CHLORINE DEMAND AND MICROBIOLOGICAL DISINFECTION IN TURBID WATER

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ABSTRACT

Nearly 780 million people lack access to an improved water source and 1.5 million children under the age of five die each year due to diarrheal disease. Chlorinating water is one form of household water treatment that has been proven to be effective at disinfecting water; however, there is uncertainty in the ability of chlorine to effectively disinfect turbid waters. Turbid waters tend to have a larger chlorine demand, and particulate matter in turbid waters can shield microorganisms from disinfection. A double dose (3.75 mg/L) of chlorine is recommended in turbid waters.

This research investigated the chlorine demand and microbiological disinfection of turbid waters with 10, 100 and 300 NTU and 0, 2, 10, and 25 mg/L of additional total organic carbon (TOC) when a double dose of chlorine was applied. Experiments were run in a laboratory using reactors with 10 L of RODI water, Kaolin clay to add turbidity, a TOC standard solution to add TOC, *E. coli* inoculated broth to add *E. coli*, and Clorox® bleach to add chlorine. Temperature, pH, TOC, free chlorine residual, total chlorine residual, turbidity, and *E. coli* were measured over a 24 hour period following chlorination.

Four major conclusions were drawn from this research: (1) a double dose of chlorine was not large enough to maintain free chlorine residual levels as recommended by the CDC SWS program over a 24 hour period, (2) waters with higher TOC concentrations have a higher chlorine demand, (3) a double dose of chlorine effectively disinfected water of 10, 100, and 300 NTU with average log reductions between 6.4 and 8.2, and (4) a double dose of chlorine did not result in all water samples meeting the WHO drinking water standard of <1 CFU/100mL when the initial concentration of *E. coli* was on the order of 10^8CFU/100mL.

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1.0 Introduction

1.1 Access to an Improved Water Source

Water is an essential part of human life, and in 2010, access to safe and clean drinking water was deemed a human right (Kiefer et al., 2012). Unfortunately, there are still 780 million people—11 percent of the global population—who lack access to an improved drinking water source, defined as a water source that is protected from outside contamination (WHO/UNICEF, 2012).

In the past decades, more than 2 billion people gained access to an improved drinking water source. However, there are still many drinking water disparities between regions and between urban and rural areas. Sub-Saharan Africa and Oceania have the highest percentages of people who lack access to an improved water source, 39 and 46 percent respectively. Almost every country that has less than 50 percent of the population with access to improved water is in Sub-Saharan Africa. Access to an improved water source also varies between urban and rural areas. The number of people in rural areas who still lack access to an improved water source is five times greater than in urban areas (WHO/UNICEF, 2012). The disparity in access to safe drinking water between rural and urban areas, and developing countries and developed countries has a large impact on the differences in human health in these areas.

1.2 The Burden of Diarrheal Disease

The World Health Organization (WHO) estimated that approximately 9.1 percent of the global burden of disease and 6.3 percent of all deaths worldwide could be prevented by improving water, sanitation, and hygiene (Pruss-Ustun et al., 2008). Unsafe drinking water alone can cause many different illnesses, one of which is diarrhea. Every year, millions of people die

from diarrheal disease, and it is especially dangerous to children. About 1.5 million children under five die every year because of diarrheal disease. People who lack access to improved drinking water are more likely to be affected by diarrheal disease (WHO/UNICEF, 2009). Most of the people who lack access to safe drinking water live in developing countries so they also carry the highest burden of diarrheal disease.

1.3 Diarrheal Disease and Natural Disasters

The risk of diarrheal disease can also increase after a natural disaster. While natural disasters themselves do not cause diarrheal disease, diarrheal diseases can often be spread after a natural disaster if there is flooding and water becomes contaminated, or if people are forced to stay in close proximity to each other for an extended period of time. Natural disasters simply exacerbate the spread of diarrheal disease that is already endemic to the area (Kouadio et al., 2012). In some cases, diarrheal disease can be brought to an area affected by a natural disaster by aid workers. Ten months after the devastating earthquake in Haiti in 2010, there was an outbreak of cholera (bacteria that causes diarrheal disease). Cholera had not been present in Haiti for nearly 100 years, and it was determined that the bacteria responsible for the outbreak was not native to Haiti, but that it had been introduced in Haiti as a result of human activity (Cravioto et al., 2010).

1.4 Water Quality Interventions

1.4.1 Differences in Water Quality Interventions in Developed and Developing Countries

There are many types of interventions used to prevent the spread of waterborne illnesses such as diarrhea. In developed countries, most water quality interventions are part of the

infrastructure of the country. The governments often set standards for water, and water treatment facilities must make sure that the water they distribute meets the governmental regulations. People have access to safe, running water in their homes so they do not have to worry about any further treatment or adverse health effects. Most developed countries have strict standards for their drinking water because they are capable of spending money on treating and distributing water.

In areas where water supply and treatment is not part of infrastructure, there are community-based interventions that target sanitation, hygiene, water supply, and water treatment such as the Safe Water System (SWS). SWS is a program that was developed by the Centers for Disease Control and Prevention (CDC) and Pan American Health Organization (PAHO) to improve water, sanitation, and hygiene (WASH) in communities that do not have access to clean, running water. To improve WASH in these communities, the SWS focuses on (1) household water treatment, (2) safe storage of treated water, and (3) behavior changes within the community that focus on improved hygiene, sanitation, and water and food handling (CDC, 2012). The standards for the water quality in areas where these community-based interventions are implemented are often not regulated, but the CDC, WHO, and other aid programs make recommendations for the quality of water in these areas. Because many of these communities cannot afford to treat and distribute their water, they will use other means to provide themselves with clean drinking water. Individuals will collect and treat their own water using different methods of household water treatment.

1.4.2 Household Water Treatment

One of the steps of the SWS program is household water treatment. There are many ways to treat water in the household. Chlorination, flocculant powder, solar disinfection, ceramic

filtration, and slow sand filtration are a few options for household water treatment. Each of these methods has advantages and disadvantages, and the best option for water treatment depends on the conditions of the existing water and sanitation, cultural acceptability, implementation feasibility, availability of technology, and other factors that vary between communities and areas (CDC, 2012). It is often hard to convince people to use the products that are necessary to keep their water safe because they are not accustomed to using them (Reller et al., 2003). However, many studies have been done that show that household water treatment improves the microbiological quality of water and reduces the burden of diarrheal disease for people who use the treatments (Clasen et al., 2007; Fewtrell & Colford, 2005).

1.4.3 Chlorination as a Water Quality Intervention

A major form of household water treatment is chlorination. The SWS often uses chlorination to treat water. The method is called point-of-use (POU) chlorination. POU chlorination is recommended in many developing countries and in emergencies to ensure safe drinking water for people (Lantagne, 2008). After collection, people add one bottle cap of a sodium hypochlorite solution to a standard sized container (10L) of clear water, or two bottle caps to a standard sized container (10 L) of turbid water, and wait thirty minutes before drinking (CDC, 2011). Chlorination is one of the easiest forms of water treatment, and studies have shown the POU chlorination effectively reduces diarrhea (Arnold & Colford, 2007). The major advantages of POU chlorination are (1) the proven reduction of bacteria and viruses in water, (2) the residual protection against recontamination, (3) the ease-of-use and user acceptability, (4) the proven reduction of diarrheal disease, and (5) the scalability and low cost (CDC, 2011). Because chlorine is so easy to use and relatively inexpensive as a form of water treatment, it is often used in emergency situations and in developing countries to provide people with clean drinking water.

1.4.4 Limitations of Chlorination as a Water Quality Intervention

While chlorine does have many advantages, there are still a few limitations to the use of chlorine as a form of water treatment. The use of chlorine is limited because (1) it is not as effective in turbid water (2) it does not make water look any cleaner and can affect the taste and odor of the water which causes people to stop using it to disinfect water, and (3) there are potential health risks from chlorination by-products (CDC, 2011; Rangel et al., 2003). Water that is turbid has particulate matter in it that will react with chlorine. This makes chlorination less effective because there is less chlorine available to kill bacteria and viruses. By adding a higher dose of chlorine, there is more chlorine available to kill bacteria and viruses, however, the addition of chlorine often makes the taste and odor of the water unfavorable and people are less likely to drink it. There are also potential long-term health effects from chlorination by-products.

1.5 Need for Further Research

It is important that research continues to investigate methods of water disinfection to help reduce the burden of diarrheal disease. Chlorination is one of the easiest and least expensive methods of household water treatment to implement. Chlorination has been proven to be effective at reducing diarrheal disease. Many people who lack access to an improved water source collect water that is very turbid, and there is little evidence on whether chlorine is effective in highly turbid water. The purpose of this research is to further investigate the use of chlorine as a disinfectant in highly turbid water.

2.0 Background

2.1 Scope

The background of this thesis addresses *E. coli* as a water quality parameter, chlorine, turbidity, total organic carbon (TOC), and the current research of chlorine disinfection of turbid water. To fulfill the objectives of this project, sufficient understanding of each of these topics is necessary.

2.2 *E. coli* as a Water Quality Parameter

Escherichia coli (*E. coli)* levels can be used to determine whether water is safe to drink. Water that is unsafe to drink can carry many different types of harmful bacteria, protozoa, and viruses. It is impractical and difficult to detect many of the microorganisms in the water; therefore, specific microbiological indicators are used to determine whether water is safe to drink.

A microbiological indicator is a bacterium that does not carry the disease, but is known to be associated with the microorganism that does. Some microbial indicators include fecal (thermotolerant) coliform, and *E. coli*. These microbial indicators are typically present in water contaminated with fecal matter. If any type of fecal indicator organism is detected in water, it indicates that the water is not suitable for human consumption without treatment (WHO, 2011).

E. coli is a widely used microbial indicator. It is naturally found in the gastrointestinal tract of mammals, and most types of *E. coli* are harmless. A single gram of feces can contain as many as one billion *E. coli*. Since *E. coli* is not normally found in uncontaminated waters, it makes it an excellent indicator that water has been contaminated with fecal matter (CDC, 2010). The WHO has created an *E. coli* risk classification scheme to set guidelines for the safety of

drinking water. Water with 0 colony forming units (CFU) of *E. coli* per 100 mL is "in conformity with WHO guidelines," 1-10 is "low risk", 10-100 is "intermediate risk", 100-1000 is "high risk", and >1000 is "very high risk" (WHO, 1997). The WHO recommends that action be taken if *E. coli* is found in water. It is important to disinfect contaminated water, especially if the water is meant for human consumption.

2.3 Chlorine

2.3.1 Chlorine Residual

Chlorine is an oxidizing agent, and when it is added to water, it reacts with the natural organic matter, ammonia, nitrogen, hydrogen sulfide, and metals like iron and manganese in the water. The amount of chlorine that reacts with these things in the water is known as the chlorine demand, and the reacted chlorine is then unavailable for disinfection. After the initial reactions between chlorine and the constituents in the water, there is leftover chlorine available, referred to as total chlorine residual (TCR). TCR consists of combined chlorine and free chlorine. Combined chlorine is the chlorine that has combined with ammonia to form chloramines (monochloramine, NH_2Cl; dichloramine, $NHCl_2$; and trichloramine, NCl_3). Free chlorine is found in the form of hypochlorous acid (HOCl) and hypochlorite (OCl^-). Free chlorine is twenty times more effective as a disinfectant than combined chlorine (Cairncross & Feachem, 1993). To ensure complete disinfection, it is important that the dose of chlorine applied to water exceeds the chlorine demand of the water and produces free chlorine residual rather than combined chlorine residual. The dose of chlorine needed to achieve the formation of free chlorine residual is called the "breakpoint" and varies depending on the content of the water (Cairncross & Feachem, 1993).

People add chlorine to water to kill harmful bacteria and viruses in the water. When the chlorine comes into contact with bacteria, the membranes of the cells are compromised which causes the cells to die (Reed, 2005). Chlorination of water started in the United States in the early 1900's. Filtration and chlorination of the drinking water systems in the United States resulted in reduced mortality over the following decades due to the decrease in waterborne diseases such as typhoid fever and diarrheal diseases (Cutler & Miller, 2005).

Chlorination is an important step in water treatment in most developed countries. Water that is treated and distributed in developed countries is chlorinated and regulated so that free chlorine residual is maintained through the distribution system to ensure there is no recontamination of the water. The standards for free chlorine residual vary between governmental entities, but the minimum standard for free chlorine residual in water systems is recommended to be between 0.2 and 0.5 mg/L (WHO, 2011). In the United States, the maximum contaminant level for free chlorine residual as defined by the Environmental Protection Agency (EPA) is 4 mg/L (USEPA, 1998).

For people who collect and store their own water, the standards and recommendations are slightly different. The WHO recommends a minimum free chlorine residual concentration of 0.2 mg/L and a maximum of 5.0 mg/L. However, the CDC SWS program recommends a maximum free chlorine residual concentration of 2.0 mg/L 30 minutes after the addition of chlorine to water (CDC, 2008). The difference in maximum standards comes from the level of user acceptability due to taste and odor. The differences are also due to the level of risk each organization places on the health effects of chlorine due to disinfection by-products (DBPs) such as trihalomethanes (THMs). The maximum recommendation of 5.0 mg/L by the WHO is "conservative, as no adverse effect level was identified" in a study done with humans and

animals that were exposed to chlorine in drinking water (WHO, 2011). DBPs are not a concern for the purpose of this research because studies have shown that "household chlorination of turbid and non-turbid waters did not create THM concentrations that exceeded health risk guidelines" (Lantagne et al., 2010).

2.3.2 Factors that Affect Chlorination

There are many variables that affect the performance of chlorine as a disinfectant. The type and concentration of the organisms being inactivated, the chlorine dosage and contact time (known as the CT factor), the pH and temperature of the water, and the presence of other constituents in the water determine the effectiveness of disinfection.

As mentioned before, hypochlorous acid (HOCl) is the most effective form of chlorine residual as a disinfectant. However, the formation of HOCl is dependent on many other factors. The pH of the water that chlorine is added to is the major determinant of the formation of HOCl because it dictates the amount of dissociation of HOCl to H^+ and OCl^- ions. As pH increases, more OCl^- ions are formed, but OCl^- is ineffective as a disinfectant because it cannot penetrate the membranes of cells due to its negative charge. Therefore, if pH is higher than 9, there is little to no disinfecting power. Temperature also affects the amount of dissociation. Lower temperatures decrease the amount of dissociation, and higher temperatures increase the amount of dissociation (White, 1999).

The amount of time that chlorine is in contact with the water also affects how effective chlorine is as a disinfectant. The minimum contact time for chlorine and water is 30 minutes in water that is 18°C and has a pH between 6.8 and 7.2 (Reed, 2005). The CT factor is a parameter used to determine the necessary dose of disinfectant and contact time in order to have effective disinfection. The CT factor is the concentration of chlorine residual (mg/L) multiplied by the

contact time (min). CT tables have been developed to allow people to determine the appropriate doses and contact times to reach certain levels of disinfection. The higher the dose, and the longer the contact time, the higher the CT value and the more effective the disinfection (Conservation, 2005).

Turbidity can also affect the performance of chlorine as a disinfectant because chlorine quickly binds to organic matter and becomes unavailable to kill other microorganisms (Crump et al., 2004). This will be discussed further in the following section.

2.4 Turbidity

2.4.1 The Composition of Turbid Water

Turbidity is a measurement of the amount of light that is absorbed or dispersed through a sample of water (WHO, 2011). The nephelometric turbidity unit (NTU) is the unit used to measure turbidity. Low turbidities are associated with clear water, and high turbidities are associated with very dirty water. Turbidity can be caused by a combination of inorganic and organic matter such as silt, sand, mud, bacteria, and chemical precipitates (WHO). Turbidity varies in its composition in different regions (LeChevallier et al., 1981).

2.4.2 Turbidity and Bacteria Shielding

Microorganisms tend to attach themselves to suspended particles in water. There are many factors that affect the amount of bonding there is between microorganisms and particulates. These factors include the type of adsorbent (quartz, sand, silt, clay), the surface charge characteristics, the chemistry of the solution (pH, temperature), and the surface features of the microorganisms (Zhao et al., 2012).

The efficiency of disinfection can be greatly affected by turbidity because many microorganisms find protection in the particulate matter in the water. Most water treatment programs are designed to remove most of the particulate matter before disinfection. The use of filtration or flocculation removes turbidity by removing particulate matter, and therefore, removes a large portion of microorganisms. If the particulate matter is not removed, disinfection is much less effective because the microorganisms that are attached to the particulate matter are also not removed, and there are fewer interactions between the disinfectant and the microorganisms due to the reaction of chlorine with other constituents in the water (WHO, 2011).

2.4.3 Turbidity as a Water Quality Parameter

Turbidity is often used as a measurement of water quality because it is a measurement of how clear the water is. Turbidity is either measured using an electric turbidity meter or a turbidity tube. Electric turbidity meters are considered to be more accurate than turbidity tubes especially when measuring turbidities less than 5 NTUs. However they are more expensive than turbidity tubes, require a power source, and are prone to damage. Turbidity tubes on the other hand, are less expensive, have a simple design, and are durable. The problem with turbidity tubes is that they cannot measure turbidities less than 5NTUs and they are not nearly as precise as an electric turbidity meter (WHO). Turbidity tubes are useful in determining the difference in turbidities at different orders of magnitude, but the variability in turbidity tube readings is significant. Therefore, for accurate and precise measurements, an electric turbidity meter should be used (Dorea & Simpson, 2011).

Turbidity is used as one standard for water quality. In the United States, all drinking water that comes from public water systems must have a turbidity of less than 1 NTU

(Protection, 2010). The WHO recommends that all drinking water have a turbidity of 5 NTU or less. The standard of 5 NTU or less applies to community-based water systems, as well as any household water treatment systems. Any drinking water that has turbidity greater than 5 NTU would be unsafe to consume because of the amount of particulate matter in the water. In water that is going to be chlorinated, the standard is stricter. The WHO recommends that any water that is going to be chlorinated should have a turbidity of less than 1 NTU in order for chlorination to be effective (WHO Fact Sheet). The stricter standard comes from the phenomenon of bacteria shielding. With a lower turbidity, there is not as much particulate matter present for bacteria to shield themselves with, which makes chlorination more effective.

Turbidity is a water quality measurement that is relatively easy to make when in the field. It is important to measure turbidity because it can indicate whether water is safe to drink. High turbidity indicates high content of bacteria due to the interaction between bacteria and particulate matter. However, it is not always true that high content of bacteria indicates high turbidity (Pronk et al., 2006). Water can have high bacteria content and a very low turbidity which is why it is important to measure other water quality parameters as well as turbidity.

2.5 Total Organic Carbon

2.5.1 TOC as a Water Quality Parameter

The total organic carbon (TOC) in a sample of water can be measured and used to determine what kind of particulate matter is in water. TOC is often used to get a sense of the amount of organic matter there is in a sample of water. Microorganisms use organic matter to shield themselves from disinfection, and organic matter also causes the formation of disinfection by-products that can potentially be harmful to humans (Volk et al., 2002). TOC concentrations

can indicate whether or not water is safe to drink, and waters with a high concentration of TOC are most likely not safe to drink.

Measuring TOC is an involved process that is difficult to measure in the field and is most effective when done in a lab. TOC is measured using heat, oxygen, ultraviolet irradiation, or chemical oxidants that convert organic carbon to carbon dioxide (APHA, 1992). In order to accurately measure TOC, all inorganic carbon must be removed, the sample must be acidified to achieve a pH of less than 2.0, and the sample must be analyzed as soon as possible after collection. All of these factors make it difficult to accurately measure TOC concentrations in the field.

2.5.2 The Importance of a Relationship between TOC and Turbidity

Because TOC is difficult to measure in the field, it would be helpful and informative if the relationship between turbidity and TOC was established so that turbidity could be used as a proxy for TOC. Turbidity is a parameter that is easily measured in the field, and if turbidity and TOC could be related, field workers would be able to determine the TOC concentration of water from a turbidity measurement. This would be important so that field workers could accurately assess the quality of water and implement the appropriate methods to prepare the water for consumption.

In a study done by Lantagne et al. (2008), when turbidity and TOC were both measured and compared, no correlation was found, possibly due to a limited data set. However, that is not always the case. LeChevallier et al. (1981) did find a correlation between TOC and turbidity ($r = 0.82$). They also found that the chlorine demand of water was almost completely associated with soluble TOC. There was a positive correlation between chlorine demand and turbidity as well (LeChevallier et al., 1981).These correlations indicate that there may be a relationship between

turbidity and TOC. A more defined relationship between TOC and turbidity will allow field workers to quickly know more about the chlorine demand of the water by simply testing the turbidity (LeChevallier et al., 1981).

2.6 Chlorine Disinfection of Turbid Waters

2.6.1 Chlorine Disinfection of Water with Turbidity 0-10 NTU

There have been many studies that have investigated using chlorine as a disinfectant in water of low turbidity (0-10NTU). For example, Crump et al. (2004) found that sodium hypochlorite achieves *E. coli* concentrations of <1 CFU/100mL in low and medium turbidity water (Figure 2.1).

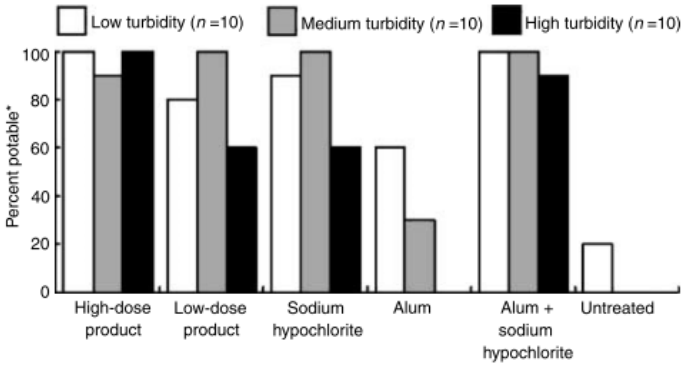

Figure 2.1 Percentage of water samples rendered potable (WHO potability standard <1 E. coli CFU/100mL) by starting turbidity category, western Kenya, 2002 (Crump, et. al., 2004)

Programs that focus on household water treatment, like the SWS program, have specific recommendations for chlorine doses in low turbidity water. The SWS program uses bottle caps for the appropriate dose. If water is clear, the recommended dose is 1 bottle cap. If the water is turbid, the recommended dose is 2 bottle caps. However it is made clear that these recommendations are only appropriate in "lower turbidity water" (CDC, 2011). The numerical recommended dose for low turbidity water is 1.875 mg/L (Lantagne, 2008).

There are concerns with dosing for lower turbidity waters. While high doses of chlorine will effectively disinfect low turbidity water, doses that are too high can adversely affect the taste and smell of the water (WHO, 2011). If the water is not aesthetically appealing, it is unlikely that people will drink it. The formation of DBPs such as THMs is also a concern of over-dosing a water source. However, studies have shown that if the appropriate dose of chlorine is used, DBPs do not reach a harmful level and should not be a concern (Lantagne, et al., 2008).

2.6.2 Chlorine Disinfection of Water with Turbidity 10-100 NTU

The research on the use of chlorine as a disinfectant in water of medium turbidity (10-100NTU) or high turbidity (>100NTU) is not extensive. The studies that have been performed call attention to the need for further research on direct chlorination of turbid waters.

