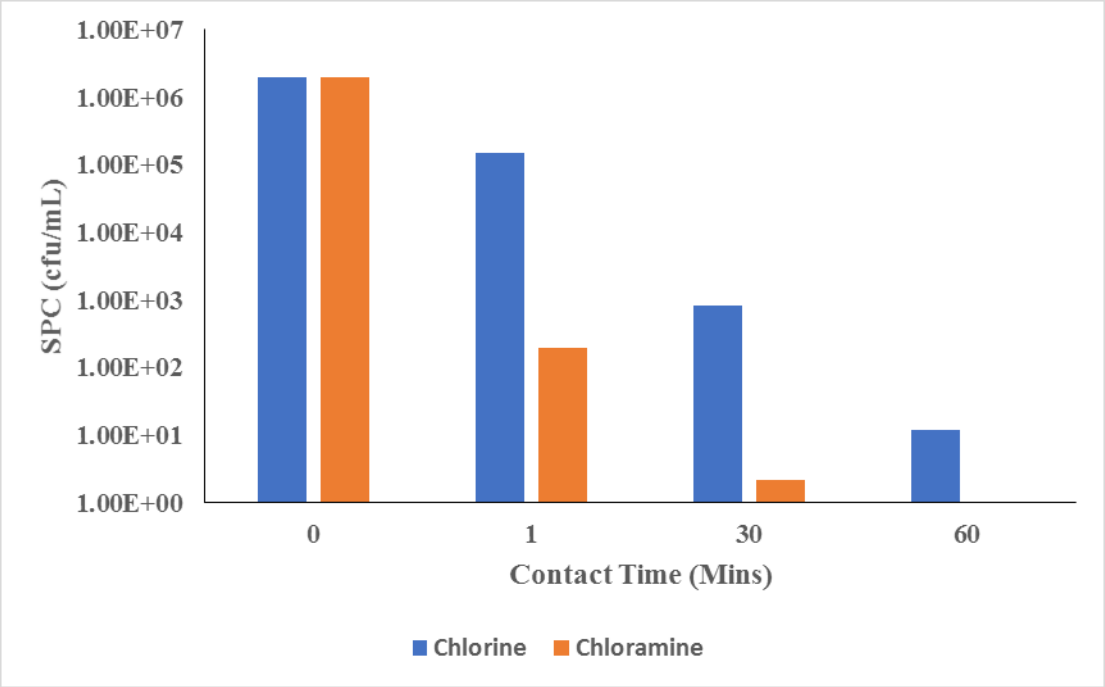


Figure 4.19: Chlorine and Chloramine Salmonella enterica inactivation comparison at 2.0 mg/L

Figure 4.20 shows the comparison of chlorine and monochloramine 2.0 mg/L disinfection dosage effectiveness on the inactivation of gram-negative *Shigella dysenteriae* within the laboratory prototype distribution network. It is clear from the figure that monochloramine shows a higher inactivation rate than chlorine. The inactivation of monochloramine is rapid and it takes less time to inactivate gram-negative *Shigella dysenteriae*. Monochloramine completely inactivates the *Shigella*

dysenteriae at 30 minutes of the contact time whereas chlorine completely inactivates the *Shigella dysenteriae* after 60 minutes of the contact time which confirms the higher inactivation efficiency of monochloramine than chlorine.