There has been research performed on household water treatment interventions that can be done prior to chlorination to reduce turbidity. In studies by Preston et al. (2010) and Kotlarz et al. (2010), the use of both physical and chemical water clarification mechanisms prior to chlorination was investigated. The studies concluded that all of the interventions studied reduced the turbidity of water (Table 2.1). However, cloth filtration and moringa flocculation did not reduce the chlorine demand while the other three interventions (sand filtration, settling and decanting, and alum flocculation) reduced the chlorine demand (Table 2.1). This data suggests that even if people are using cloth filtration or moringa flocculation as pre-chlorination water treatment, they will still need to add a dose of 3.75 mg/L of chlorine to ensure adequate disinfection (Kotlarz et al., 2009; Preston et al., 2010).

Table 2.1 Effect of interventions on chlorine demand and turbidity (Preston et al., 2010) and (Kotlarz et al., 2010)

Intervention Type	Intervention	Chlorine Demand Reduction?	Turbidity Reduction?
Physical	Cloth Filtration	**No**	Yes
	Sand Filtration	Yes	Yes
	Settling and Decanting	Yes	Yes
Chemical	Moringa Flocculation	**No (Increased)**	Yes
	Alum Flocculation	Yes	Yes

Lantagne (2008) investigated the levels of chlorine residual in different drinking water sources from 13 countries. In her studies she found that only 41.7 percent of unimproved water sources of turbidity 10-100NTU met the criteria for free chlorine residual after the addition of a dose of 3.75 mg/L of sodium hypochlorite. Furthermore, none of the three water sources with turbidity greater than 100NTU had any chlorine residual present after the addition of a dose of 3.75 mg/L of sodium hypochlorite (Lantagne, 2008). The lack of free chlorine residual in the water after 24 hours raises concerns of the microbiological safety of the water. This data suggests that water with turbidity 10-100NTU has a high chlorine demand and may not be microbiologically safe. However, this is dependent on the characteristics of the source water.

Crump et al. (2004) found that direct chlorination of waters with turbidity 10-100NTU resulted in *E. coli* concentrations of <1 CFU/100mL 30 minutes after chlorination. It was also found that in 6 of 10 water sources with turbidity greater than 100 NTU the *E. coli* concentration was <1 CFU/100mL 30 minutes after chlorination. This data suggests that chlorine disinfection can be effective in turbid waters.

2.7 Objectives

The objective of this research is to verify the recommended double dose (3.75 mg/L) of chlorine in turbid waters by determining the microbiological efficacy of a double dose of chlorine to reduce microbial contamination in waters with turbidity of 10-100 NTU. The relationship between TOC and turbidity in determining the efficacy of chlorine in reducing microbial contamination will also be investigated.

3.0 Methods

3.1 Experimental Methods

In this study, five parameters were measured before and after the addition of 3.75 mg/L of NaOCl to 10 L water samples of varying turbidities and TOC concentrations. The five parameters measured were (1) free chlorine residual, (2) total chlorine residual, (3) *E. coli* concentration, (4) turbidity, and (5) TOC. Water samples were prepared with turbidity of 10, 100, or 300 NTU and spiked with 0, 2, 10, or 25 mg/L of TOC (Table 3.1).

Table 3.1 Reactor Numbers and Characteristics

Sample Reactor #	Corresponding Control Reactor # No NaOCl Added	Turbidity (NTU)	TOC Added (mg/L)
1	13A	10	2
2	13B	100	2
3	13C	300	2
4	13D	10	10
5	13E	100	10
6	13F	300	10
7	13G	10	25
8	13H	100	25
9	13I	300	25
10	13J	10	0
11	13K	100	0
12	13L	300	0

The samples were prepared in the laboratory using reverse osmosis distilled (RODI) water. Varying levels of turbidity were created using white Kaolin clay (Table 3.2).

Table 3.2 Amount of Kaolin Clay Added to Create Turbidity

Target Turbidity	Amount of Kaolin Clay Added to 10L of Water (g)
10NTU	0.32
100NTU	2.86
300NTU	5.80

Varying levels of TOC were created using a TOC standard solution. A high concentration of *E. coli* (10^8 CFU/100mL) was added to each water sample. The water was spiked with such a large concentration of *E. coli* so a complete analysis of log reduction could be performed. Natural waters would likely have concentrations of *E. coli* on the order of 10^4 CFU/100mL. The *E. coli* was grown from a frozen stock of ATCC 25922. The *E. coli* was first grown on an agar plate using the streak plate method. After 24 hours of incubation, a single CFU was isolated and used to inoculate LB Broth. The broth was then incubated and agitated for 24-48 hours. To enumerate the concentration of *E. coli* in the broth, a GE GeneQuant 100 spectrophotometer was used and an OD measurement was taken. The broth was then added to the water sample and mixed using a sterile glass stirring rod to allow the *E. coli* to interact with the Kaolin clay in the water.

To chlorinate the water, sodium hypochlorite solution was used. Clorox® bleach (5.7%-6% available chlorine) was the source of the NaOCl. To determine the concentration of chlorine in the bleach solution, the bleach was titrated three times using a HACH digital titrator. The average of the three titrations was used to determine the volume of bleach to add to each 10 L reactor to reach a dose of 3.75 mg/L of NaOCl.

Free chlorine residual and total chlorine residual were measured 1, 2, 4, 10, and 24 hours after the addition of NaOCl using the LaMotte 1200 Chlorine Colorimeter and N,N-diethylparaphenylene diamine (DPD) tablets.

E. coli concentration was measured at 1, 10, and 24 hours after the addition of NaOCl using the IDEXX most-probable-number (MPN) testing method.

Turbidity was measured at 1, 2, and 24 hours after the addition of NaOCl using the HACH 2100 Portable Turbidimeter.

TOC was measured at 1, 2, and 24 hours after the addition of NaOCl using a Shimazdu TOC analyzer.

The protocol was replicated 3 to 5 times for each reactor. Each replication is referred to as a trial.

Please refer to Appendix A for the detailed protocol, Appendix B for a list of laboratory materials and equipment, and Appendix C for a detailed experimental procedure for each parameter measured.

3.2 Quality Control

Several quality assurance and control measures were utilized during each experiment. Temperature and pH values were measured in each reactor at 0, 1, and 24 hours after the addition of NaOCl in order to account for any potential differences between samples.

A control reactor was used for each different sample in order to account for any natural growth or die off of *E. coli* (Table 3.1). The control reactor had the same characteristics as the sample, however no NaOCl was added.

Furthermore, in order to test the precision of the laboratory equipment, a duplicate sample was taken every ten tests. The relative percent difference was calculated and averaged to determine how precise each laboratory device was.

4.0 Results

4.1 Reactor 1 – 10 NTU, 2 mg/L TOC

Reactor 1 contained 10 NTU turbidity, and an additional 2 mg/L of TOC. Four trials of Reactor 1 were performed.

Temperature and pH, Turbidity, TOC

The average temperature and pH of the water in Reactor 1 were 19°C (min=19; max=20; stdev=0.50) and 7.5 (min=7.5; max=7.5; stdev=0), respectively (Table 4.1). The average initial turbidity of the Reactor 1 water was 13 NTU (min=11, max=15, stdev=1.9) (Table 4.1). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 1 was 34 mg/L (min=32; max=36; stdev=1.7) (Table 4.1). The TOC concentration remained relatively constant over time.

Table 4.1 Reactor 1 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	19	19	19
	Trial 2	20	20	20
	Trial 3	19	19	19
	Trial 4	19	19	19
	Average (stdev)	**19 (0.50)**	**19 (0.50)**	**19 (0.50)**
pH	Trial 1	7.5	7.5	7.5
	Trial 2	7.5	7.5	7.5
	Trial 3	7.5	7.5	7.5
	Trial 4	7.5	7.5	7.5
	Average (stdev)	**7.5 (0.0)**	**7.5 (0.0)**	**7.5 (0.0)**
Turbidity (NTU)	Trial 1	14	12	2.3
	Trial 2	11	9.6	2.4
	Trial 3	13	11	3.1
	Trial 4	15	9.9	2.9
	Average (stdev)	**13 (1.9)**	**11 (1.1)**	**2.7 (0.40)**
TOC (mg/L)	Trial 1	32	34	32
	Trial 2	36	35	35
	Trial 3	33	33	32
	Trial 4	33	33	32
	Average (stdev)	**34 (1.7)**	**34 (1.1)**	**33 (1.6)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.1). The average remaining free chlorine residual after 24 hours was 0.15 mg/L (min=0.01; max=0.25; stdev=0.11). The average remaining total chlorine residual after 24 hours was 0.47 (min=0.4; max=0.63; stdev=0.11).

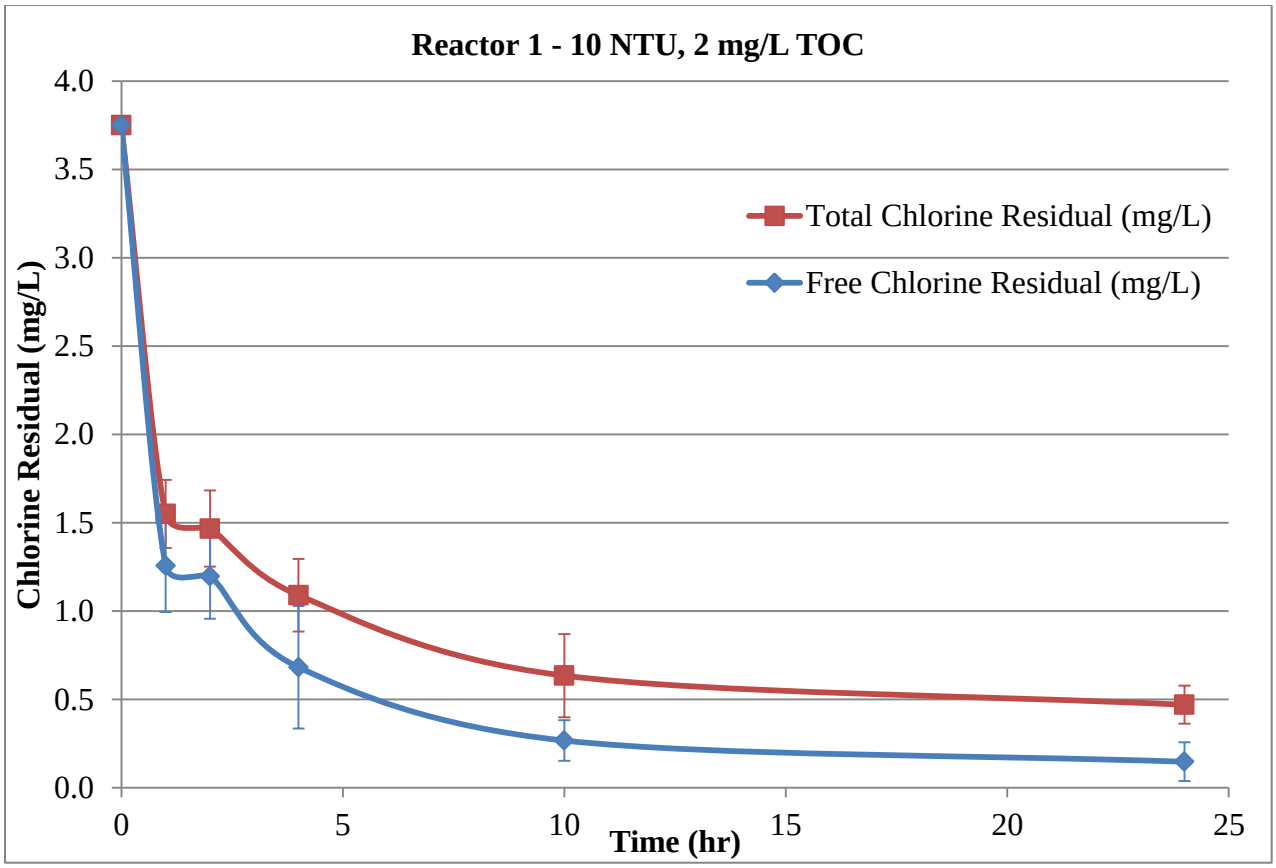

Figure 4.1 Total chlorine residual and free chlorine residual in Reactor 1. Data points represent the average of four trials, and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of E. coli was 2.0×10^8 CFU/100mL (min=1.4×10^8; max=2.4×10^8; stdev=5.4×10^7) (Table 4.2). After 24 hours, the average log reduction was 8.3 (min=8.2; max=8.4; stdev=0.12) (Table 4.3). Log reduction was calculated using the following equation:

$$\log_{10} \frac{Concentration\ of\ E.coli\ before\ chlorination\ (\frac{CFU}{100mL})}{Concentration\ of\ E.coli\ after\ chlorination\ (\frac{CFU}{100mL})}$$

Table 4.2 *E. coli* Counts for Reactor 1

E. coli Count (CFU/100mL)	Time (hr)			
	0	1	10	24
Trial 1	1.6E+08	40	<1	<1
Trial 2	2.4E+08	52	<1	<1
Trial 3	1.4E+08	<10	<1	<1
Trial 4	2.4E+08	240	9.8	1
Average (stdev)	**2.0E+08 (5.4E+07)**	**86 (110)**	**3.2 (4.4)**	**1.0 (0.0)**

Table 4.3Log Reduction of *E. coli* in Reactor 1

Log Reduction	Time (hr)		
	1	10	24
Average (stdev)	**6.6 (0.47)**	**8.0 (0.44)**	**8.3 (0.12)**

4.2 Reactor 2 – 100 NTU, 2 mg/L TOC

Reactor 2 contained 100 NTU turbidity, and an additional 2 mg/L of TOC. Four trials of Reactor 2 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 2 ranged from 19 to 20℃ (min=19; max=20) and was relatively constant over time. The average pH of the water ranged from 7.5 to 7.6 (min=7.4; max=7.6) and was also constant over time (Table 4.4). The average initial turbidity of the Reactor 2 water was 100 NTU (min=82, max=120, stdev=17) (Table 4.4). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 2 was 34 mg/L (min=32; max=37; stdev=2.4) (Table 4.4). The TOC concentration remained constant over time.

Table 4.4Reactor 2 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	20	19	19
	Trial 2	20	20	20
	Trial 3	19	19	19
	Trial 4	19	19	19
	Average (stdev)	**20 (0.58)**	**19 (0.50)**	**19 (0.50)**
pH	Trial 1	7.6	7.6	7.6
	Trial 2	7.6	7.6	7.6
	Trial 3	7.4	7.4	7.4
	Trial 4	7.6	7.5	7.6
	Average (stdev)	**7.6 (0.10)**	**7.5 (0.10)**	**7.6 (0.10)**
Turbidity (NTU)	Trial 1	96	68	7
	Trial 2	82	64	18
	Trial 3	110	110	19
	Trial 4	120	94	14
	Average (stdev)	**100 (17)**	**84 (22)**	**15 (5.4)**
TOC (mg/L)	Trial 1	32	31	31
	Trial 2	37	36	35
	Trial 3	33	33	32
	Trial 4	33	32	31
	Average (stdev)	**34 (2.4)**	**33 (2.2)**	**33 (2.0)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.2). The average remaining free chlorine residual after 24 hours was 0.17 mg/L (min=0.01; max=0.33; stdev=0.15). The average remaining total chlorine residual after 24 hours was 0.53 (min=0.40; max=0.67; stdev=0.13).

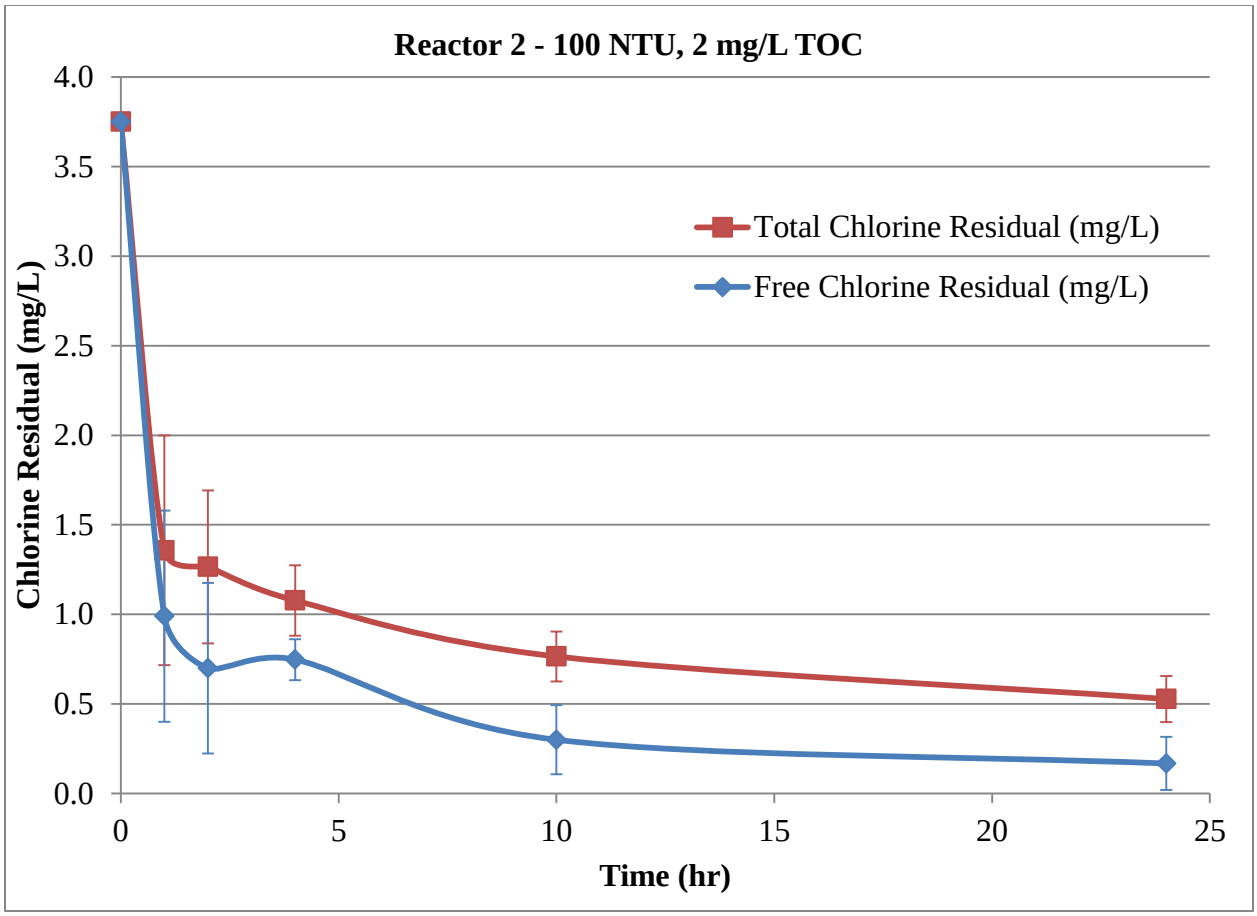

Figure 4.2 Total chlorine residual and free chlorine residual in Reactor 2. Data points represent the average of four trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of E. coli was 2.2×10^8 CFU/100mL (min=1.7×10^8; max=2.4×10^8; stdev=4.0×10^7) (Table 4.5). After 24 hours, the average log reduction was 8.3 (min=8.2; max=8.4; stdev=0.08) (Table 4.6).

Table 4.5 *E. coli* Counts for Reactor 2

E. coli Count (CFU/100mL)	Time (hr)			
	0	1	10	24
Trial 1	TNTC	<1	<1	<1
Trial 2	2.4E+08	52	<1	<1
Trial 3	2.4E+08	24000	<1	<1
Trial 4	1.7E+08	<10	<1	<1
Average (stdev)	**2.2E+08 (4.0E+07)**	**6000 (12000)**	**1.0 (0.0)**	**1.0 (0.0)**

TNTC – Too numerous to count

Table 4.6Log Reduction of *E. coli* in Reactor 2

Log Reduction	Time (hr)		
	1	10	24
Average (stdev)*	**6.0 (1.7)**	**8.3 (0.08)**	**8.3 (0.08)**

*Trial 1 not included because *E. coli* at time 0hr was too numerous to count

4.3 Reactor 3 – 300 NTU, 2 mg/L TOC

Reactor 3 contained 300 NTU turbidity, and an additional 2 mg/L of TOC. Three trials of Reactor 3 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 3 was 19°C (min=19; max=20; stdev=0.58) and was constant over time. The average pH of the water was 7.60 (min=7.5; max=7.7) and was also constant over time (Table 4.7). The average initial turbidity of the Reactor 3 water was 270 NTU (min=160, max=350, stdev=100) (Table 4.7). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 3 was 32 mg/L (min=31; max=32; stdev=0.88) (Table 4.7). The TOC concentration remained constant over time.

Table 4.7 Reactor 3 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	20	20	20
	Trial 2	19	19	19
	Trial 3	19	19	19
	Average (stdev)	**19 (0.58)**	**19 (0.58)**	**19 (0.58)**
pH	Trial 1	7.7	7.7	7.6
	Trial 2	7.5	7.5	7.6
	Trial 3	7.6	7.5	7.6
	Average (stdev)	**7.6 (0.10)**	**7.6 (0.12)**	**7.6 (0.0)**
Turbidity (NTU)	Trial 1	160	76	36
	Trial 2	350	170	28
	Trial 3	310	150	29
	Average (stdev)	**270 (100)**	**130 (50)**	**31 (4.4)**
TOC (mg/L)	Trial 1	31	30	31
	Trial 2	32	33	33
	Trial 3	32	32	31
	Average (stdev)	**32 (0.88)**	**32 (1.5)**	**32 (1.5)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.3). The average remaining free chlorine residual after 24 hours was 0.17 mg/L (min=0.03; max=0.26; stdev=0.12). The average remaining total chlorine residual after 24 hours was 0.51 (min=0.43; max=0.59; stdev=0.08).

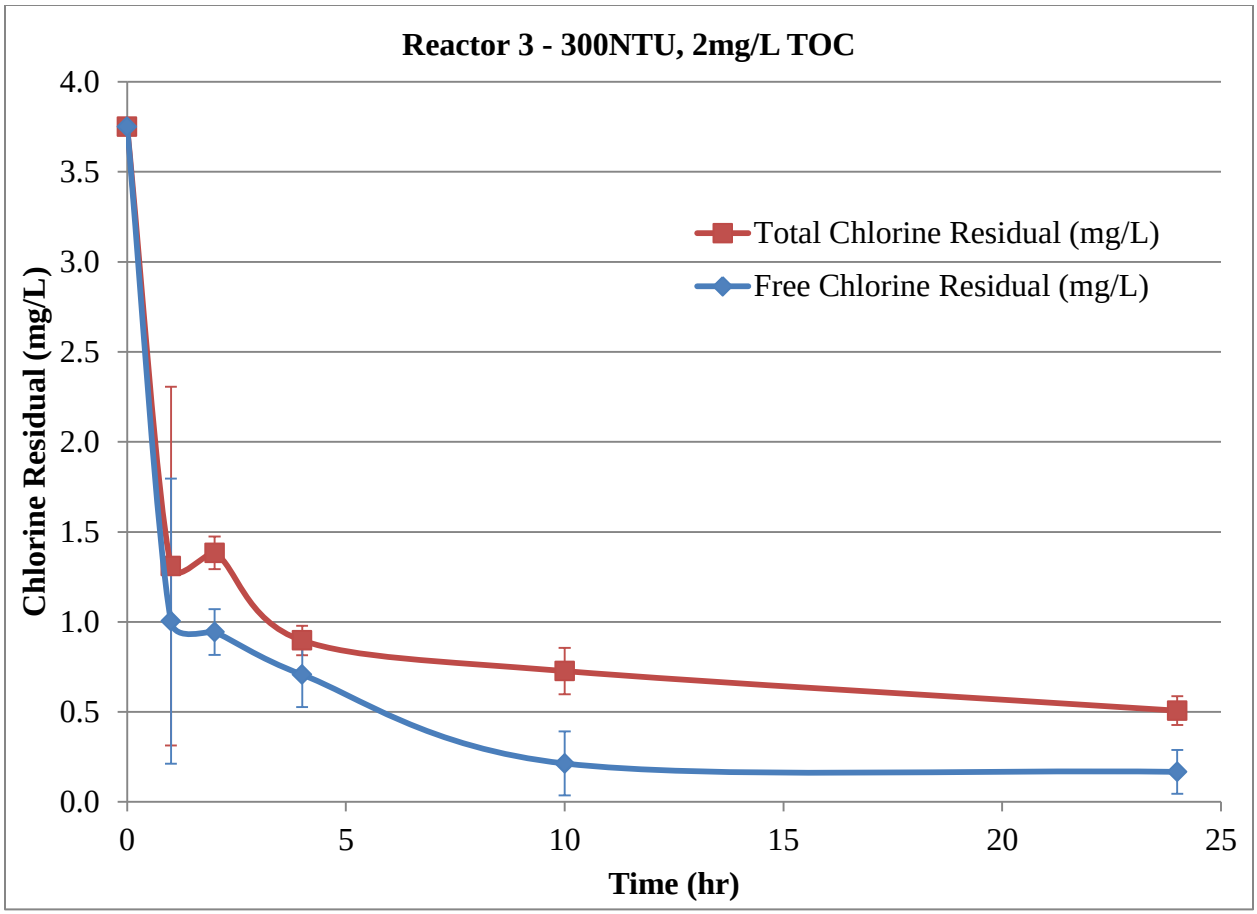

Figure 4.3 Total chlorine residual and free chlorine residual in Reactor 3. Data points represent the average of three trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of E. coli was 1.9×10^8 CFU/100mL (min=1.3×10^8; max=2.4×10^8; stdev=7.9×10^7) (Table 4.8). After 24 hours, the average log reduction was 8.3 (min=8.1; max=8.4; stdev=0.19) (Table 4.9).

Table 4.8 *E. coli* Counts for Reactor 3

E. coli Count (CFU/100mL)	Time (hr)			
	0	1	10	24
Trial 1	TNTC	1	<1	<1
Trial 2	2.4E+08	270	<1	<1
Trial 3	1.3E+08	<10	<1	<1
Average (stdev)	**1.9E+08 (7.9E+07)**	**94 (150)**	**1.0 (0.0)**	**1.0 (0.0)**

TNTC – Too numerous to count

Table 4.9 Log Reduction of *E. coli* in Reactor 3

Log Reduction	Time (hr)		
	1	10	24
Average (stdev)*	**6.5 (0.82)**	**8.3 (0.19)**	**8.25 (0.19)**

*Trial 1 not included because *E. coli* at time 0hr was too numerous to count

4.4 Reactor 4 – 10 NTU, 10 mg/L TOC

Reactor 4 contained 10 NTU turbidity, and an additional 10 mg/L of TOC. Three trials of Reactor 4 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 4 was 19°C (min=19; max=19; stdev=0) and was constant over time. The average pH of the water was 7.5 (min=7.4; max=7.6) and was also constant over time (Table 4.10). The average initial turbidity of the Reactor 4 water was 13 NTU (min=12, max=14, stdev=1.1) (Table 4.10). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 4 was 43 mg/L (min=42; max=45; stdev=1.5) (Table 4.10). The TOC concentration remained constant over time.

Table 4.10 Reactor 4 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	19	19	19
	Trial 2	19	19	19
	Trial 3	19	19	19
	Average (stdev)	**19 (0.0)**	**19 (0.0)**	**19 (0.0)**
pH	Trial 1	7.6	7.6	7.6
	Trial 2	7.5	7.4	7.5
	Trial 3	7.4	7.4	7.5
	Average (stdev)	**7.5 (0.10)**	**7.5 (0.12)**	**7.5 (0.06)**
Turbidity (NTU)	Trial 1	13	11	2.4
	Trial 2	12	12	3.4
	Trial 3	14	10	3.1
	Average (stdev)	**13 (1.1)**	**11 (0.70)**	**2.9 (0.52)**
TOC (mg/L)	Trial 1	44	45	44
	Trial 2	42	41	41
	Trial 3	45	44	43
	Average (stdev)	**43 (1.5)**	**43 (2.3)**	**43 (1.8)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.4). The average remaining free chlorine residual after 24 hours was 0.28 mg/L (min=0.04; max=0.48; stdev=0.22). The average remaining total chlorine residual after 24 hours was 0.60 (min=0.54; max=0.67; stdev=0.07).

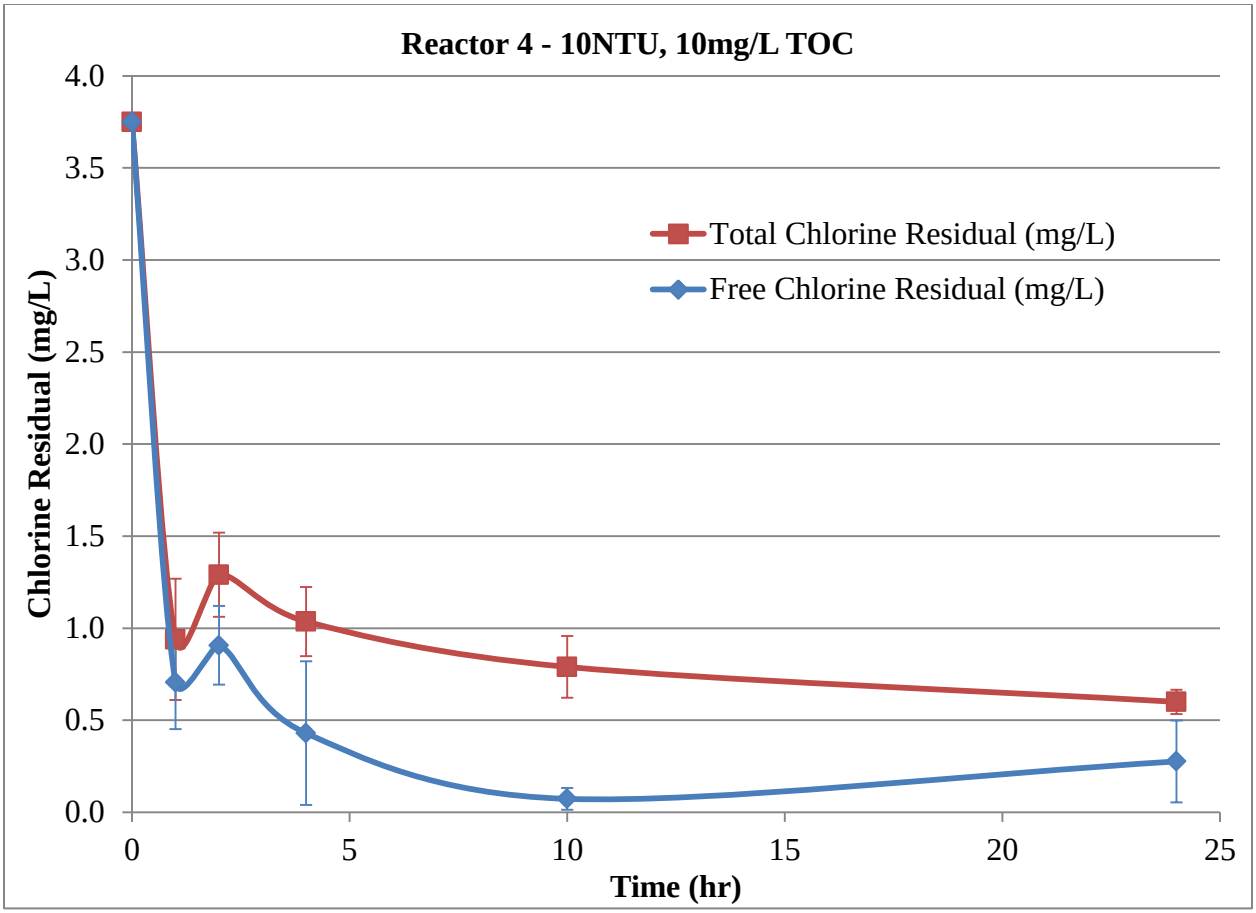

Figure 4.4 Total chlorine residual and free chlorine residual in Reactor 4. Data points represent the average of three trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of E. coli was $2.3x10^8$ CFU/100mL (min=$2.0x10^8$; max=$2.4x10^8$; stdev=$2.5x10^7$) (Table 4.11). After 24 hours, the average log reduction was 8.4 (min=8.3; max=8.4; stdev=0.05) (Table 4.12).

Table 4.11 *E. coli* Counts for Reactor 4

	Time (hr)			
E. coli Count (CFU/100mL)	0	1	10	24
Trial 1	2.4E+08	20	<1	<1
Trial 2	2.0E+08	<10	<1	<1
Trial 3	2.4E+08	<10	<1	<1
Average (stdev)	**2.3E+08 (2.5E+07)**	**13 (5.8)**	**1.0 (0.0)**	**1.0 (0.0)**

Table 4.12 Log Reduction of *E. coli* in Reactor 4

	Time (hr)		
Log Reduction	1	10	24
Average (stdev)	**7.3 (0.16)**	**8.4 (0.05)**	**8.4 (0.05)**

4.5 Reactor 5 – 100 NTU, 10 mg/L TOC

Reactor 5 contained 100 NTU turbidity, and an additional 10 mg/L of TOC. Four trials of Reactor 5 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 5 ranged from 19 to 20℃ (min=19; max=20) and was constant over time. The average pH of the water ranged from 7.5 to 7.6 (min=7.4; max=7.7) and was also constant over time (Table 4.13). The average initial turbidity of the Reactor 5 water was 120 NTU (min=110, max=120, stdev=5.0) (Table 4.13). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 5 was 40 mg/L (min=37; max=43; stdev=2.4) (Table 4.13). The TOC concentration remained constant over time.

Table 4.13 Reactor 5 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	20	20	19
	Trial 2	20	20	20
	Trial 3	19	19	19
	Trial 4	19	19	19
	Average (stdev)	**20 (0.58)**	**20 (0.58)**	**19 (0.50)**
pH	Trial 1	7.6	7.5	7.7
	Trial 2	7.6	7.5	7.6
	Trial 3	7.5	7.4	7.4
	Trial 4	7.5	7.5	7.5
	Average (stdev)	**7.6 (0.06)**	**7.5 (0.05)**	**7.6 (0.13)**
Turbidity (NTU)	Trial 1	110	63	11
	Trial 2	120	110	19
	Trial 3	120	110	17
	Trial 4	120	91	14
	Average (stdev)	**120 (5.0)**	**94 (22)**	**15 (3.6)**
TOC (mg/L)	Trial 1	37	39	39
	Trial 2	43	42	42
	Trial 3	41	41	40
	Trial 4	39	39	38
	Average (stdev)	**40 (2.4)**	**40 (1.5)**	**40 (1.8)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.5). The average remaining free chlorine residual after 24 hours was 0.11 mg/L (min=0.01; max=0.18; stdev=0.07). The average remaining total chlorine residual after 24 hours was 0.35 (min=0.15; max=0.64; stdev=0.21).

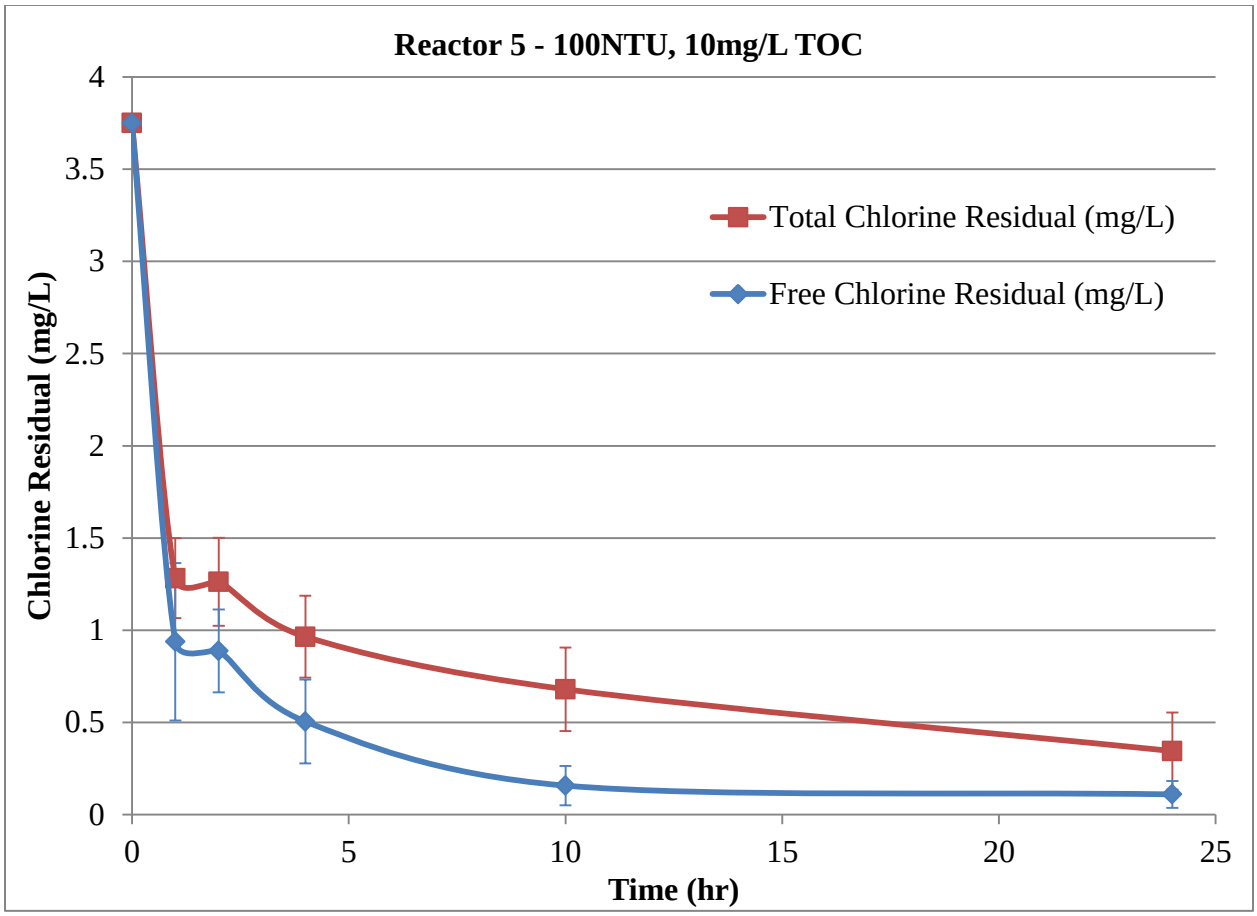

Figure 4.5 Total chlorine residual and free chlorine residual in Reactor 5. Data points represent the average of four trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of *E. coli* was 2.4×10^8 CFU/100mL (min=2.4×10^8; max=2.4×10^8; stdev=0.0) (Table 4.14). After 24 hours, the average log reduction was 7.9 (min=6.8; max=8.4; stdev=0.90) (Table 4.15).

Table 4.14 *E. coli* Counts for Reactor 5

E. coli Count (CFU/100mL)	Time (hr)			
	0	1	10	24
Trial 1	TNTC	<1	<1	<1
Trial 2	2.4E+08	20	<1	<1
Trial 3	2.4E+08	<10	<1	<1
Trial 4	2.4E+08	<10	<1	36.4
Average (stdev)	**2.4E+08 (0.0)**	**10 (7.8)**	**1.0 (0.0)**	**9.9 (18)**

TNTC – Too numerous to count

Table 4.15 Log Reduction of *E. coli* in Reactor 5

Log Reduction	Time (hr)		
	1	10	24
Average (stdev)*	**7.3 (0.17)**	**7.9 (0.90)**	**7.9 (0.90)**

*Trial 1 not included because *E. coli* at time 0hr was too numerous to count

4.6 Reactor 6 – 300 NTU, 10 mg/L TOC

Reactor 6 contained 300 NTU turbidity, and an additional 10 mg/L of TOC. Three trials of Reactor 6 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 6 was 19°C (min=19; max=20; stdev=0.58) and was constant over time. The average pH of the water ranged from 7.5 to 7.60 (min=7.5; max=7.6) and was also constant over time (Table 4.16). The average initial turbidity of the Reactor 6 water was 290 NTU (min=200, max=344, stdev=81) (Table 4.16). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 6 was 39 mg/L (min=38; max=41; stdev=1.2) (Table 4.16). The TOC concentration remained constant over time.

Table 4.16 Reactor 6 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	20	20	20
	Trial 2	19	19	19
	Trial 3	19	19	19
	Average (stdev)	**19 (0.58)**	**19 (0.58)**	**19 (0.58)**
pH	Trial 1	7.5	7.6	7.6
	Trial 2	7.5	7.6	7.6
	Trial 3	7.6	7.6	7.6
	Average (stdev)	**7.5 (0.58)**	**7.6 (0.0)**	**7.6 (0.0)**
Turbidity (NTU)	Trial 1	200	150	27
	Trial 2	340	180	21
	Trial 3	340	180	39
	Average (stdev)	**290 (81)**	**170 (17)**	**29 (8.8)**
TOC (mg/L)	Trial 1	38	39	38
	Trial 2	41	41	41
	Trial 3	39	39	39
	Average (stdev)	**39 (1.2)**	**40 (1.3)**	**40 (1.3)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.6). The average remaining free chlorine residual after 24 hours was 0.29 mg/L (min=0.21; max=0.35; stdev=0.07). The average remaining total chlorine residual after 24 hours was 0.47 (min=0.44; max=0.52; stdev=0.04).

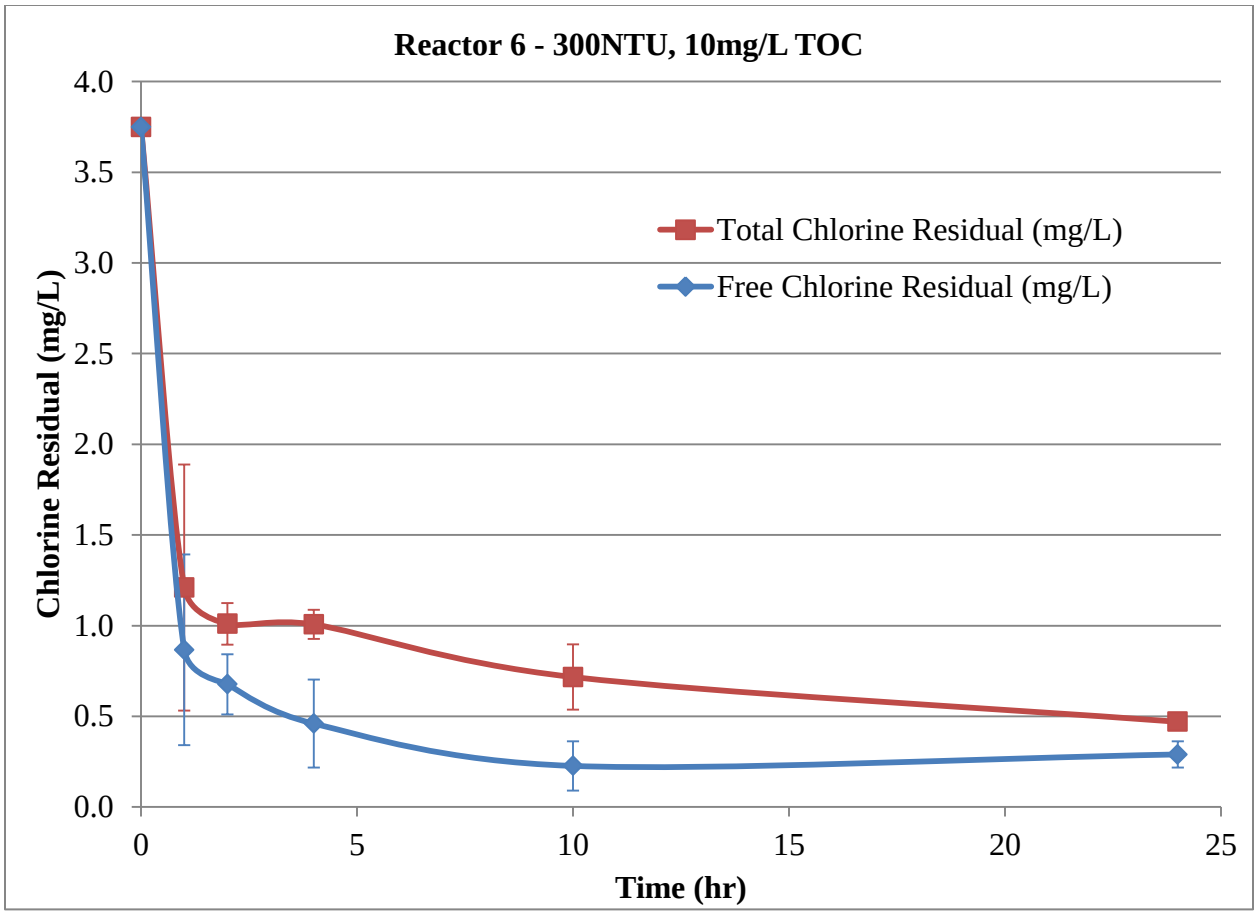

Figure 4.6 Total chlorine residual and free chlorine residual in Reactor 6. Data points represent the average of three trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of *E. coli* was 2.4×10^8 CFU/100mL (min=2.4×10^8; max=2.4×10^8; stdev=0.0) (Table 4.17). After 24 hours, the average log reduction was 8.4 (min=8.4; max=8.4; stdev=0.0) (Table 4.18).

Table 4.17 *E. coli* Counts for Reactor 6

E. coli Count (CFU/100mL)	Time (hr)			
	0	1	10	24
Trial 1	TNTC	<1	<1	<1
Trial 2	2.4E+08	60	<1	<1
Trial 3	2.4E+08	<10	<1	<1
Average (stdev)	**2.4E+08 (0.0)**	**24 (32)**	**1.0 (0.0)**	**1.0 (0.0)**

TNTC – Too numerous to count

Table 4.18 Log Reduction of *E. coli* in Reactor 6

Log Reduction	Time (hr)		
	1	10	24
Average (stdev)	**7.0 (0.55)**	**8.4 (0.0)**	**8.4(0.0)**

*Trial 1 not included because *E. coli* at time 0hr was too numerous to count

4.7 Reactor 7 – 10 NTU, 25 mg/L TOC

Reactor 7 contained 10 NTU turbidity, and an additional 25 mg/L of TOC. Three trials of Reactor 7 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 7 ranged was 19℃ (min=19; max=20) and was constant over time. The average pH of the water ranged from 7.5 to 7.6 (min=7.5; max=7.7) and was also constant over time (Table 4.19). The average initial turbidity of the Reactor 7 water was 15 NTU (min=10, max=22, stdev=6.3) (Table 4.19). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 7 was 58 mg/L (min=57; max=60; stdev=1.3) (Table 4.19). The TOC concentration remained constant over time.