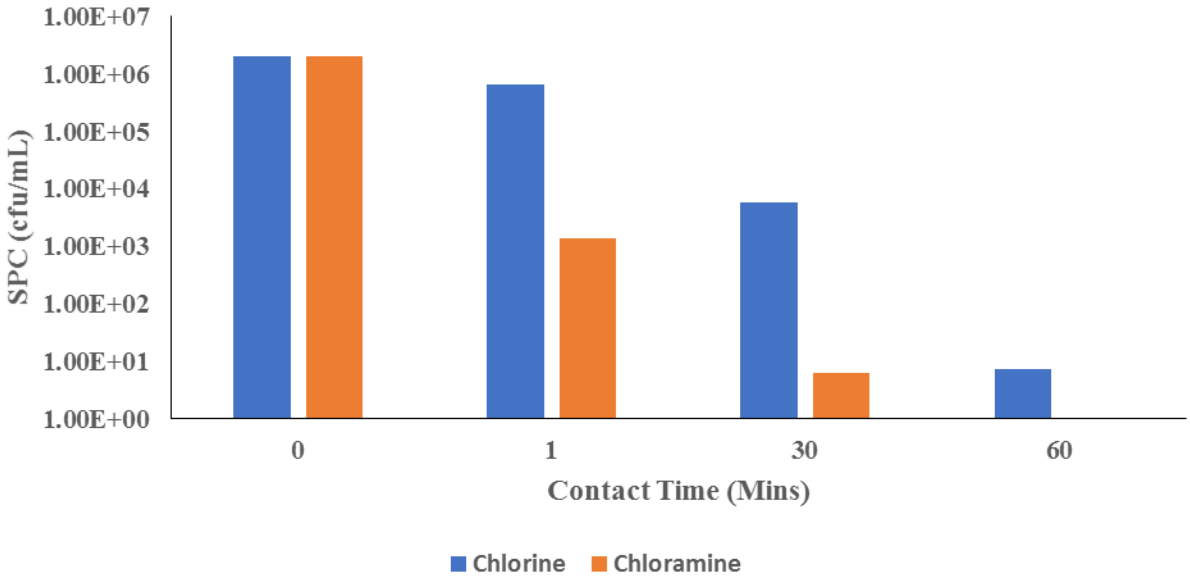


Figure 4.20: Chlorine and Chloramine *Shigella dysenteriae* inactivation comparison at 2.0 mg/L

Parameters	SP-1	SP-2	SP-3	WHO
pH	7.7	7.7	7.7	6.5-8.5
EC	997	995	994	1000
Teperature	30	30	29.5	15-35
TDS	517	517	516	<1000
Hardness	308	308	311	<500
Alkalainity	327	329	340	<500
Turbidity	0.5	0.5	0.6	<1
DO	6.8	6.8	6.8	5-9.5
R.Cl	0.6	0.6	0.8	<1

Table 4.14: Physicochemical parameters analysis of prototype study

Chapter 5

Conclusions and Recommendations

Drinking water quality mainly depends upon the technique implemented for the treatment of the supply water and on the quality of the supply water. It not only depends upon the physicochemical parameters but also on the microbial population present within the water. Poor management along with the inappropriate treatment of the drinking water supplies lead to the outbreak of water borne diseases. In dried out pipeline, microbial specie may multiply into trillions in a week. The disinfection process of the drinking water is shifting towards chloramine due to its less volatility, long term disinfection and less reactivity. The main aim of the study was to measure the most suitable optimum dose for the microbial inactivation within the distribution network with a residual chlorine within the safe limits at the consumers end.

Conclusions

Phase-1: Bench Scale

i. Microbial inactivation study shows that the *Shigella dysenteriae* was more resistant to chlorination because higher dose was required for the complete inactivation.

ii. Monochloramine shown greater rate of inactivation of both gram-negative *Salmonella enterica* and *Shigella dysenteriae*. Inactivation efficiency of the monochloramine is higher than the chlorine.

iii. The optimum dose found was 2.0 mg/L for complete inactivation of both gram-negative microbial strains.

Phase-2: Prototype study

i. 2.0 mg/L of the optimum dose was applied within the laboratory prototype distribution network to compare the inactivation efficiency of chlorine and chloramine.

ii. In laboratory prototype distribution network, chloramine inactivation of both gram-negative *Salmonella enterica* and *Shigella dysenteriae* was higher than the chlorine at all the sampling points 1,2 and 3.

iii. Microbial inactivation with chloramine was achieved within 30 minutes of the contact time within the laboratory prototype distribution network.

iv. Chloramine shows more effectiveness than chlorine by rapid inactivation of microbial strains within the laboratory prototype distribution network.

Recommendations

i. Comparative study between ultraviolet disinfection technique and chloramination could be carried out to monitor the more effectiveness of chloramine

ii. Gram positive resistant bacterial inactivation by chloramine disinfection could be carried out

iii. Monitoring disinfection by-products (Haloacetonitriles) study could be carried for chloramine disinfection

Chapter 6

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