Table 4.19 Reactor 7 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	19	19	19
	Trial 2	19	20	20
	Trial 3	19	19	19
	Average (stdev)	**19 (0.0)**	**19 (0.58)**	**19 (0.58)**
pH	Trial 1	7.6	7.5	7.5
	Trial 2	7.7	7.7	7.6
	Trial 3	7.5	7.5	7.5
	Average (stdev)	**7.6 (0.10)**	**7.6 (0.12)**	**7.5 (0.06)**
Turbidity (NTU)	Trial 1	22	12	3.1
	Trial 2	10	10	3.0
	Trial 3	13	10	3.0
	Average (stdev)	**15 (6.3)**	**11 (0.93)**	**3.1 (0.08)**
TOC (mg/L)	Trial 1	58	57	58
	Trial 2	60	60	60
	Trial 3	57	58	57
	Average (stdev)	**58 (1.3)**	**58 (1.6)**	**59 (1.4)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.7). The average remaining free chlorine residual after 24 hours was 0.12 mg/L (min=0.01; max=0.25; stdev=0.12). The average remaining total chlorine residual after 24 hours was 0.53 (min=0.33; max=0.73; stdev=0.20).

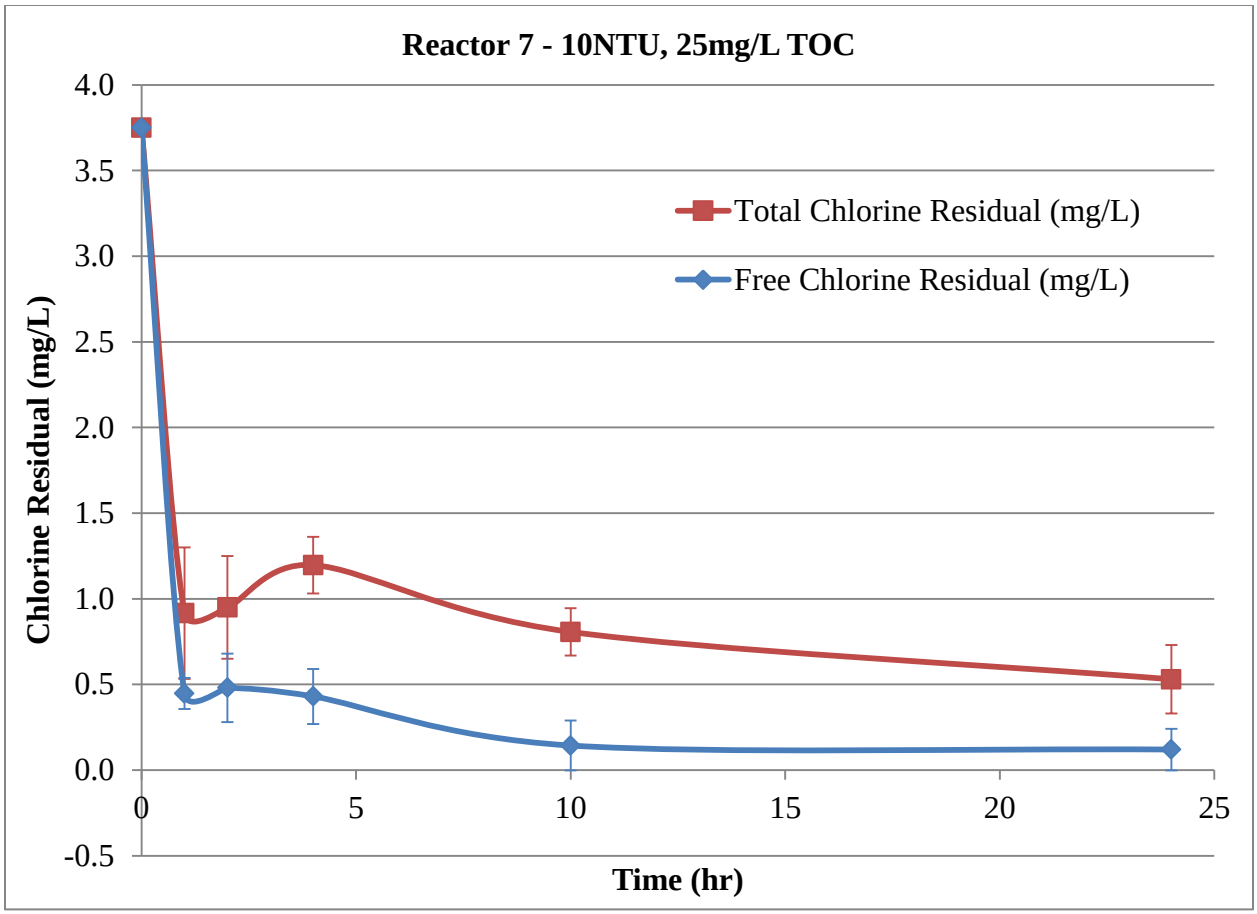

Figure 4.7 Total chlorine residual and free chlorine residual in Reactor 7. Data points represent the average of three trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of E. coli was 1.9×10^8 CFU/100mL (min=1.2×10^8; max=2.4×10^8; stdev=6.2×10^7) (Table 4.20). After 24 hours, the average log reduction was 8.3 (min=8.1; max=8.4; stdev=0.16) (Table 4.21).

Table 4.20 *E. coli* Counts for Reactor 7

	Time (hr)			
E. coli Count (CFU/100mL)	0	1	10	24
Trial 1	2.0E+08	<20	<1	<1
Trial 2	1.2E+08	52	<1	<1
Trial 3	2.4E+08	<10	<1	<1
Average (stdev)	**1.9E+08 (6.2E+07)**	**27 (22)**	**1.0 (0.0)**	**1.0 (0.0)**

Table 4.21 Log Reduction of *E. coli* in Reactor 7

	Time (hr)		
Log Reduction	1	10	24
Average (stdev)	**6.9 (0.51)**	**8.3 (0.16)**	**8.3 (0.16)**

4.8 Reactor 8 – 100 NTU, 25 mg/L TOC

Reactor 8 contained 100 NTU turbidity, and an additional 25 mg/L of TOC. Three trials of Reactor 8 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 8 ranged from 19 to 20℃ (min=19; max=20) and was constant over time. The average pH of the water was 7.5 (min=7.4; max=7.5; stdev=0.06) and was also constant over time (Table 4.22). The average initial turbidity of the Reactor 8 water was 110 NTU (min=91, max=120, stdev=15) (Table 4.22). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 8 was 57 mg/L (min=55; max=59; stdev=2.3) (Table 4.22). The TOC concentration remained constant over time.

Table 4.22 Reactor 8 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	19	19	20
	Trial 2	20	20	20
	Trial 3	19	19	19
	Average (stdev)	**19 (0.58)**	**19 (0.58)**	**20 (0.58)**
pH	Trial 1	7.5	7.5	7.5
	Trial 2	7.5	7.5	7.5
	Trial 3	7.4	7.4	7.4
	Average (stdev)	**7.5 (0.58)**	**7.5 (0.58)**	**7.5 (0.58)**
Turbidity (NTU)	Trial 1	110	ND	7.3
	Trial 2	120	82	13.0
	Trial 3	91	78	17.6
	Average (stdev)	**110 (15)**	**80 (2.5)**	**13 (5.1)**
TOC (mg/L)	Trial 1	55	52	53
	Trial 2	59	57	58
	Trial 3	57	57	57
	Average (stdev)	**57 (2.3)**	**56 (2.8)**	**56 (2.6)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.8). The average remaining free chlorine residual after 24 hours was 0.18 mg/L (min=0.02; max=0.32; stdev=0.15). The average remaining total chlorine residual after 24 hours was 0.47 (min=0.23; max=0.66; stdev=0.22).

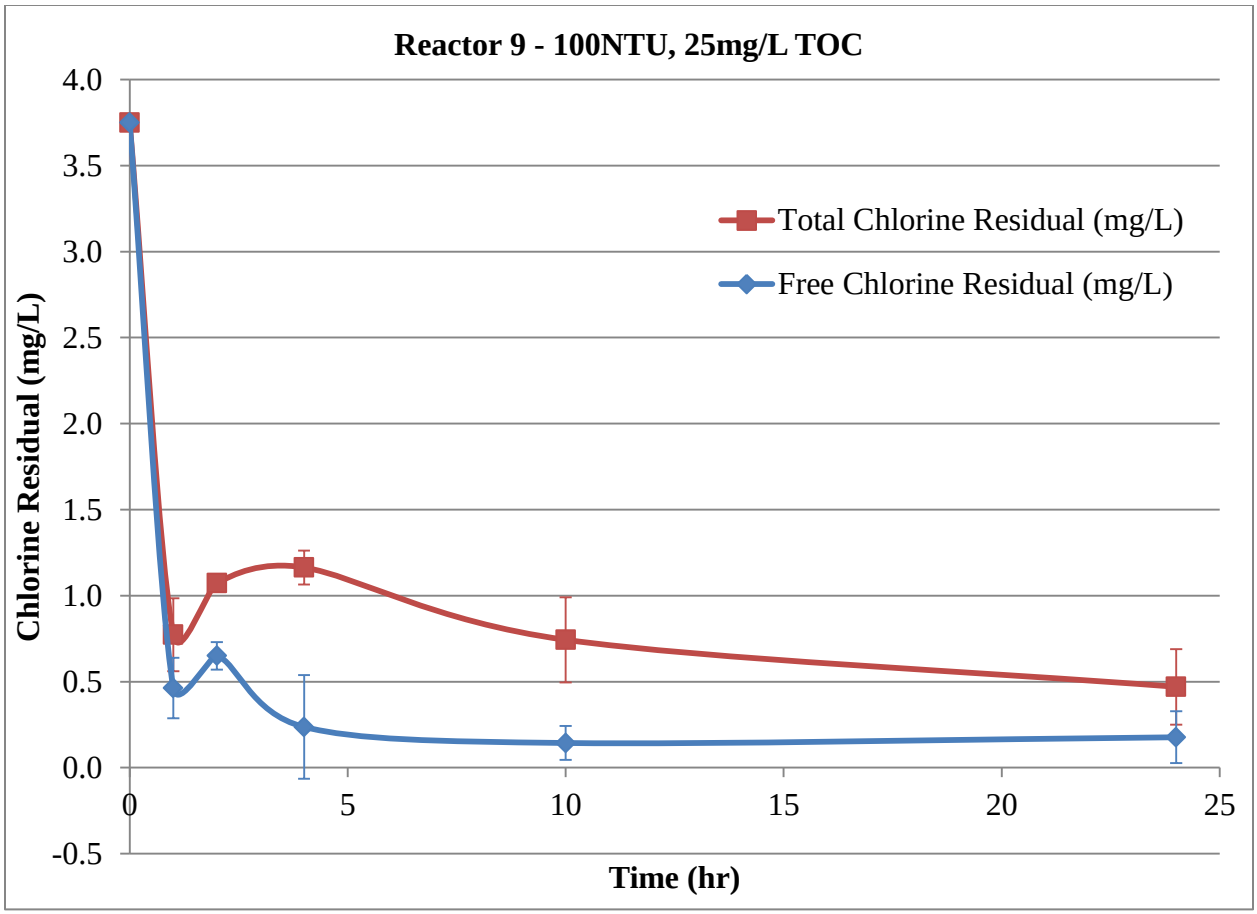

Figure 4.8 Total chlorine residual and free chlorine residual in Reactor 8. Data points represent the average of three trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of *E. coli* was 2.2×10^8 CFU/100mL (min=2.0×10^8; max=2.4×10^8; stdev=3.1×10^7) (Table 4.23). After 24 hours, the average log reduction was 8.3 (min=8.3; max=8.4; stdev=0.06) (Table 4.24).

Table 4.23 *E. coli* Counts for Reactor 8

E. coli Count (CFU/100mL)	Time (hr)			
	0	1	10	24
Trial 1	TNTC	<1	<1	<1
Trial 2	2.0E+08	150	100	<1
Trial 3	2.4E+08	5200	<1	<1
Average (stdev)	**2.2E+08 (3.1E+07)**	**1800 (3000)**	**34 (57)**	**1.0 (0.0)**

TNTC – Too numerous to count

Table 4.24 Log Reduction of *E. coli* in Reactor 8

Log Reduction	Time (hr)		
	1	10	24
Average (stdev)	**5.4 (1.0)**	**7.3 (1.5)**	**8.3 (0.06)**

*Trial 1 not included because *E. coli* at time 0hr was too numerous to count

4.9 Reactor 9 – 300 NTU, 25 mg/L TOC

Reactor 9 contained 300 NTU turbidity, and an additional 25 mg/L of TOC. Three trials of Reactor 9 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 9 was 19°C (min=19; max=20) and was constant over time. The average pH of the water ranged from 7.5 to 7.6 (min=7.5; max=7.6) and was also constant over time (Table 4.25). The average initial turbidity of the Reactor 9 water was 280 NTU (min=220, max=310, stdev=52) (Table 4.25). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 9 was 56 mg/L (min=54; max=58; stdev=1.7) (Table 4.25). The TOC concentration remained constant over time.

Table 4.25 Reactor 9 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	20	19	19
	Trial 2	19	19	19
	Trial 3	19	19	19
	Average (stdev)	**19 (0.58)**	**19 (0.0)**	**19 (0.0)**
pH	Trial 1	7.6	7.6	7.6
	Trial 2	7.6	7.5	7.5
	Trial 3	7.5	7.5	7.5
	Average (stdev)	**7.6 (0.06)**	**7.5 (0.06)**	**7.5 (0.06)**
Turbidity (NTU)	Trial 1	310	ND	31
	Trial 2	310	100	18
	Trial 3	220	100	21
	Average (stdev)	**280 (52)**	**100 (0.0)**	**23 (6.7)**
TOC (mg/L)	Trial 1	54	54	54
	Trial 2	58	58	57
	Trial 3	55	54	54
	Average (stdev)	**56 (1.7)**	**55 (2.2)**	**55 (1.8)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.9). The average remaining free chlorine residual after 24 hours was 0.14 mg/L (min=0.10; max=0.18; stdev=0.04). The average remaining total chlorine residual after 24 hours was 0.40 (min=0.32; max=0.53; stdev=0.11).

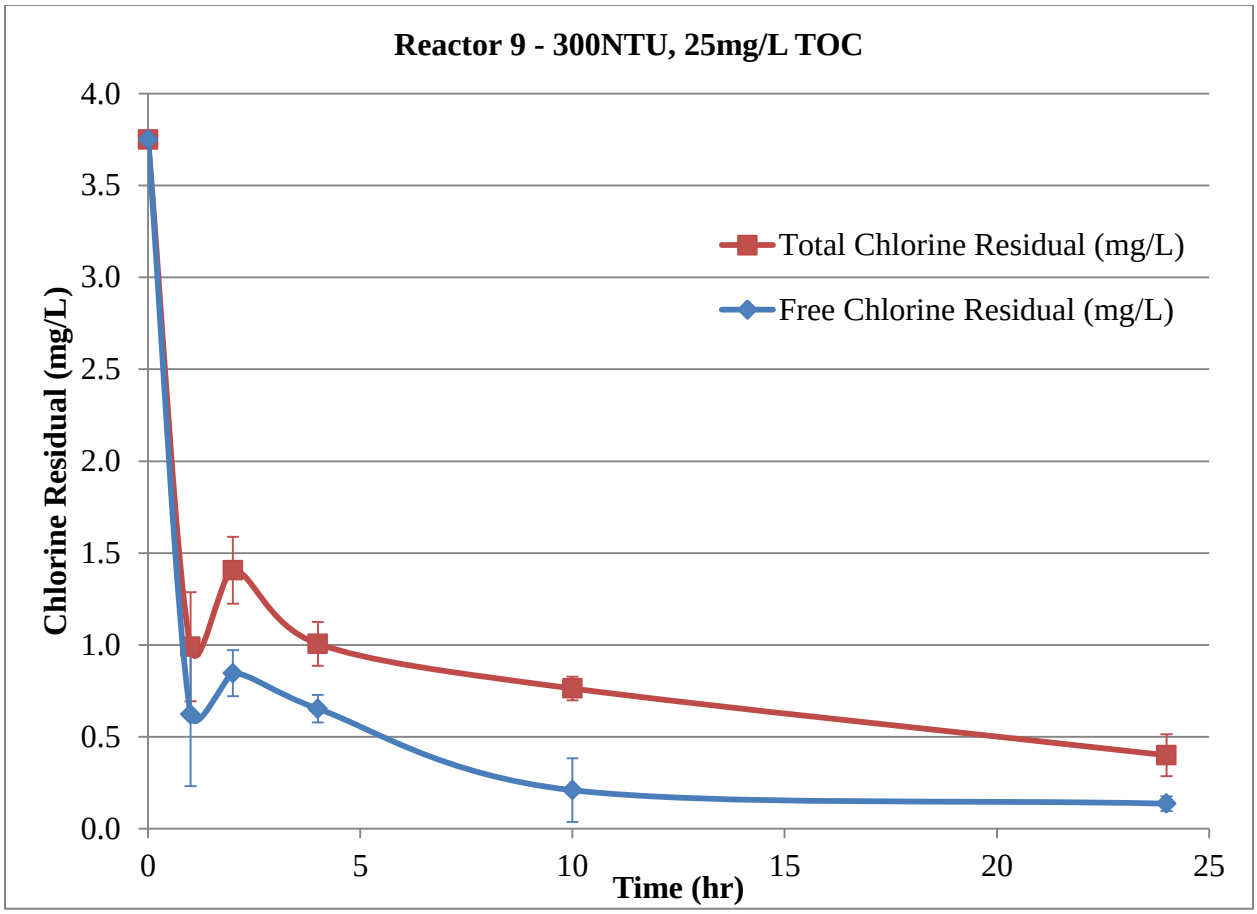

Figure 4.9 Total chlorine residual and free chlorine residual in Reactor 9. Data points represent the average of three trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of *E. coli* was 2.1×10^8 CFU/100mL (min=1.7×10^8; max=2.4×10^8; stdev=4.9×10^7) (Table 4.26). After 24 hours, the average log reduction was 8.2 (Table 4.27).

Table 4.26 *E. coli* Counts for Reactor 9

E. coli Count (CFU/100mL)	Time (hr)			
	0	1	10	24
Trial 1	TNTC	8.5	1	<1
Trial 2	2.4E+08	<20	345	TNTC
Trial 3	1.7E+08	<10	<1	<1
Average (stdev)	**2.1E+08 (4.9E+07)**	**13 (6.3)**	**116 (200)**	**1.0 (0.0)**

TNTC – Too numerous to count

Table 4.27 Log Reduction of *E. coli* in Reactor 9

Log Reduction	Time (hr)		
	1	10	24
Average (stdev)	**7.2 (0.11)***	**7.0 (1.69)**	**8.2 (0.0)****

*Trial 1 not included because *E. coli* at 0hr was too numerous to count

**Trial 1 and Trial 2 not included because *E. coli* at 0hr was too numerous to count

4.10 Reactor 10 – 10 NTU, No TOC Addition

Reactor 10 contained 10 NTU turbidity, and no additional TOC. Four trials of Reactor 10 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 10 was 20℃ (min=20; max=20; stdev=0.0) and was constant over time. The average pH of the water was 7.6 (min=7.5; max=7.7) and was also constant over time (Table 4.28). The average initial turbidity of the Reactor 10 water was 14 NTU (min=11, max=18, stdev=3.1) (Table 4.28). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 10 was 39 mg/L (min=37; max=41; stdev=1.6) (Table 4.28). The TOC concentration remained constant over time.

Table 4.28 Reactor 10 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	20	20	20
	Trial 2	20	20	20
	Trial 3	20	20	20
	Trial 4	20	20	20
	Average (stdev)	**20 (0.0)**	**20 (0.0)**	**20 (0.0)**
pH	Trial 1	7.5	7.5	7.5
	Trial 2	7.6	7.5	7.6
	Trial 3	7.6	7.5	7.6
	Trial 4	7.7	7.7	7.6
	Average (stdev)	**7.6 (0.08)**	**7.6 (0.10)**	**7.6 (0.05)**
Turbidity (NTU)	Trial 1	13	9.3	3.4
	Trial 2	13	7.5	3.2
	Trial 3	18	12	3.5
	Trial 4	11	11	3.7
	Average (stdev)	**14 (3.1)**	**10 (2.0)**	**3.5 (0.22)**
TOC (mg/L)	Trial 1	41	39	41
	Trial 2	40	39	40
	Trial 3	39	38	38
	Trial 4	37	36	36
	Average (stdev)	**39 (1.6)**	**38 (1.4)**	**39 (1.9)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.10). The average remaining free chlorine residual after 24 hours was 0.02 mg/L (min=0.00; max=0.04; stdev=0.02). The average remaining total chlorine residual after 24 hours was 0.52 (min=0.50; max=0.58; stdev=0.04).

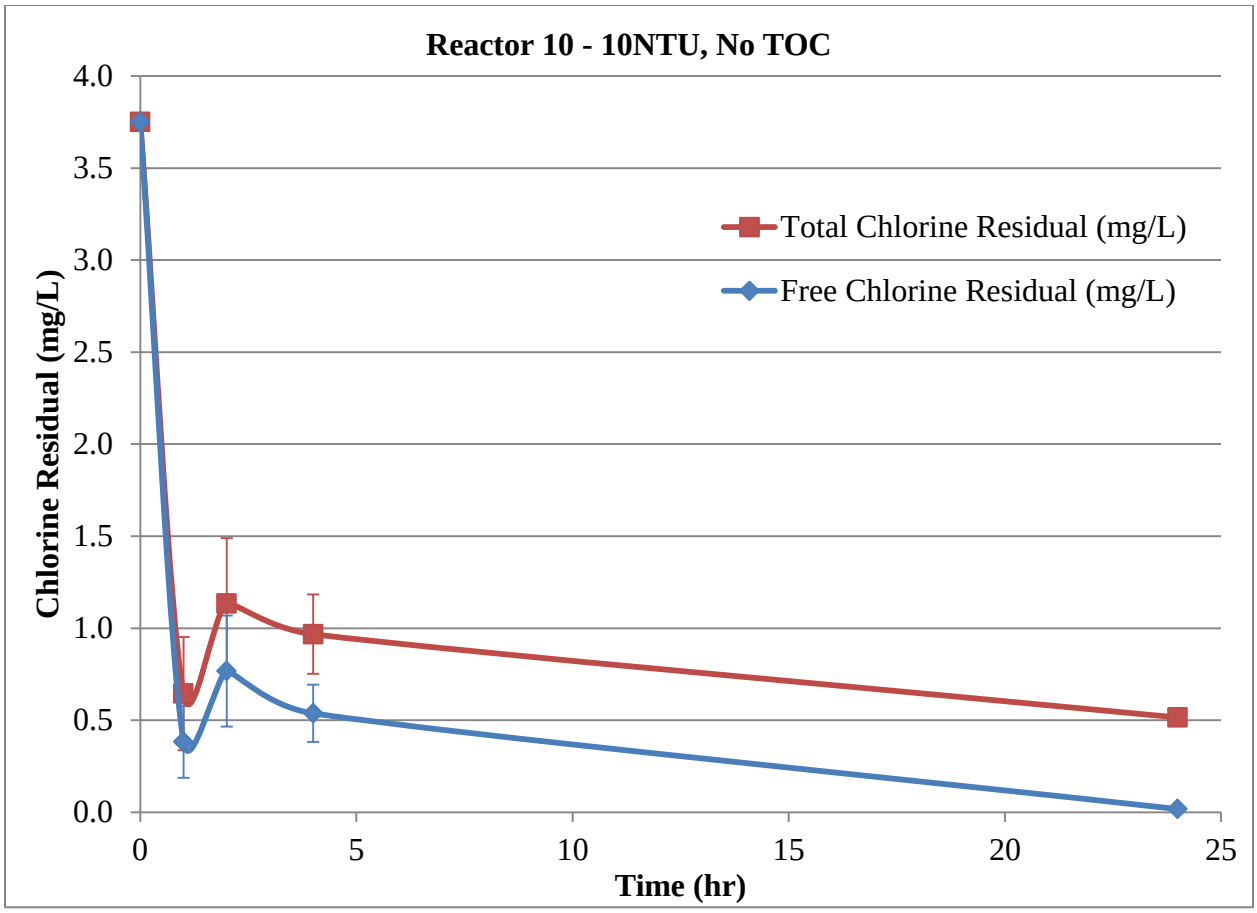

Figure 4.10 Total chlorine residual and free chlorine residual in Reactor 10. Data points represent the average of four trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of E. coli was 2.2×10^8 CFU/100mL (min=1.7×10^8; max=2.4×10^8; stdev=3.4×10^7) (Table 4.29). After 24 hours, the average log reduction was 6.6 (min=5.4; max=7.6; stdev=1.1) (Table 4.30).

Table 4.29 *E. coli* Counts for Reactor 10

E. coli Count (CFU/100mL)	Time (hr)			
	0	1	10*	24
Trial 1	2.0E+08	TNTC	ND	440
Trial 2	2.4E+08	TNTC	ND	980
Trial 3	2.4E+08	TNTC	ND	34
Trial 4	1.7E+08	1200	ND	4.1
Average (stdev)	**2.1E+08 (3.4E+07)**	**1200 (0.0)**	**ND**	**360 (460)**

*No data was collected at 10 hours

TNTC - Too numerous to count

Table 4.30 Log Reduction of *E. coli* in Reactor 10

Log Reduction	Time (hr)		
	1	10*	24
Average (stdev)	**5.2 (0.0)****	**ND**	**6.6 (1.1)**

*No data was collected at 10 hours

**Trials 1, 2, and 3 were too numerous to count at 1hr so they are not included in the average and standard deviation calculation

4.11 Reactor 11 – 100 NTU, No TOC Addition

Reactor 11 contained 100 NTU turbidity, and no additional TOC. Three trials of Reactor 11 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 11 was 19°C (min=19; max=19; stdev=0.0) and was constant over time. The average pH of the water was 7.6 (min=7.5; max=7.7) and was also constant over time (Table 4.31). The average initial turbidity of the Reactor 11 water was 100 NTU (min=90, max=110, stdev=10) (Table 4.31). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 11 was 32 mg/L (min=30; max=34; stdev=1.8) (Table 4.31). The TOC concentration remained constant over time.

Table 4.31 Reactor 11 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	19	19	19
	Trial 2	19	19	19
	Trial 3	19	19	19
	Average (stdev)	**19 (0.0)**	**19 (0.0)**	**19 (0.0)**
pH	Trial 1	7.7	7.7	7.6
	Trial 2	7.6	7.6	7.6
	Trial 3	7.5	7.5	7.5
	Average (stdev)	**7.6 (0.10)**	**7.6 (0.10)**	**7.6 (0.06)**
Turbidity (NTU)	Trial 1	90	ND	14
	Trial 2	100	81	10
	Trial 3	110	92	13
	Average (stdev)	**100 (10)**	**87 (7.3)**	**12 (1.8)**
TOC (mg/L)	Trial 1	30	30	29
	Trial 2	34	34	34
	Trial 3	32	32	31
	Average (stdev)	**32 (1.8)**	**32 (2.0)**	**31 (2.6)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.11). The average remaining free chlorine residual after 24 hours was 0.30 mg/L (min=0.21; max=0.36; stdev=0.08). The average remaining total chlorine residual after 24 hours was 0.53 (min=0.51; max=0.56; stdev=0.03).

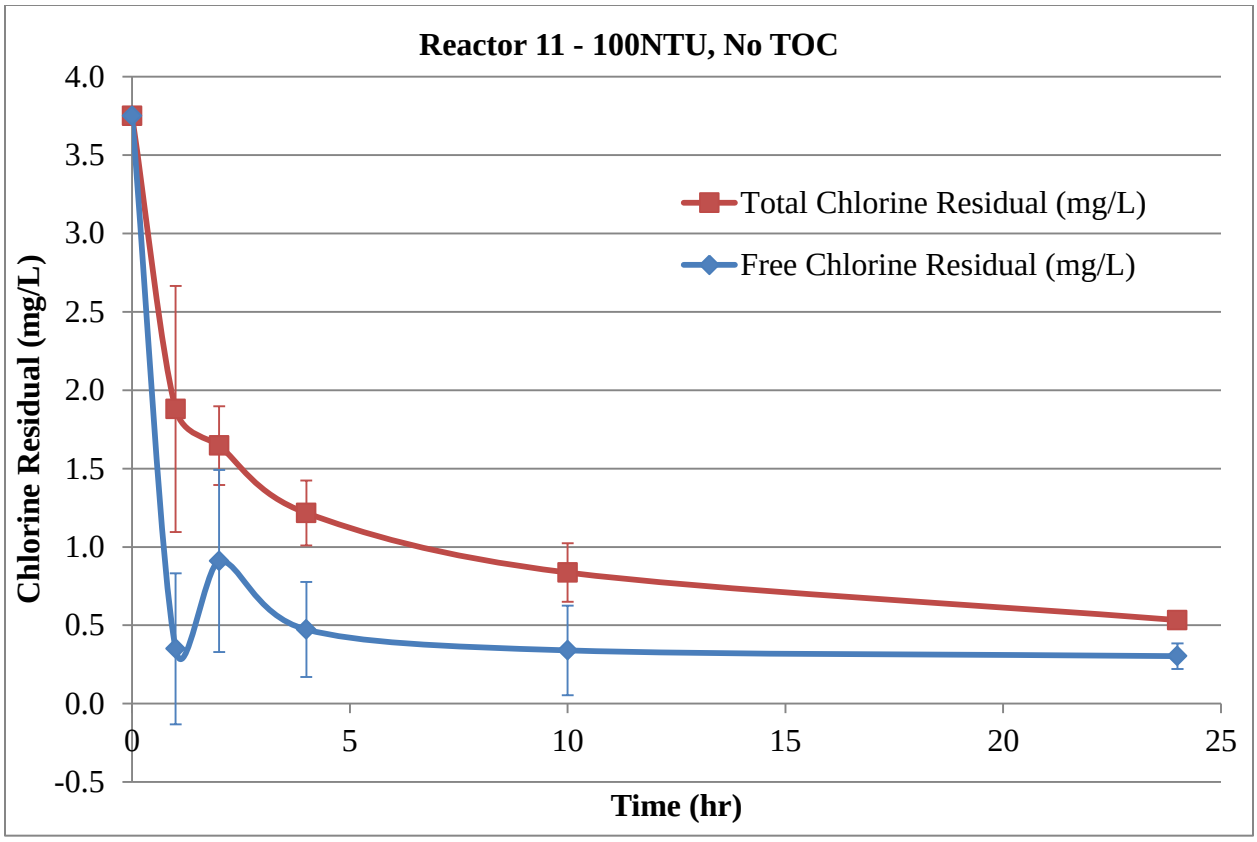

Figure 4.11 Total chlorine residual and free chlorine residual in Reactor 11. Data points represent the average of three trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of *E. coli* was 2.0×10^8 CFU/100mL (min=1.6×10^8; max=2.4×10^8; stdev=6.1×10^7) (Table 4.32). After 24 hours, the average log reduction was 8.3 (min=8.2; max=8.4; stdev=0.14) (Table 4.33).

Table 4.32 *E. coli* Counts for Reactor 11

	Time (hr)			
E. coli Count (CFU/100mL)	0	1	10	24
Trial 1	TNTC	2	4.1	<1
Trial 2	1.6E+08	<20	<1	<1
Trial 3	2.4E+08	31	8.6	<1
Average (stdev)	**2.0E+08 (6.1E+07)**	**18 (15)**	**4.6 (3.8)**	**1.0 (0.0)**

TNTC – Too numerous to count

Table 4.33 Log Reduction of *E. coli* in Reactor 11

Log Reduction	Time (hr)		
	1	10	24
Average (stdev)*	**6.9 (0.0)**	**7.8 (0.52)**	**8.3 (0.14)**

*Trial 1 not included because *E. coli* at time 0hr was too numerous to count

4.12 Reactor 12 – 300 NTU, No TOC Addition

Reactor 12 contained 300 NTU turbidity, and no additional TOC. Five trials of Reactor 12 were performed.

Temperature and pH, Turbidity, TOC

The average temperature of the water in Reactor 12 was 20°C (min=20; max=20; stdev=0.0) and was constant over time. The average pH of the water ranged from 7.5 to 7.6 (min=7.5; max=7.7) and was also constant over time (Table 4.34). The average initial turbidity of the Reactor 12 water was 270 NTU (min=200, max=310, stdev=58) (Table 4.34). Turbidity decreased over time as the clay settled. The average initial TOC concentration for Reactor 12 was 36 mg/L (min=29; max=38; stdev=3.7) (Table 4.34). The TOC concentration remained constant over time.

Table 4.34 Reactor 12 Summary Data

Parameter		Time (hr)		
		0	1	24
Temperature (°C)	Trial 1	20	20	20
	Trial 2	20	20	20
	Trial 3	20	20	20
	Trial 4	20	20	20
	Trial 5	20	20	20
	Average (stdev)	**20 (0.0)**	**20 (0.0)**	**20 (0.0)**
pH	Trial 1	7.6	7.5	7.5
	Trial 2	7.5	7.5	7.5
	Trial 3	7.6	7.6	7.6
	Trial 4	7.7	7.7	7.5
	Trial 5	7.5	7.5	7.6
	Average (stdev)	**7.6 (0.08)**	**7.6 (0.09)**	**7.5 (0.05)**
Turbidity (NTU)	Trial 1	310	ND	34
	Trial 2	200	150	38
	Trial 3	310	130	27
	Trial 4	310	170	31
	Trial 5	210	140	34
	Average (stdev)	**270 (58)**	**150 (17)**	**33 (4.1)**
TOC (mg/L)	Trial 1	29	29	29
	Trial 2	37	38	39
	Trial 3	38	39	38
	Trial 4	36	36	37
	Trial 5	38	37	38
	Average (stdev)	**36 (3.7)**	**36 (4.1)**	**36 (4.1)**

Chlorine Residual

FCR and TCR declined over the 24 hours of testing (Figure 4.12). The average remaining free chlorine residual after 24 hours was 0.04 mg/L (min=0.00; max=0.13; stdev=0.05). The average remaining total chlorine residual after 24 hours was 0.55 (min=0.46; max=0.59; stdev=0.05).

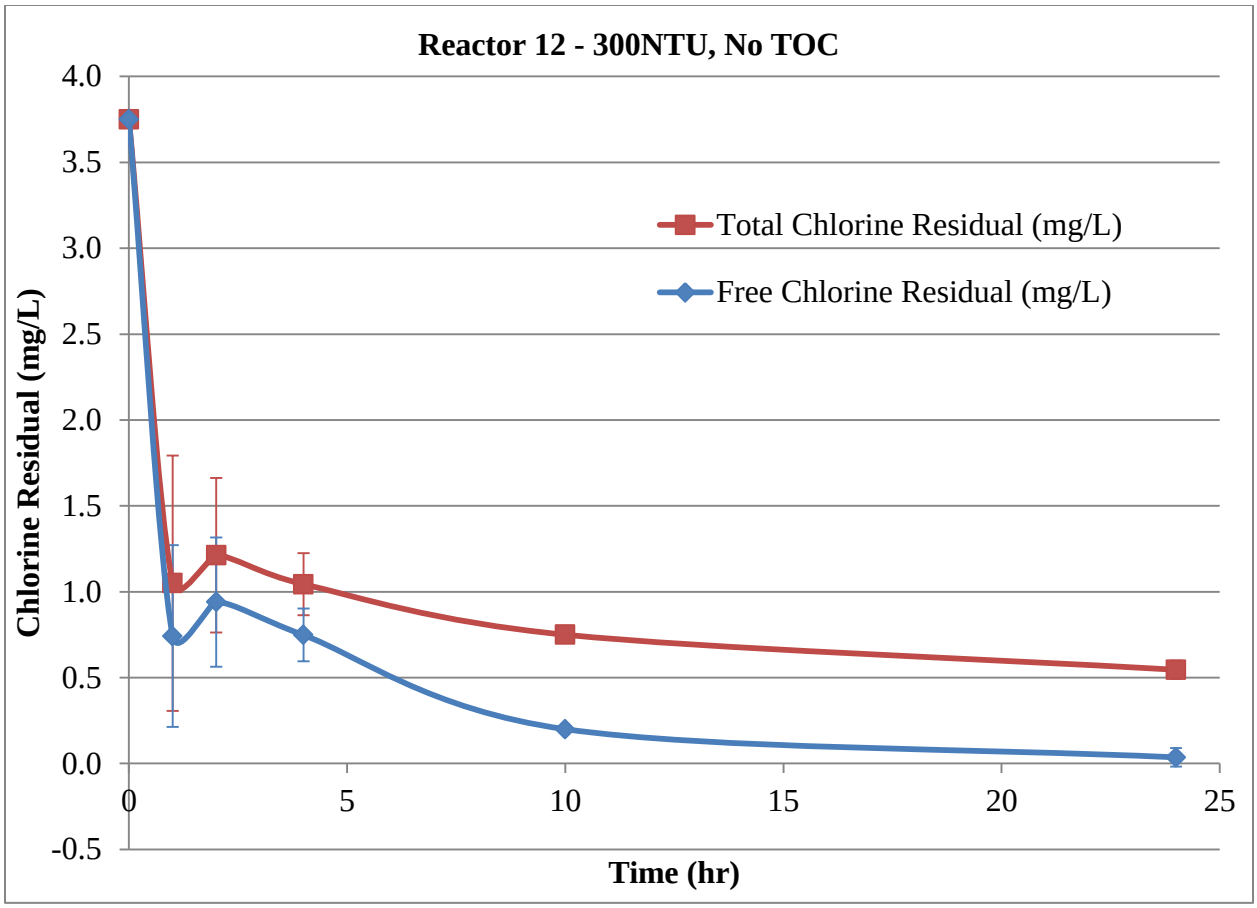

Figure 4.12 Total chlorine residual and free chlorine residual in Reactor 12. Data points represent the average of five trials and error bars show one standard deviation from the mean.

E. coli

The average initial concentration of *E. coli* was 1.6×10^8 CFU/100mL (min=1.0×10^8; max=2.4×10^8; stdev=5.9×10^7) (Table 4.35). After 24 hours, the average log reduction was 6.7 (min=5.8; max=7.4; stdev=0.67) (Table 4.36).

Table 4.35 *E. coli* Counts for Reactor 12

E. coli Count (CFU/100mL)	Time (hr)			
	0	1	10	24
Trial 1	TNTC	48	<1	<1
Trial 2	1.4E+08	TNTC	ND	13
Trial 3	2.4E+08	TNTC	ND	51
Trial 4	1.6E+08	820	ND	6.3
Trial 5	1.0E+08	TNTC	ND	150
Average (stdev)	**1.6E+08 (5.9E+07)**	**430 (550)**	**1.0 (0.0)**	**44 (62)**

TNTC - Too numerous to count

Table 4.36 Log Reduction of *E. coli* in Reactor 12

Log Reduction	Time (hr)		
	1	10*	24
Average (stdev)	**5.3 (0.0)****	**ND**	**6.7 (0.67)**

*No data was collected at 10 hours

**Trials 1, 2, 3, and 5 not included because *E. coli* at 0hr and/or 1hr was too numerous to count

4.13 Reactors 13A to13L – Controls

One control trial was performed for each reactor (Table 4.37). The control reactors were prepared identically to the sample reactors; however, chlorine was not added to the control reactors.

Table 4.37 Reactor Characteristics

Sample Reactor #	Corresponding Control Reactor #	Turbidity (NTU)	TOC Added (mg/L)
1	13A	10	2
2	13B	100	2
3	13C	300	2
4	13D	10	10
5	13E	100	10
6	13F	300	10
7	13G	10	25
8	13H	100	25
9	13I	300	25
10	13J	10	0
11	13K	100	0
12	13L	300	0

Temperature and pH, Turbidity, TOC

The temperature in the control reactors ranged from 19 to 20℃ and remained constant over time. The pH ranged from 7.5 to 7.7 and remained relatively constant over time in each reactor (Table 4.38).

Table 4.38 Temperature and pH Data for Control Reactors

Reactor #	Temp (°C)			pH		
	Time (hr)			Time (hr)		
	0	1	24	0	1	24
13A	19	19	19	7.5	7.5	7.5
13B	19	19	19	7.6	7.6	7.6
13C	20	20	20	7.6	7.6	7.6
13D	20	20	20	7.7	7.7	7.5
13E	20	19	19	7.6	7.6	7.5
13F	20	19	20	7.5	7.5	7.5
13G	19	19	19	7.5	7.5	7.5
13H	19	19	19	7.5	7.5	7.5
13I	19	19	19	7.5	7.5	7.5
13J	19	19	19	7.6	7.6	7.6
13K	20	20	20	7.6	7.6	7.6
13L	20	20	20	7.6	7.6	7.6

The average initial turbidity for reactors with an initial target turbidity of 10 NTU was 12 NTU (min=9.9; max=17; stdev=2.8). The average initial turbidity for reactors with an initial target turbidity of 100 NTU was 110NTU (min=84; max=140; stdev=24). The average initial turbidity for reactors with an initial target turbidity of 300 NTU was 250NTU (min=140; max=310; stdev=78) (Table 4.39).

Table 4.39 Turbidity Data for Control Reactors

Initial Turbidity	Reactor #	Time (hr)		
		0	1	24
10NTU	13A	17	9.2	2.4
	13D	9.9	10	2.0
	13G	12	8.8	3.2
	13J	11	7.0	2.6
	Average (stdev)	**12 (2.8)**	**9.0 (1.3)**	**2.6 (0.51)**
100NTU	13B	140	66	5.7
	13E	98	ND	7.8
	13H	100	52	8.8
	13K	84	85	4.6
	Average (stdev)	**110 (24)**	**68 (17)**	**7.0 (1.9)**
300NTU	13C	260	120	9.7
	13F	140	130	19
	13I	300	120	23
	13L	310	150	17
	Average (stdev)	**250 (78)**	**130 (14)**	**17 (5.7)**

The average initial TOC concentration for reactors with an additional 2 mg/L TOC was 35 (min=34; max=36; stdev=0.84) and the TOC concentration remained relatively constant over time. The average initial TOC concentration for reactors with an additional 10 mg/L TOC was 38 (min=28; max=44; stdev=9.3). The average initial TOC concentration for reactors with an additional 25 mg/L TOC was 56 (min=53; max=59; stdev=2.9) and the TOC concentration remained relatively constant over time. The average initial TOC concentration for reactors with no additional TOC was 37 (min=30; max=47; stdev=8.7) (Table 4.40).

Table 4.40 TOC Data for Control Reactors

TOC Added	Reactor #	Time (hr)		
		0	1	24
2mg/L	13A	36	36	34
	13B	34	36	31
	13C	35	35	31
	Average (stdev)	**35 (0.84)**	**36 (0.76)**	**32 (1.9)**
10mg/L	13D	44	39	41
	13E	28	28	20
	13F	43	43	40
	Average (stdev)	**38 (9.3)**	**37 (7.9)**	**34 (12)**
25mg/L	13G	59	59	56
	13H	58	58	55
	13I	53	54	52
	Average (stdev)	**56 (2.9)**	**57 (2.6)**	**54 (2.3)**
No	13J	30	30	28
	13K	34	34	30
	13L	47	45	42
	Average (stdev)	**37 (8.7)**	**36 (7.5)**	**33 (7.2)**

Chlorine Residual

The average initial FCR in the control reactors was 0.02 mg/L (min=0; max=0.08; stdev=0.02) and decreased to 0mg/L after 24 hours. The average initial TCR in the control reactors was 0.07 mg/L (min=0.01; max=0.15; stdev=0.04) and decreased to 0mg/L after 24 hours (Table 4.41).

Table 4.41 Chlorine Residual Data for Control Reactors

Chlorine Residual of Control Reactors		Time (hr)					
		0	1	2	4	10	24
Free Chlorine Residual (mg/L)	**Average (stdev)**	**0.02 (0.02)**	**0.01 (0.01)**	**0.01 (0.01)**	**0.0 (0.0)**	**0.0 (0.0)**	**0.0 (0.0)**
	Min	0.0	0.0	0.0	0.0	0.0	0.0
	Max	0.08	0.02	0.03	0.01	0.01	0.01
Total Chlorine Residual (mg/L)	**Average (stdev)**	**0.07 (0.04)**	**0.02 (0.01)**	**0.02 (0.01)**	**0.01 (0.01)**	**0.0 (0.0)**	**0.0 (0.0)**
	Min	0.01	0.0	0.0	0.0	0.0	0.0
	Max	0.15	0.04	0.03	0.02	0.01	0.01

E. coli

The *E. coli* counts remained relatively constant over time (Table 4.42). There was no log reduction in any of the control reactors (Table 4.43).

Table 4.42 *E. coli* Counts for Control Reactors (CFU/100mL)

Reactor #	Time (hr)			
	0	1	10	24
13A	2.4E+08	2.4E+08	2.4E+08	2.4E+08
13B	2.4E+08	2.4E+08	2.4E+08	2.4E+08
13C	1.3E+08	2.4E+08	2.4E+08	2.4E+08
13D	2.4E+08	2.4E+08	2.4E+08	2.4E+08
13E	TNTC	TNTC	TNTC	TNTC
13F	2.4E+08	2.4E+08	2.4E+08	2.4E+08
13G	2.4E+08	2.4E+08	2.4E+08	2.4E+08
13H	2.4E+08	2.4E+08	2.4E+08	2.4E+08
13I	2.4E+08	2.4E+08	2.4E+08	2.4E+08
13J	2.4E+08	2.4E+08	2.4E+08	2.4E+08
13K	2.4E+08	2.4E+08	2.4E+08	2.4E+08
13L	2.4E+08	2.4E+08	ND	2.4E+08

TNTC – Too numerous to count

Table 4.43 Log Reduction of *E. coli* in Control Reactors

Reactor #	Time (hr)		
	1	10	24
13A	0.00	0.00	0.00
13B	0.00	0.00	0.00
13C	-0.27	-0.27	-0.27
13D	0.00	0.00	0.00
13E	ND	ND	ND
13F	0.00	0.00	0.00
13G	0.00	0.00	0.00
13H	0.00	0.00	0.00
13I	0.00	0.00	0.00
13J	0.00	0.00	0.00
13K	0.00	0.00	0.00
13L	0.00	ND	0.00

4.14 Quality Control

A duplicate sample was taken every 10 samples for each parameter. The relative percent difference was calculated for each parameter.

Temperature

The average relative percent difference (RPD) in the temperature data was 0.4 percent (min=0; max=5.1; stdev=1.5) (Table 4.44).

pH

The average RPD in the pH data was 0 percent (min=0; max=0; stdev=0) (Table 4.45).

Turbidity

The average RPD in the turbidity data was 0.7 percent (min=0; max=2.2; stdev=0.9) (Table 4.46).

TOC

The average RPD in the TOC data was 0.85 percent (min=0.06; max=2.62; stdev=0.79) (Table 4.47).

Table 4.44 Temperature Quality Control

Time (hr)	Initial (°C)	Duplicate (°C)	RPD
1	19	19	0.0
	20	20	0.0
	19	19	0.0
	20	20	0.0
	19	19	0.0
	19	19	0.0
24	20	19	5.1
	20	20	0.0
	19	19	0.0
	20	20	0.0
	19	19	0.0
	19	19	0.0
Average (stdev)			**0.4 (1.5)**

Table 4.45 pH Quality Control

Time (hr)	Initial	Duplicate	RPD
1	7.6	7.6	0.0
	7.5	7.5	0.0
	7.5	7.5	0.0
	7.5	7.5	0.0
	7.5	7.5	0.0
	7.5	7.5	0.0
24	7.6	7.6	0.0
	7.6	7.6	0.0
	7.6	7.6	0.0
	7.6	7.6	0.0
	7.4	7.4	0.0
	7.6	7.6	0.0
Average (stdev)			**0.0 (0.0)**

Table 4.46 Turbidity Quality Control

Time (hr)	Initial (NTU)	Duplicate (NTU)	RPD
1	67.9	66.9	1.5
	9.34	9.34	0.0
	11.8	12.1	2.2
	9.64	9.59	0.5
	11.4	11.3	0.9
	9.86	9.86	0.1
24	13.5	13.2	2.2
	3.22	3.22	0.1
	27.7	27.7	0.0
	18.3	18.3	0.0
	18.5	18.6	0.2
	14.0	14.2	1.2
Average (stdev)			**0.74 (0.85)**

Table 4.47 TOC Quality Control

Time (hr)	Initial (mg/L)	Duplicate (mg/L)	RPD
1	31.04	31.22	0.58
	39.21	40.25	2.62
	34.18	34.05	0.38
	34.91	35.02	0.31
	33.04	32.83	0.64
	32.5	32.48	0.06
24	31.06	30.96	0.32
	39.91	39.72	0.48
	33.4	32.89	1.54
	35.43	35.87	1.23
	32.07	32.13	0.19
	31.1	31.69	1.88
Average (stdev)			**0.85 (0.79)**

Chlorine Residual

The average RPD in FCR data was 14.1 percent (min=0; max=200; stdev=37.8). The average RPD in TCR data was 3.2 percent (min=0; max=30.8; stdev=5.8) (Table 4.48).

Table 4.48 Free Chlorine Residual and Total Chlorine Residual Quality Control

Free Chlorine Residual			
Time (hr)	Initial (mg/L)	Duplicate (mg/L)	RPD
1	0.46	0.47	2.2
	0.25	0.25	0.0
	1.02	1.07	4.8
	1.04	1.04	0.0
	1.50	1.48	1.3
	1.47	1.53	4.0
2	0.83	0.85	2.4
	0.40	0.38	5.1
	0.92	0.93	1.1
	0.65	0.66	1.5
	0.82	0.82	0.0
	1.24	1.24	0.0
4	0.82	0.79	3.7
	0.68	0.69	1.5
	0.43	0.43	0.0
	0.28	0.27	3.6
	0.04	0.05	22.2
	0.82	0.81	1.2
10	0.31	0.34	9.2
	0.30	0.29	3.4
	0.08	0.05	46.2
	0.01	0.00	200.0
	0.03	0.02	40.0
24	0.32	0.32	0.0
	0.00	0.00	0.0
	0.10	0.11	9.5
	0.19	0.22	14.6
	0.01	0.01	0.0
	0.11	0.15	30.8
Average (stdev)			**14 (38)**

Total Chlorine Residual			
Time (hr)	Initial (mg/L)	Duplicate (mg/L)	RPD
1	0.52	0.52	0.0
	0.45	0.44	2.2
	1.58	1.64	3.7
	1.28	1.29	0.8
	1.74	1.69	2.9
	1.6	1.61	0.6
2	1.47	1.47	0.0
	1.6	1.63	1.9
	1.29	1.3	0.8
	0.85	0.85	0.0
	0.95	0.96	1.0
	1.57	1.5	4.6
4	0.93	0.9	3.3
	0.95	0.92	3.2
	0.82	0.82	0.0
	0.76	0.78	2.6
	1.15	1.15	0.0
	0.99	1	1.0
10	0.8	0.81	1.2
	0.51	0.49	4.0
	0.86	0.84	2.4
	0.89	0.9	1.1
	0.94	0.92	2.2
24	0.52	0.55	5.6
	0.5	0.5	0.0
	0.33	0.3	9.5
	0.23	0.23	0.0
	0.73	0.78	6.6
	0.15	0.11	30.8
Average (stdev)			**3.2 (5.8)**

E. coli

The average RPD in log reduction of *E. coli* was 0.99 (min=0; max=5.86; stdev=1.79) (Table 4.49).

Table 4.49 *E. coli* Log Reduction Quality Control

Time (hr)	Initial	Duplicate	RPD
1	6.59	6.41	2.71
	6.67	6.77	1.54
	7.15	7.15	0.00
	6.00	6.10	1.74
10	8.38	7.91	5.86
	8.38	8.38	0.00
	8.38	8.38	0.00
	8.24	8.24	0.00
24	8.38	8.38	0.00
	8.38	8.38	0.00
	8.30	8.30	0.00
	8.11	8.11	0.00
Average (stdev)			**0.99 (1.8)**

5.0 Discussion

5.1 Chlorine Residual

TOC and Chlorine Residual

The average free and total chlorine residual decreased over the 24 hour period, and waters with higher concentrations of TOC experienced a larger initial decrease in both FCR and TCR (Figures 5.1 and 5.2). Data from the 1 hour time sample was removed from Figures 5.1 and 5.2 to highlight the overall trend. Reactors with 0 and 2 mg/L of additional TOC showed similar levels of FCR and TCR at each sample time. This suggests that there is a threshold to the effect

of TOC on chlorine residual and that there is no major difference when only 2 mg/L of TOC is added to the water.

After 24 hours, the average FCR for all TOC levels did not vary widely (0.10 to 0.21 mg/L), and the average TCR for all TOC levels did not vary widely (0.46 to 0.50 mg/L). This trend suggests that the free chlorine was used and degraded in the first few hours of exposure to the water. The free chlorine was either being used to disinfect the water, reacting with the organic carbon in the water, or it had evaporated. Loss to evaporation was assumed to be minimal because the reactors were covered.

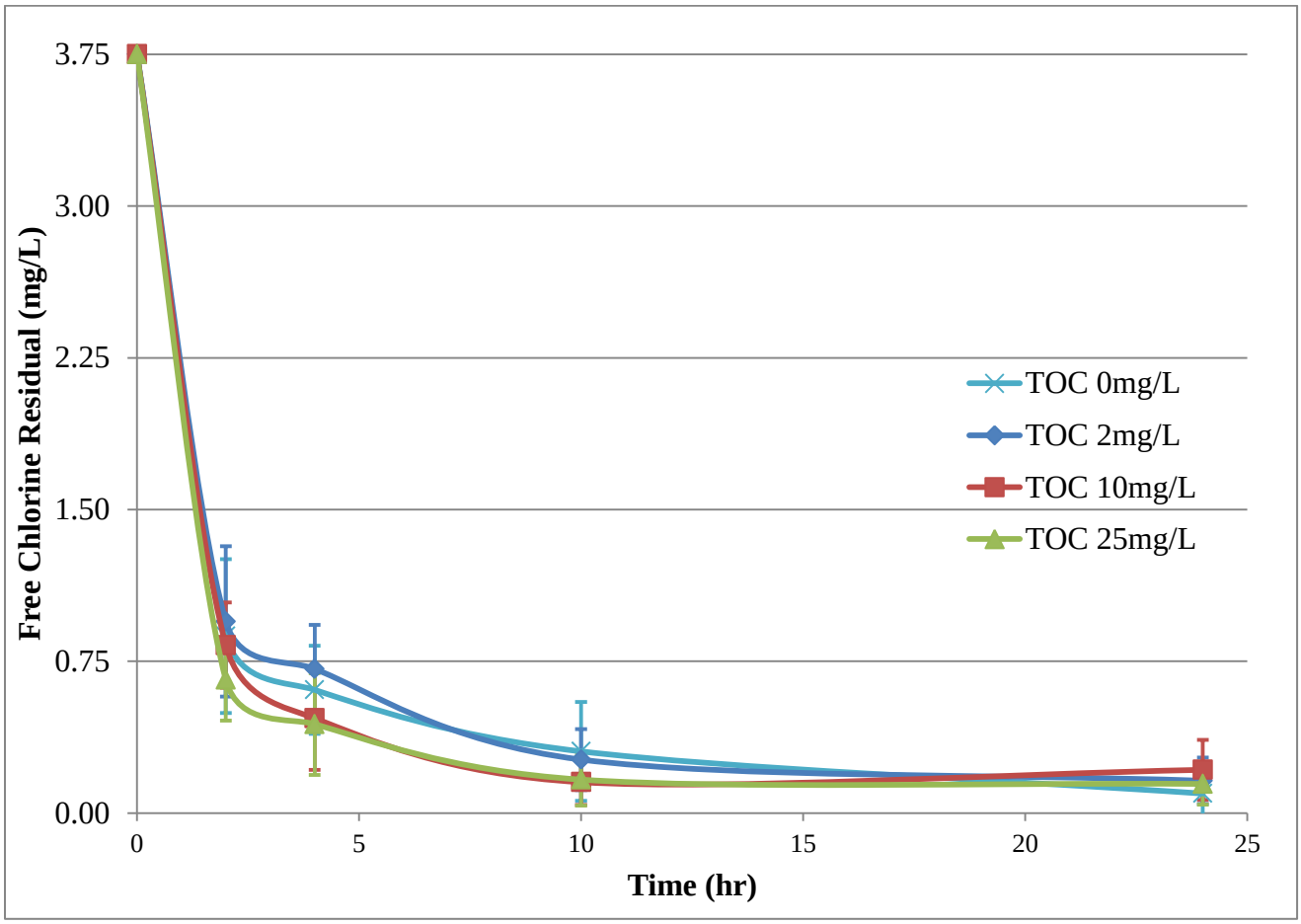

Figure 5.1 Average free chlorine residual in waters with 0, 2, 10, and 25 mg/L of additional TOC

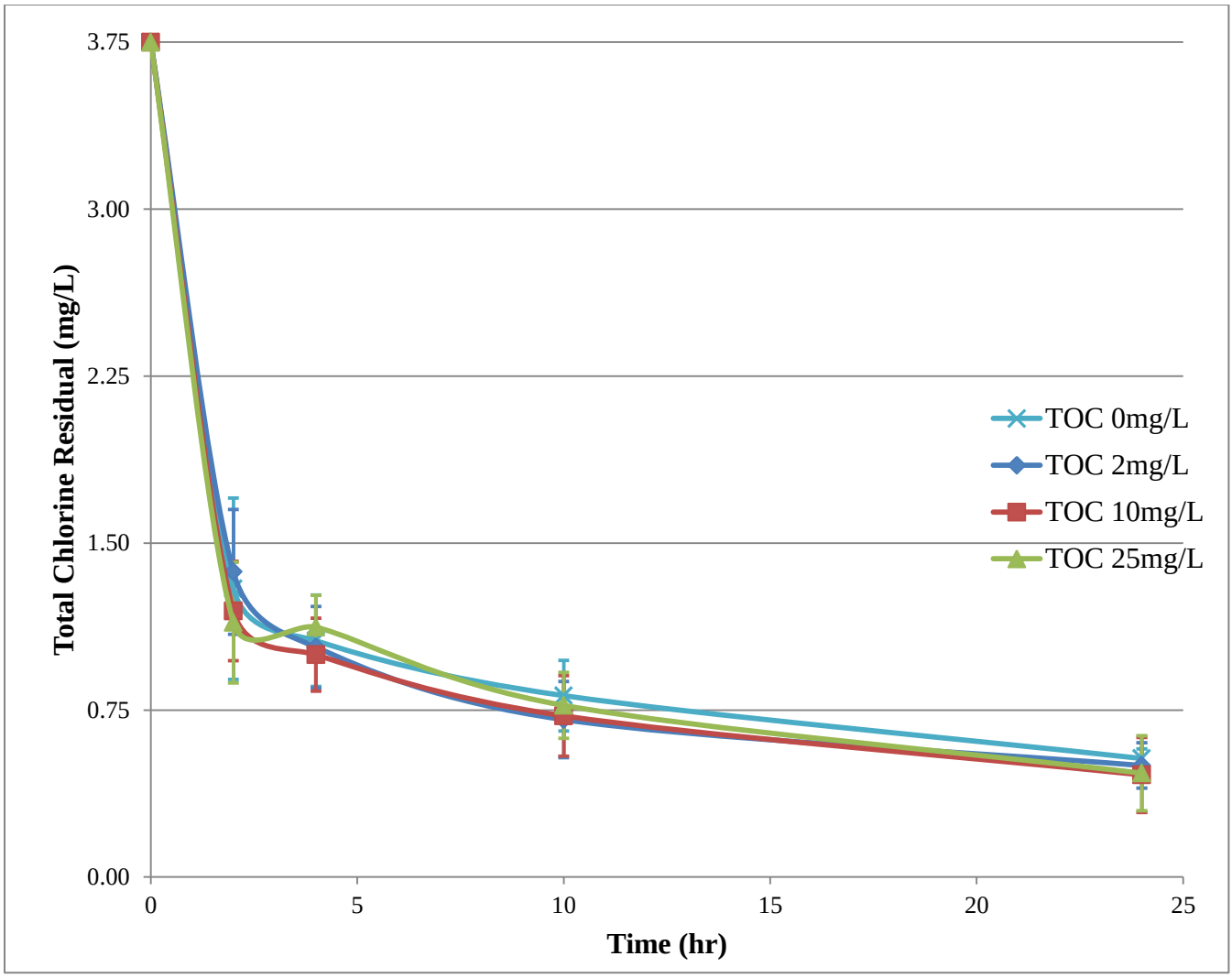

Figure 5.2 Average total chlorine residual in waters with 0, 2, 10, 25 mg/L additional TOC

Turbidity and Chlorine Residual

There was little variation in the levels of FCR and TCR in waters with different turbidities (Figures 5.3 and 5.4). This suggests that the free and total chlorine in the water was not affected by the Kaolin clay used to create turbidity. In natural waters this trend may change because the particles that create turbidity often contain organic carbon and other constituents that may react with chlorine. In the artificial water in this experiment, the only constituent added to create turbidity was the Kaolin clay. Kaolin clay does not contain any organic carbon and does

not exert any chlorine demand. Measurements of TOC in waters with only Kaolin clay were taken prior to every experiment and were always <1 mg/L.

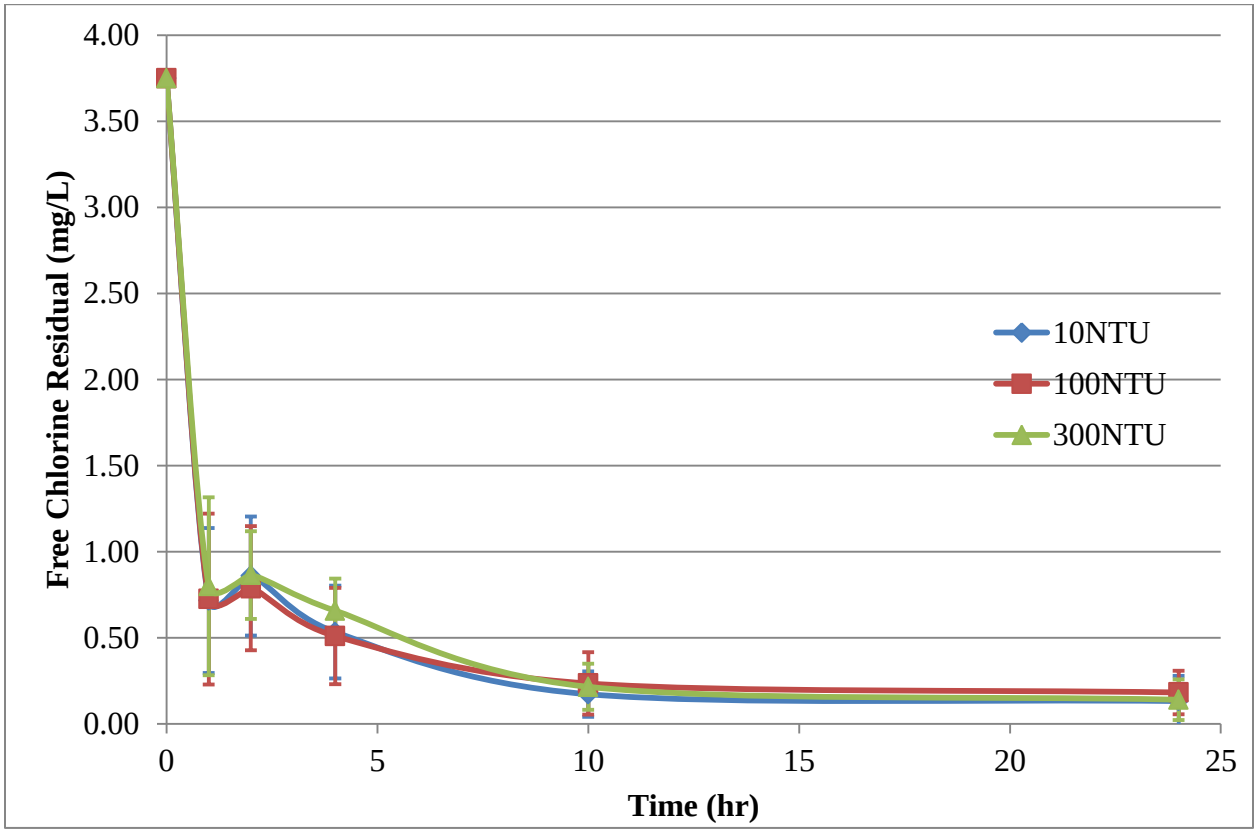

Figure 5.3 Average free chlorine residual in waters with turbidity of 10, 100 and 300 NTU

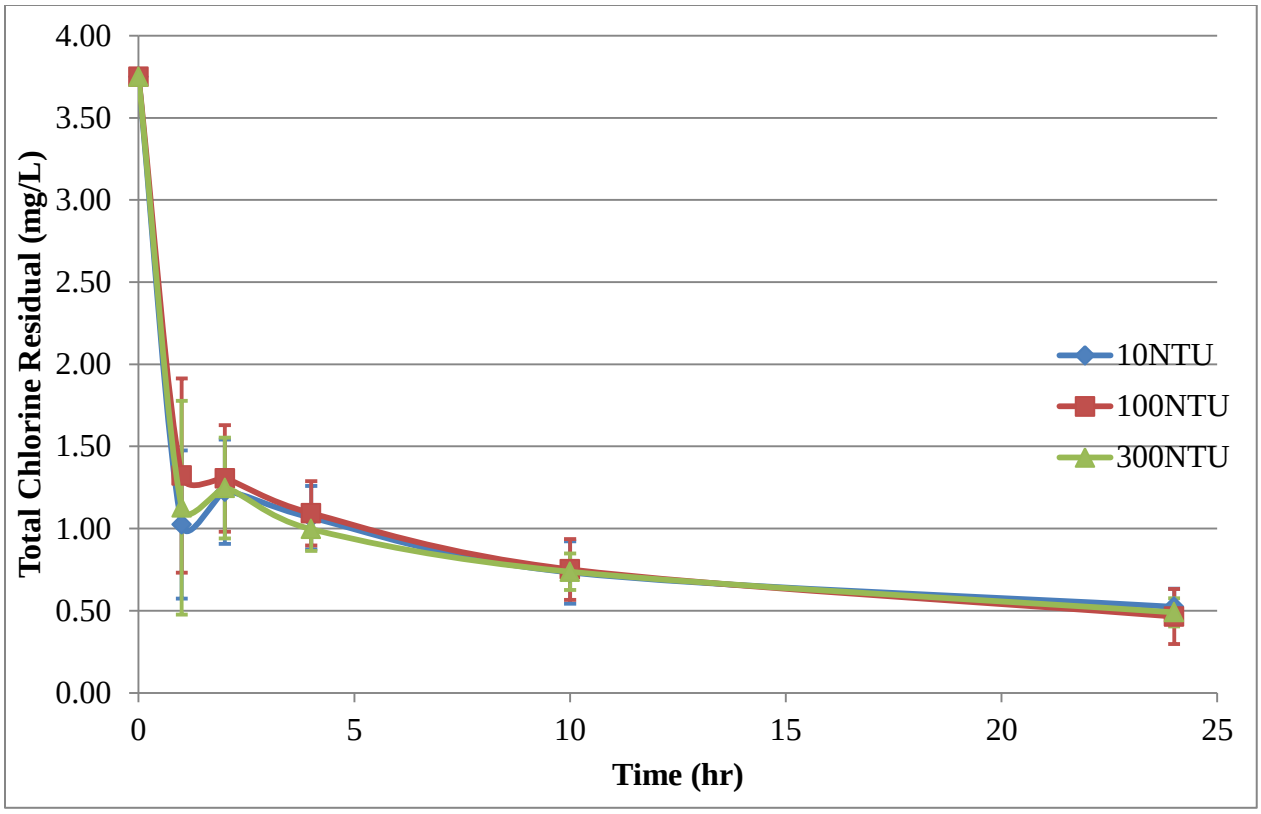

Figure 5.4 Average total chlorine residual in waters with turbidity 10, 100, and 300 NTU

Health Standards

The CDC SWS program recommends that FCR is not greater than 2.0 mg/L 30 minutes after the addition of chlorine, and not less than 0.2 mg/L 24 hours after the addition of chlorine (CDC, 2008). Only 38.1 percent of the samples across all reactors met the CDC SWS criteria after 24 hours (Figure 5.5).

In reactors with an additional 25 mg/L of TOC, only 22.2 percent of the samples met the criteria after 24 hours, whereas in reactors with only an additional 2 mg/L or 10 mg/L of TOC, 54.5 percent and 50.0 percent of the samples met the criteria after 24 hours (Figure 5.6). This suggests that water with high concentrations of TOC has a larger chlorine demand than water with low concentrations of TOC, and may not be considered safe after a dose of 3.75 mg/L of NaOCl.

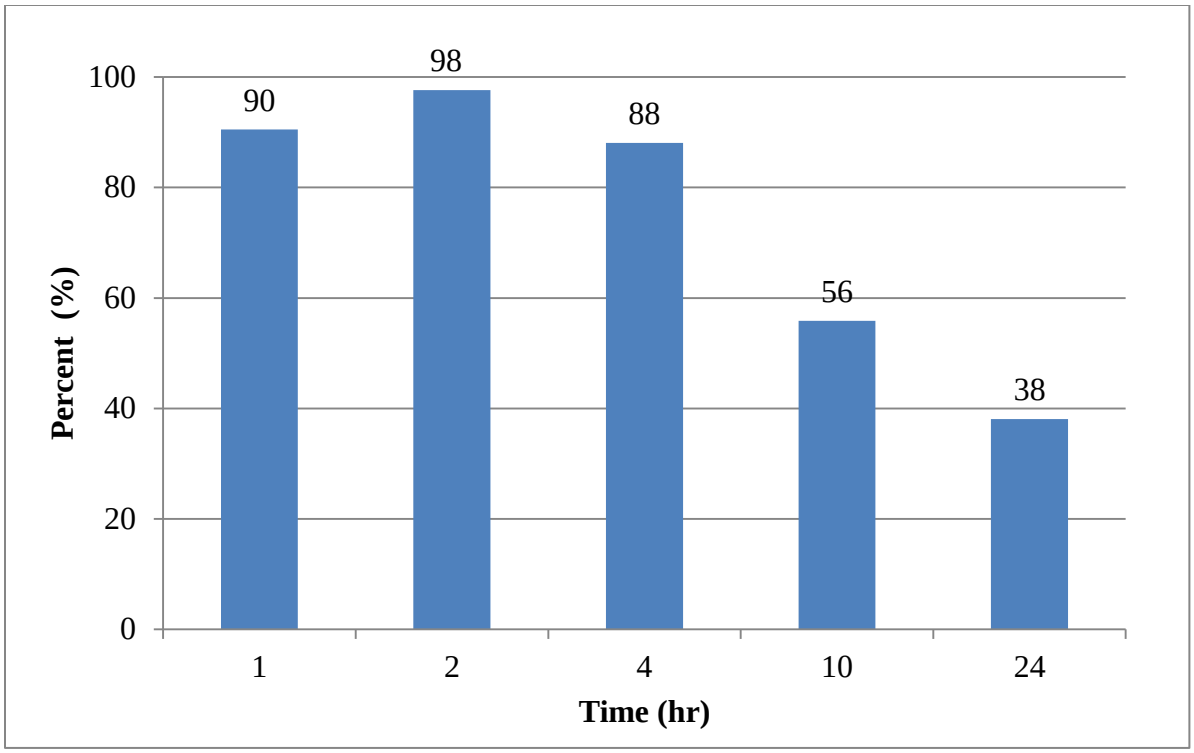

Figure 5.5 Percent of samples that met the CDC SWS criteria for free chlorine residual (0.2 to 2.0 mg/L)

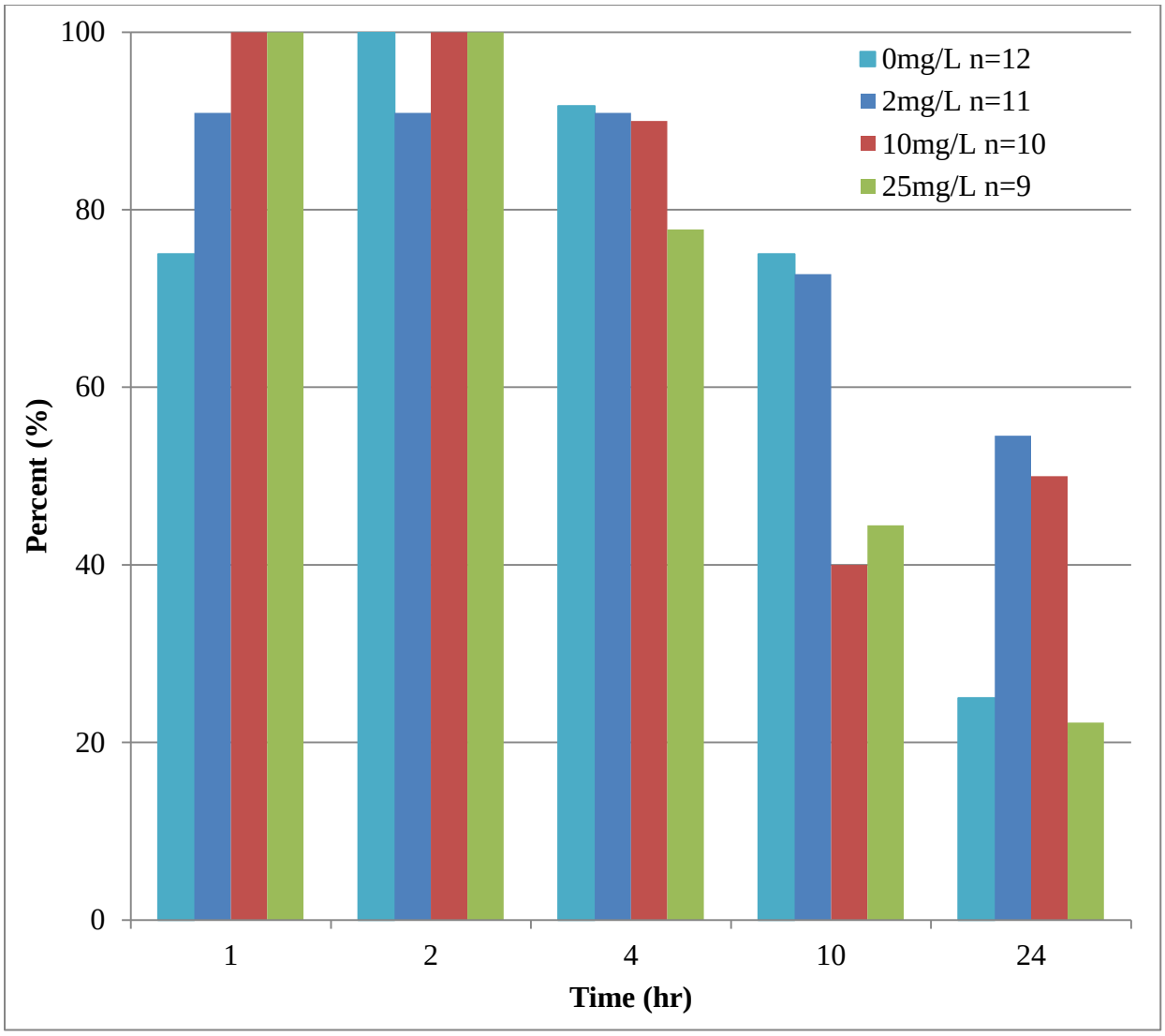

Figure 5.6 Percent of samples in waters with 0, 2, 10, and 25 mg/L additional TOC that met the CDC SWS criteria for free chlorine residual (0.2 to 2.0 mg/L)

5.2 *E. coli*

TOC and Log Reduction

The average log reduction was not affected by the concentration of TOC in the water (Figure 5.7). The log reduction increased over the 24 hour period, however there is no striking trend in how log reduction varied based on TOC concentration.

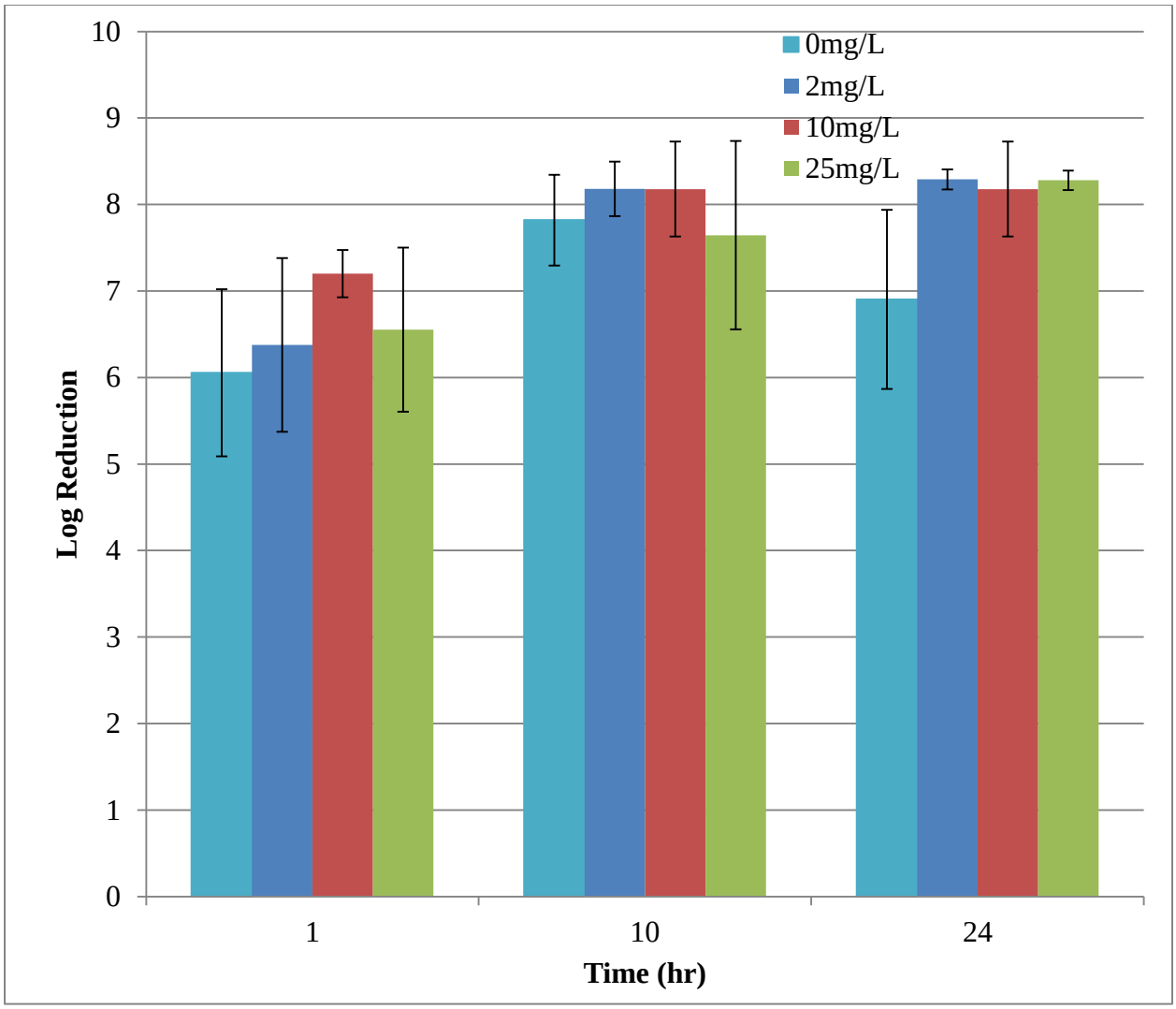

Figure 5.7 Log reduction in waters with 0, 2, 10, and 25 mg/L additional TOC

Turbidity and Log Reduction

The average log reduction was not affected by varying turbidity (Figure 5.8). The average log reduction at each sample time was similar ranging by only 0.30 at 1 hour, 0.30 at 10 hours, and 0.58 at 24 hours. The data suggests that turbidity did not have an effect on the log reduction and that a dose of 3.75 mg/L was sufficient in water of 10, 100, and 300 NTU turbidity.

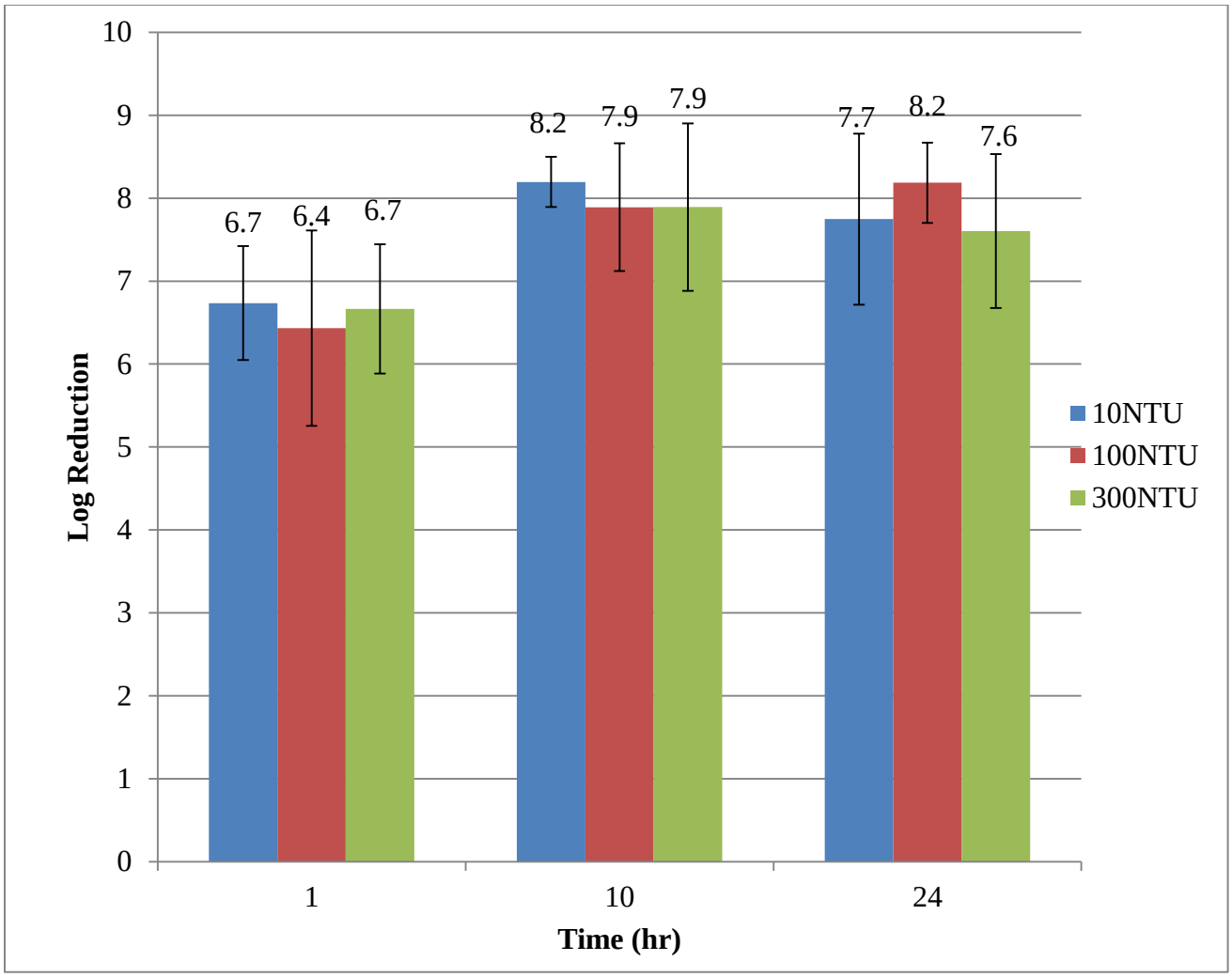

Figure 5.8 Log reduction in waters with 10, 100 and 300 NTU

Health Standards

The WHO standard for drinking water is <1 CFU/100mL. After 10 hours, 82 percent of the samples met the WHO standard (n=34), and after 24 hours, 74 percent of the samples met the WHO standard (n=42) (Figure 5.9). The data suggests that there may have been some recontamination or regrowth of *E. coli* between 10 and 24 hours, possibly due to the lack of free chlorine available for disinfection after 10 hours.

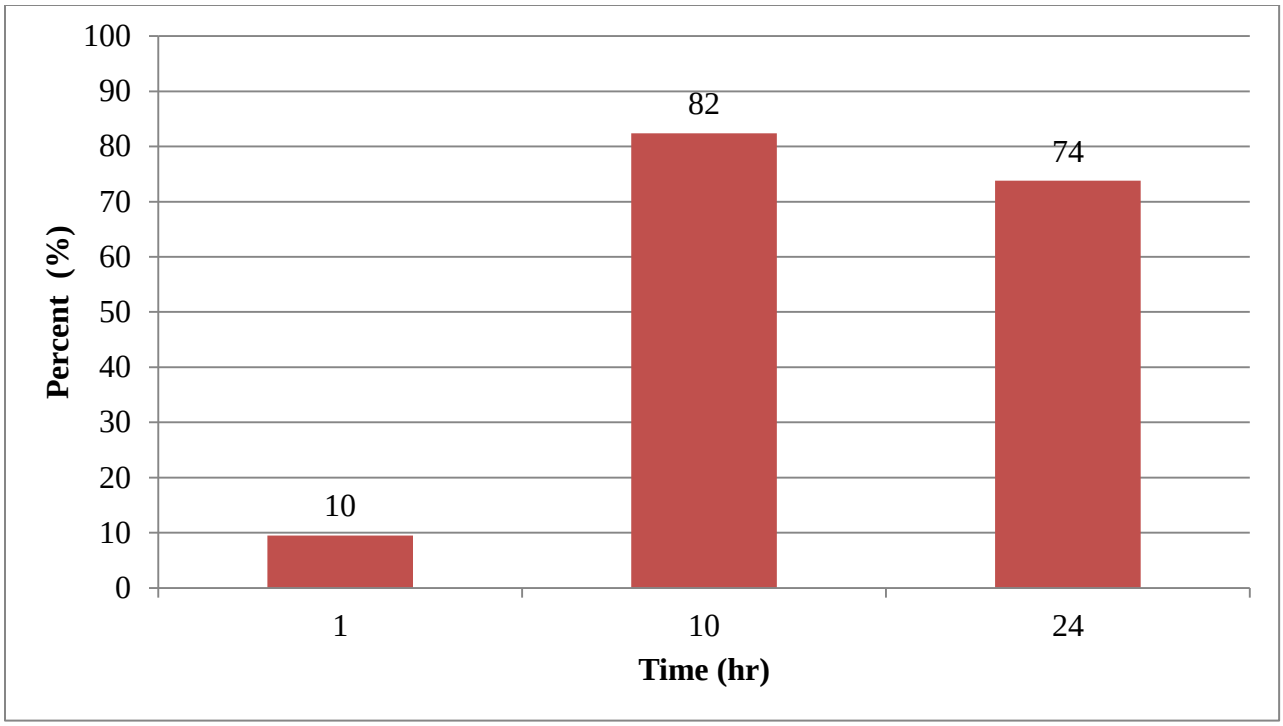

Figure 5.9 Percent of samples that met the WHO drinking water standard (<1 CFU/100mL)

5.3 Turbidity and TOC

No relationship was found between turbidity and TOC due to the experimental methods. The water used in this research was created artificially. Turbidity was created using Kaolin clay which contains no TOC, and the TOC was added using a standard solution. In order to find a relationship between turbidity and TOC, the research would need to be conducted with natural water. In natural waters, the particulate matter that creates turbidity may contain different concentrations of TOC. The research could also be conducted using RODI water and sediment from a river bed as a source of both turbidity and TOC.

5.4 Quality Control

The average RPD for each parameter was calculated and is summarized in Table 5.1.

The largest RPD between duplicate samples was seen in the FCR testing with an average RPD of 14.1 (stdev=37.8). This suggests that the LaMotte 1200 Chlorine Colorimeter was not as precise when measuring smaller values of free chlorine residual. It is important to note that because the values associated with FCR were so small (0.00 to 0.32 mg/L) at 24 hours, the RPD between two small values is going to be very large. For example, the RPD between 0.00 and 0.01 is 200 (Table 4.48) which increased the average RPD for FCR. If the RPD of 200 was removed from the average in the FCR data, the average RPD would be 7.4 and would be within an acceptable range of error. The true difference between 0.00 and 0.01 is not large and would not have greatly affected the data. Every other parameter falls within an acceptable range of error.

Table 5.1 Summary of RPD for each parameter

Parameter	Average RPD (%)	Standard Deviation	n
Temperature	0.4	1.5	12
pH	0	0	12
Turbidity	0.7	0.9	12
TOC	0.85	0.79	12
FCR	14.1	37.8	29
TCR	3.2	5.8	29
Log Reduction	0.99	1.79	12

5.5 Research Limitations

The research and data presented here has several limitations. These experiments were conducted in the laboratory using artificial water. Therefore, the direct application of the findings from this research to natural waters is not recommended without further investigation. There are several limitations that arise from the fact that the water was artificial and they include (1) the lack of natural turbidity, (2) the lack of natural TOC, and (3) the lack of natural microbiological

contamination. Natural waters may have different characteristics than the water used in this research that would affect both the chlorine demand and the many factors that affect disinfection.

There are also limitations that arise because the experiments were conducted in the laboratory rather than in a natural environment. A temperature of 19 to 20°C was maintained throughout the experiment, whereas the temperature in a real-world environment may vary based on the time of day, as well as the exposure of the container of water. The variation in temperature would affect the disinfection capability of the chlorine. Chlorine is most effective at temperatures ≥18°C and if the temperature falls below 18°C during the 24 hour period, the capability of chlorine to disinfect the water may decrease (Cairncross & Feachem, 1993).

The high concentrations of *E. coli* (10^8 CFU/100mL) used in this experiment also limit the applicability of this research. Naturally turbid waters are likely to have much lower concentrations of *E. coli* (10^4 CFU/100mL). If concentrations of *E. coli* on the order of 10^4 CFU/100mL were used in this research, a higher percent of samples meeting the WHO drinking water standard of <1 CFU/100mL would be expected. Preliminary experiments when lower concentrations of *E. coli* were used showed that this may be the case; however, the data is not reported here.

It is also unrealistic to try to chlorinate water with such a high concentration of *E. coli* because of the potential disinfection by-products that might be formed. Waters with concentrations of 10^8 CFU/100mL of *E. coli* are likely to have many other compounds in the water that could form harmful disinfection by-products like THMs (trihalomethanes), HAAs (haloacetic acids), and NDMA (N-nitrosodimethylamine).

5.6 Further Research

In order to further understand the chlorine demand and microbiological disinfection of turbid water there are several experiments that could be performed.

In order to determine the relationship, if there is one, between turbidity and TOC, experiments with natural waters need to be performed. Measuring the turbidity and TOC in different natural waters from different countries and sources could provide data that would help determine if there is a relationship between turbidity and TOC in natural waters, and if so, what that relationship is. By measuring other parameters such as flow rate, pH, and temperature, along with turbidity and TOC, and characterizing the waters based on these parameters, a model of chlorine demand and disinfection efficiency could be created.

By evaluating the effect of chlorine in natural waters with varying levels of natural turbidity and natural TOC, a more extensive conclusion could be drawn about the chlorine demand and microbiological disinfection in turbid waters. In order to do this, a similar experimental method could be followed using natural water instead of artificial water.

This research could also be performed in different natural environments in order to replicate how chlorine would be used in the field to disinfect turbid water. Doing this research with reactors placed outdoors or in an environment without temperature control would provide data on how temperature might affect the chlorine demand and microbiological efficiency in turbid waters.

Temperature controlled environments with higher or lower temperatures could also be used to conduct this research to determine how temperature affects chlorine demand and microbiological efficiency in turbid waters.

6.0 Conclusions

Four major conclusions were drawn from this research: (1) a double dose (3.75 mg/L) of chlorine was not large enough to maintain FCR levels as recommended by the CDC SWS program over a 24 hour period, (2) waters with higher TOC concentrations have a higher chlorine demand (Figure 5.1), (3) a double dose of chlorine effectively disinfected water of 10, 100, and 300 NTU with average log reductions between 6.4 and 8.2, and (4) a double dose of chlorine did not result in all water samples meeting the WHO drinking water standard of <1 CFU/100mL when the initial concentration of *E. coli* was on the order of 10^8CFU/100mL.

The results of this research give support to the recommended double dose of chlorine in turbid waters; however there is a risk of recontamination and regrowth because of the low levels of FCR after 24 hours. The use of a double dose of chlorine to disinfect waters in emergency situations and in developing countries is recommended to protect against diarrheal disease.

7.0 Acknowledgements

This research would not have been completed without the support and guidance from many individuals. I would like to thank my thesis advisor and committee chair, Professor Daniele Lantagne, and my thesis committee member, Professor John Durant. I would also like to thank Ebenezer Annan, Steve Fratto, Anna Murray, Julie Napotnik, and Justine Rayner for their support in the laboratory. This research was funded by the Cataldo Fellowship and Tufts University Summer Scholars.

Appendix A: Experimental Protocol

1. Fill plastic buckets with 10 L of RODI water. Buckets should always remain lidded.
2. Set up the 3x4 matrix shown below. Add one reactor as a control (Reactor 13).

Table A0.1 Reactor Numbers and Experimental Set-Up

	10 NTU	100 NTU	300 NTU
2 mg/L TOC	1	2	3
10 mg/L TOC	4	5	6
25 mg/L TOC	7	8	9
Control	10	11	12

Control	x NTU x mg/L TOC
No Chlorine Addition	13

3. Spike the water with *E. coli* and mix using an autoclaved glass stirring rod.
4. Measurements at 0 hour before chlorine addition:
    A. Free and total chlorine residual
    B. TOC
    C. *E. coli*
    D. Turbidity
    E. pH
    F. Temperature
5. Use titration to determine the concentration of NaOCl in the Clorox ® liquid bleach solution (take the average of three trials).
6. Add the appropriate volume of bleach needed to add a dose of 3.75 mg/L NaOCl to each bucket. Do not add bleach to the control bucket that is not in the matrix. Start the timer.
7. Measurements at 1 hour:
    A. Free and total chlorine residual
    B. TOC
    C. *E. coli*
    D. Turbidity

E. pH
F. Temperature

8. Measurements after 2 hours:
    A. Free and total chlorine residual.
9. Measurements after 4 hours:
    A. Free and total chlorine residual
10. Measurements after 10 hours:
    A. Free and total chlorine residual
    B. *E. coli*
11. Measurements after 24 hours:
    A. Free and total chlorine residual
    B. *E. coli*
    C. TOC
    D. Turbidity
    E. pH
    F. Temperature
12. Clean up:
    A. If water was not completely disinfected after 24 hours, add 1.875 mg/L of NaOCl to the bucket, mix, and wait at least 30 minutes.
    B. Pour water from buckets down the drain and clean buckets and lids using hot water and anti-bacterial soap.
13. Repeat experiment until 3 to 4 replicates have been done for each reactor.

Appendix B: Equipment List

General Materials and Equipment

- Clorox ® bleach
- RODI water (reverse osmosis distilled water)
- Stopwatch
- Tape and permanent marker
- 13 plastic buckets with lids (10 L)
- Pipettes (10 mL, 25 mL)
- Pipette bulbs (10 mL, 25 mL)
- Micropipettes (10 µL - 1000µL)
- Pipette Tips
- Waste beaker
- Latex Gloves
- 70% Ethanol solution
- Autoclave Gloves
- Safety glasses
- Lab Coat
- Hanna HI 9812-5 Portable pH/EC/TDS/℃ Meter
- Glass stir rods

Artificial Turbidity Creation

- Scale
- RODI water
- White Kaolin Clay (Lion China Clay USP)

Turbidity Testing

- 2020we Lamotte Portable Turbidimeter
- Lamotte Turbidimeter Standard Calibration Solutions
- Tubes with caps (10 mL)
- KimWipes

Total Chlorine Digital Titration

- HACH Portable digital Titrator
- Erlenmeyer flasks (125ml, glass)
- Sodium Thiosulfate Cartridge 2.00N
- Potassium iodide
- DO 3 reagent powder pillows
- DI water
- Delivery tubes for digital Titrator
- Starch indicator solution

Free and Total Chlorine Residual Testing

- LaMotte Colorimeter 1200-CL (Code 3670-01)

- LaMotte Standard Calibration Solutions
- Chlorine DPD#1 (Free Chlorine) Instrument Grade Tablets
- Chlorine DPD #3 (Total Chlorine) Instrument Grade Tablets
- Tablet Crushers
- Colorimeter tubes with caps (10 mL)

TOC Testing

- Shimadzu TOC-L CPH/CPN Analyzer
- DI water
- 9ml glass test tubes
- Test tube rack
- TOC Stock Standard Solution

***E. coli* Testing**

- Incubator
- IDEXX Quanti-Tray/2000
- Colilert-18 *E. coli* and total coliform media
- WhirlPak™ bags with de-chlorinating sodium thiosulfate
- IDEXX Quanti-Tray Sealer
- IDEXX rubber sealer insert (orange for Quanti-Tray/2000)
- UV light for sample analysis
- Milli-Q Water

***E. coli* Spiking**

- Frozen stock of *E. coli* (ATCC 25922)
- Petri dishes (100 mm x 15 mm)
- Milli-Q Water
- LB Agar
- LB Broth
- 1 L glass bottle
- 1 L glass beaker
- 500 mL Erlenmeyer flask
- Rotator/Agitator
- Incubator
- Stir plates with heat
- Stir bars
- Aluminum foil
- Autoclave
- Autoclave tape
- Inoculating loops
- GeneQuant 100 Spectrophotometer
- Spectrophotometer cuvettes

Appendix C: Protocol for Specific Experimental Methods

Creation of Artificial Turbidity

1. Add the appropriate amount of white Kaolin clay to 10 L of water (Table B.1).

Table B.1 Amount of Kaolin Clay Added to Create Turbidity

Target Turbidity	Amount of Kaolin Clay Added to 10L of Water (g)
10NTU	0.32
100NTU	2.86
300NTU	5.80

2. Mix water and clay using an autoclaved glass stirring rod.

Turbidity Measurements

1. Calibrate the Lamotte 2020 Turbidimeter with the Lamotte Standard Calibration Solution at the beginning of each experiment day.
2. Rinse an empty turbidity tube 3 times with the sample water.
3. Fill the turbidity tube to the line on the tube. Cap the tube and wipe it dry. Wipe the tube with a KimWipe.
4. Insert the tube into the turbidimeter and take the measurement.
5. Repeat the measurement 3 times and take the average.
6. Every ten turbidity tests, take one duplicate measurement with a new sample of water.

Temperature and pH Measurements

1. Calibrate the Hanna HI 9812-5 Portable pH/EC/TDS/°C meter.
2. Pipette 50 ml of the sample into a 100 ml glass beaker.
3. Insert probe and hold (without touching the side or bottom of the beaker) until the temperature stabilizes. Record the temperature.
4. Press the pH button. Allow the pH to stabilize. Record pH and remove probe from the beaker.
5. After measurements have been taken, pour the water into the waste beaker.
6. For every ten tests, take one duplicate measurement with a new sample of water.

E. coli Concentration Measurements

Follow the Most Probable Number Testing with IDEXX Procedure
(Adapted from CDC 2010, pg. 22 - 28)

1. Label the WhirlPak™ bags appropriately (date, time, dilution, initials, reactor number) and pipette samples into WhirlPak™ bags containing thiosulfate. Close WhirlPak™ bags.
2. Let samples sit in the bags for 15 minutes to deactivate the chlorine. If testing is not immediate, store samples at 4 degrees Celsius. Samples must be tested within 8 hours of collection.
3. Open the WhirlPak™ bags and add the Colilert-18 *E. coli* and total coliform media. Reseal the WhirlPak™ bags and shake until media is dissolved.

4. Open the WhirlPak™ bags and pour the water from the bags into labeled IDEXX trays and put the trays through the IDEXX sealer.
5. Place the sealed IDEXX trays in an incubator (35ºC) for 24-28 hours.
6. Remove the IDEXX trays from the incubator and place under fluorescent light to count the number of fluorescing *E. coli* colonies.
7. For every ten tests, take one duplicate measurement with a new sample of water.

Chlorine Addition

Source of NaOCl = Clorox® Liquid Bleach (6% or 60,000 mg/L NaOCl solution)

1. Use titration to determine the concentration of NaOCl in the Clorox Bleach following the *HACH Method 8209: Digital Titration for Total Chlorine Concentration*, Iodometric Method Using Sodium Thiosulfate (20–70,000 mg/L).
2. Repeat the HACH Digital Titration Method 8209 three times and use the average concentration measured for all subsequent calculations.
3. Calculate the volume of Clorox® Liquid Bleach (V_1) needed to add a dose of 3.75 mg/L NaOCl to each reactor using the following equation:

$$C_1 \times V_1 = C_2 \times V_2$$

C_1 = Concentration of Clorox® Liquid Bleach [mg/L]
V_1 = Volume of Clorox® Liquid Bleach to add to the Sample [L]
C_2 = 3.75 mg/L dose of NaOCl [mg/L]
V_2 = Volume of the Sample [L]

4. Pipette the appropriate volume (V_1) of Clorox® Liquid Bleach into each reactor.

Total and Free Chlorine Residual Measurements

1. Calibrate the LaMotte 1200 Colorimeter with the LaMotte Standard Calibration Solution at the beginning of each experiment day.
2. Use a pipette (10mL) to extract water from the reactor.
3. Rinse each colorimeter tube three times with the sample.
4. Follow the *Chlorine Test Procedure – DPD (diethyl-p-phenylene diamine) Method* for the LaMotte 1200 Colorimeter Chlorine DPD Tablet Test (Model 1200-CL, Code 3670-01).
5. Pour water from the colorimeter tubes into the waste beaker after collecting free and total chlorine residual measurement.
6. Every ten chlorine residual tests, take one duplicate measurement with a new sample of water.

TOC Addition

1. After turbidity has been added to the water, measure the TOC in each reactor.
2. Calculate the volume of TOC Stock Standard Solution (V_1) needed to add a dose of 2, 10, or 25 mg/L of TOC to each reactor using the following equation:

$$C_1 \times V_1 = C_2 \times V_2$$

C_1 = Concentration of TOC Stock Standard Solution [mg/L]

V_1 = Volume of TOC Stock Standard Solution to add to the Sample [L]
C_2 = 2, 10, or 25 mg/L dose of NaOCl [mg/L]
V_2 = Volume of the Sample [L]

3. Pipette the appropriate volume (V_1) of the TOC Stock Standard Solution into each reactor.

TOC Measurements

1. Make all appropriate checks before analysis.
    a. Check the remaining dilution water.
    b. Check the remaining acid.
    c. Drain the vessel water.
    d. Check the humidifier water level.
    e. Open air tank.
2. Turn on the Shimadzu TOC-L CPH/CPN Analyzer by making sure both the main power switch and the power switch are on.
3. Open the software on the computer and create a sample table.
4. Set up and run machine to create the calibration curve.
    a. Add appropriate concentration of standard solution to a 9ml glass vial and place in the appropriate spot in the carousel.
5. Set up machine to run samples from each reactor in the matrix and the control reactor. Pipette sample into the 9ml glass vial and place in the carousel (make sure each sample is labeled appropriately in the table and in the right position in the carousel).
6. Run samples. Every ten samples, take one duplicate measurement with a new sample of water.
7. When the test is finished, remove the samples from the carousel and dispose of properly.
8. Shutdown the machine and close air tank.

E. coli Spiking Procedure

At Least 3 Days before Spiking
Make agar plates:

1. Add appropriate amount of LB Agar and Milli-Q water to 1 L beaker on a heat and stir plate. Label the beaker and cover the beaker loosely with aluminum foil.
2. Heat and stir LB Agar and Milli-Q water using an autoclaved stir bar at 500rpm until solution reaches a rolling boil. Allow solution to boil for 1 minute, then remove the beaker from the heat and stir plate.
3. Loosely tape the aluminum foil onto the beaker using autoclave tape.
4. Place the beaker into an autoclavable stainless steel tray and autoclave the beaker with the agar solution at 121°C for 15 minutes.
5. Using autoclave gloves, remove the tray from the autoclave.
6. Place the beaker onto the stir plate (no heat) and stir agar solution at 200rpm until it is cool enough to handle.
7. Label petri dishes with initials and date.

8. Pour agar solution into petri dish until the bottom of the petri dish is covered. Be careful not to move the petri dishes quickly once the agar has been poured so that the agar does not slosh onto the side of the petri dish.
9. Allow petri dishes to sit for 8 hours to harden.
10. After 8 hours, seal the side of each petri dish with parafilm.
11. Agar plates can be stored for 4-6 weeks at 4°C.

2 Days before Spiking

Make broth:

1. Add appropriate amount of LB Broth and Milli-Q water to 1 L glass bottle on a heat and stir plate. Label the glass bottle and cover the bottle loosely with aluminum foil.
2. Heat and stir LB Broth and Milli-Q water using an autoclaved stir bar at 500rpm until solution reaches a rolling boil. Allow solution to boil for 1 minute, then remove the bottle from the heat and stir plate.
3. Loosely cap the bottle and place autoclave tape on the bottle.
4. Place the bottle into an autoclavable stainless steel tray and autoclave the bottle with the broth at 121°C for 15 minutes.
5. Using autoclave gloves, remove the tray from the autoclave.
6. Tighten the cap on the bottle and store the broth at room temperature.

Make a streak plate:

1. Remove a 1.5 mL cryo-vial of frozen *E. coli* stock from the -80°C freezer and let thaw.
2. Remove 3 to 5 agar plates from the 4°C refrigerator and bring to room temperature.
3. Wipe down countertops with 70% ethanol.
4. Remove the cap from the cryo-vial.
5. Dip a new, sterile, inoculating loop into the cryo-vial. Stir with the inoculating loop. Remove the loop.
6. Lift agar plate lid, streak with the ½ - ¼ - ¼ streak plate method (Figure B.1).

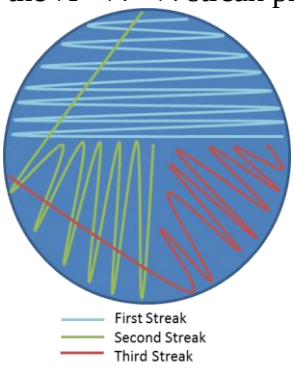

Figure B.1 Streak plate method

   a. Use loop to streak ½ of the plate. Dispose of loop in biohazard bag.
   b. Using one side of a new, sterile, inoculating loop, drag through the first streak, and streak ¼ of the plate.
   c. Using the other side of the loop, drag through the previous streak, and streak the last ¼ of the plate. Dispose of loop in biohazard bag.
7. Close the petri dish and place upside down.
8. Incubate petri dish at 35°C for 12-24 hours.

9. Dispose of cryo-vial in biohazard bag.

1 Day before Spiking

Inoculate broth:

1. Wipe down countertops with 70% ethanol.
2. Pour broth into appropriate sized containers for the rotator in the incubator.
3. Remove streak plate from incubator.
4. Locate an isolated colony.
5. Open the broth container. Using a new, sterile inoculating loop, pluck the isolated colony from the plate. Dip the inoculating loop into the broth and mix. Take the inoculating loop out of the broth and dispose in a biohazard bag. Close the broth container.
6. Place the container of inoculated broth on the rotator in the incubator. Turn on the rotator and incubate at 35°C for 24 hours.

Day of Spiking

Spike water with *E. coli*:

1. Pipette 1 mL of regular, not-inoculated broth into a cuvette. Take a reference reading on the GeneQuant 100 spectrophotometer.
2. Remove inoculated broth from incubator.
3. Pipette 1 mL of inoculated broth into a cuvette. Take an OD reading of inoculated broth.
4. Estimate concentration of inoculated broth using the following equation:

$$OD\ Reading\ \times 2 \times 8x10^{8} = CFU/100mL$$

5. Using a pipette, add the inoculated broth to each reactor.

References

APHA. (1992). Part 5000: Aggregate organic constituents. *Standard Methods for the Examination of Waters and Wastewater, 18*, 5.1-5.50.

Arnold, B. F., & Colford, J. M., Jr. (2007). Treating water with chlorine at point-of-use to improve water quality and reduce child diarrhea in developing countries: a systematic review and meta-analysis. *Am J Trop Med Hyg, 76*(2), 354-364.

Cairncross, S., & Feachem, R. (1993). *Environmental Health Engineering in the Tropics: An Introductory Text* (2nd ed.). New York: John Wiley & Sons.

CDC. (2008). *Chlorine Residual Testing*. Atlanta, Georgia. Retrieved from http://www.cdc.gov/safewater/publications_pages/chlorineresidual.pdf

CDC. (2010). *Microbiological Indicator Testing in Developing Countries: A Fact Sheet for the Field Practitioner*. Retrieved from www.hip.watsan.net/content/download/4848/28138/file/

CDC. (2011). Household Water Treatment, Chlorination, The Safe Water System. In C. f. D. C. a. P. (CDC) (Ed.): Center for Disease Control and Prevention (CDC).

CDC. (2012). *CDC and the Safe Water System*. Retrieved from http://www.cdc.gov/safewater/PDF/SWS-Overview-factsheet508c.pdf

Clasen, T., Schmidt, W. P., Rabie, T., Roberts, I., & Cairncross, S. (2007). Interventions to improve water quality for preventing diarrhoea: systematic review and meta-analysis. *BMJ, 334*(7597), 782.

Conservation, D. o. E. a. (2005). *Guidelines for the Design, Construction and Operation of WAter and Sewerage Systems*. Newfoundland.

Cravioto, A., Lanata, C. F., Lantagne, D. S., & Balakrish Nair, G. (2010). *Final Report of the Independent Panel of Experts on the Cholera Outbreak in Haiti*. Retrieved from http://www.un.org/News/dh/infocus/haiti/UN-cholera-report-final.pdf

Crump, J. A., Okoth, G. O., Slutsker, L., Ogaja, D. O., Keswick, B. H., & Luby, S. P. (2004). Effect of point-of-use disinfection, flocculation and combined flocculation-disinfection on drinking water quality in western Kenya. *J Appl Microbiol, 97*(1), 225-231.

Cutler, D., & Miller, G. (2005). The role of public health improvements in health advances: the twentieth-century United States. *Demography, 42*(1), 1-22.

Dorea, C. C., & Simpson, M. R. (2011). Turbidity tubes for drinking water quality assessments. *Journal of Water, Sanitation and Hygiene for Development, 1*(4), 233.

Fewtrell, L., & Colford, J. M., Jr. (2005). Water, sanitation and hygiene in developing countries: interventions and diarrhoea--a review. *Water Sci Technol, 52*(8), 133-142.

HACH Company. (2003). *HACH water analysis handbook: Method 8209 - total chlorine digital titrator, idiometric method using sodium thiosulfate.* USA: HACH Company.

Kiefer, T., Lande, L., Adler-Michaelson, H., Neumeyer, H., Khalfan, A., Roaf, V., et al. (2012). *The human right to safe drinking water and sanitation in law and policy - a sourcebook.*

Kotlarz, N., Lantagne, D., Preston, K., & Jellison, K. (2009). Turbidity and chlorine demand reduction using locally available physical water clarification mechanisms before household chlorination in developing countries. *J Water Health, 7*(3), 497-506.

Kouadio, I. K., Aljunid, S., Kamigaki, T., Hammad, K., & Oshitani, H. (2012). Infectious diseases following natural disasters: prevention and control measures. *Anti Infect. Ther., 10*(1), 95-104.

LaMotte Company.*1200 colorimeter: Chlorine test procedure – DPD method.* Chestertown, Maryland: LaMotte Company.

Lantagne, D. S. (2008). Sodium hypochlorite dosage for household and emergency water treatment. *American Water Works Association, 100*(8).

Lantagne, D. S., Blount, B. C., Cardinali, F., & Quick, R. (2008). Disinfection by-product formation and mitigation strategies in point-of-use chlorination of turbid and non-turbid waters in western Kenya. *J Water Health, 6*(1), 67-82.

Lantagne, D. S., Cardinali, F., & Blount, B. C. (2010). Disinfection by-product formation and mitigation strategies in point-of-use chlorination with sodium dichloroisocyanurate in Tanzania. *Am J Trop Med Hyg, 83*(1), 135-143.

LeChevallier, M. W., Evans, T. M., & Seidler, R. J. (1981). Effect of Turbidity on Chlorination Efficiency and Bacterial Persistence in Drinking Water. *Appl Environ Microbiol, 42*(1), 159-167.

Preston, K., Lantagne, D., Kotlarz, N., & Jellison, K. (2010). Turbidity and chlorine demand reduction using alum and moringa flocculation before household chlorination in developing countries. *J Water Health, 8*(1), 60-70.

Pronk, M., Goldscheider, N., & Zopfi, J. (2006). Dynamics and interation of organic carbon, turbidity and bacteria in a karst aquifer system. *Hydrogeology Journal, 14*(4), 473-484.

Drinking Water (2010).

Pruss-Ustun, A., Bos, R., Gore, F., & J., B. (2008). *Safe water, better health: costs, benefits and sustainability of interventions to protect and promote health*. Geneva, Switzerland.

Rangel, J. M., Lopez, B., Mejia, M. A., Mendoza, C., & Luby, S. (2003). A novel technology to improve drinking water quality: a microbiological evaluation of in-home flocculation and chlorination in rural Guatemala. *J Water Health, 1*(1), 15-22.

Reed, R. A. (2005). *How to measure chlorine residual*. New Delhi: World Health Organization.

Reller, M. E., Medoza, C. E., Lopex, M. B., Alvarez, M., Hoekstra, R. M., Olson, C. A., et al. (2003). A randomized controlled trial of household-based flocculat-disinfectant drinking water treatment for diarrhea prevention in rural Guatemala. *Am J Trop Med Hyg, 69*(4), 411-419.

USEPA. (1998). *National Primary Drinking Water Regulations: Disinfectants and Disinfection Byproducts*.

Volk, C., Wood, L., Johnson, B., Robinson, J., Zhu, H. W., & Kaplan, L. (2002). Monitoring dissolved organic carbon in surface and drinking waters. *Journal of Environmental Monitoring, 4*(1), 43-47.

White, G. C. (1999). *Handbook of Chlorination and Alternative Disinfectants* (4 ed.). New York: John Wiley & Sons, Inc.

WHO. *Fact Sheet 2.33: Turbidity Measurement*. Retrieved from http://www.who.int/water_sanitation_health/hygiene/emergencies/fs2_33.pdf

WHO. (1997). *Guidelines for drinking-water qualtiy*. Geneva: World Health Organization (WHO).

WHO. (2011). *Guidelines for Drinking-water Quality*. Geneva: World Health Organization (WHO).

WHO/UNICEF. (2009). *Diarrhoea: Why children are still dying and what can be done*.

WHO/UNICEF. (2012). *Progress on Drinking Water and Sanitation*.

Zhao, W., Liu, X., Huang, Q., Walker, S. L., & Cai, P. (2012). Interactions of pathogens Escherichia coli and Streptococcus suis with clay minerals. *Applied Clay Science, 69*, 37-42.