DETERMINING THE EFFECTIVENESS OF WATER TREATMENT PROCESS BARRIERS FOR THE REMOVAL OF VIRUSES IN DRINKING WATER

DISSERTATION SUBMITTED FOR THE DEGREE

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KHOMOTSO CHARITY SETLHARE

210059389

Supervisor: **Dr. CC Ssemakalu**

Co-Supervisors: **Dr. N Leat**

Prof. M Pillay

DECLARATION

I, Khomotso Charity Setlhare hereby declare that Determining the effectiveness of water treatment process barriers for the removal of viruses in drinking water is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. The thesis has not been submitted or will not be submitted to a university or any institution for the award of a degree.

Signed (Author) **Date**

Signed (Supervisor) **Date**

Signed (Co-supervisor) **Date**

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Thank you

DEDICATION

It is with deepest and warmest affection that I dedicate this book to my late mother (Moselantja Jerminah Setlhare), a strong and gentle soul who taught me to trust in God and believe in hard work.

ABSTRACT

The presence of enteric viruses in drinking water poses a health risk to consumers. It is therefore very important for drinking water suppliers to provide water that is pathogen free and fit for human consumption. This can be achieved by an effective water treatment system that ensures the safety of water from the treatment plant until the water reaches the consumer. This study assessed the ability of a conventional water treatment system to remove viruses. The system consisted of three unit processes, namely, clarification, sand filtration and disinfection. These processes were simulated on a bench-scale to determine the effectiveness of each one at removing viruses. Clarification was conducted using a Phipps and Bird jar testing system and three different chemical treatments: (i) Polyelectrolyte (SUDFLOC 3835), (ii) a combination of lime and activated silica and (iii) a combination of lime, activated silica and ferric chloride. Sand filtration was simulated using a Phipps and Bird column filtration system. Disinfection was conducted using free chlorine. The findings from this study showed that the removal or inactivation of viruses increased with an increase in the concentration of chemicals added. For clarification, the combination of lime, activated silica and ferric chloride was the most effective treatment for the removal or inactivation of viruses. Sand filtration was found to be ineffective for the removal of viruses. Disinfection was shown to be the most effective process for the removal or inactivation of viruses. While clarification, sand filtration and disinfection did not remove or inactivate viruses equally, the entire treatment chain is still essential. This is because even if a barrier does not directly remove viruses it ensures that subsequent processes can function effectively. Overall the treatment processes should not be considered as discrete barriers but rather an integrated system that must function throughout to avoid a risk to customers.

KEY WORDS: Enteric viruses, drinking water, water treatment, clarification, sand filtration, disinfection

ABBREVIATIONS AND ACRONYMS

DALY	Disability Adjusted Life Year
DWS	Department of Water and Sanitation
HBTs	Health-Based Targets
L	Liter
mg	milligram
min	minutes
ml	milliliter
ML/d	Mega liters per day
NTU	Nephelometric Turbidity Units
PFU	Plaque Forming Unit
SABS	South African Bureau of Standards
SANS	South African National Drinking Water Standard
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

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CHAPTER 1.

General background of the study

1.1. Introduction

Water is a crucial element for sustainable socio-economic development and the elimination of poverty. Therefore, it is essential to conserve water and ensure the water is sustainable for future generations. According to the Department of Water and Sanitation (DWS), South Africa is ranked the 30th driest country worldwide (Kohler, 2016). In South Africa, 62% of the water is used for agriculture, 27% for domestic purposes, 8% for power generation, mining and industrial processes, and 3% for commercial forestry plantation (Fig.1).

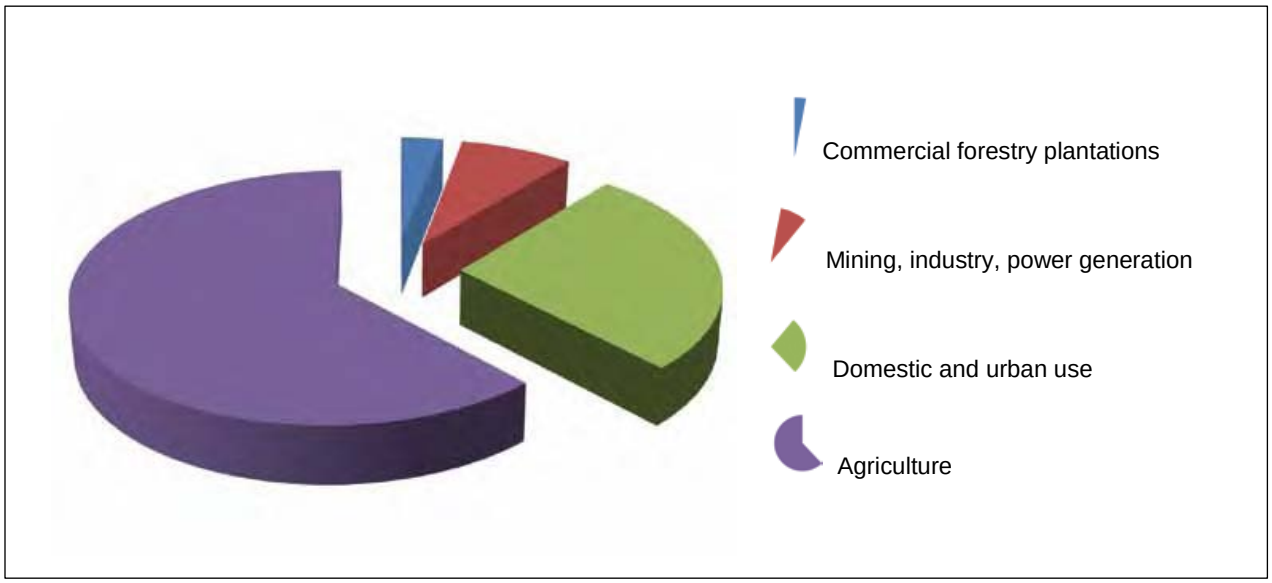

Figure 1: Distribution of Water Use in South Africa (Strydom & Oelofse, 2010).

The increasing population growth rate and water usage patterns are straining South Africa's existing water resources. As a consequence, the South African government set aside funds to build more dams to keep up with the water requirements (Pearson, 2010a).

South Africa relies on rainfall to replenish its water reserves. The average rainfall received in South Africa is approximately 497 mm per annum, compared to a world average of 860 mm (Alex & Pouris, 2016). However, rainfall is not evenly distributed in South Africa. For instance, the Eastern part of the country tends to receive more rain as opposed to the Western part (Fig.2). This is due to the nature of the weather conditions.

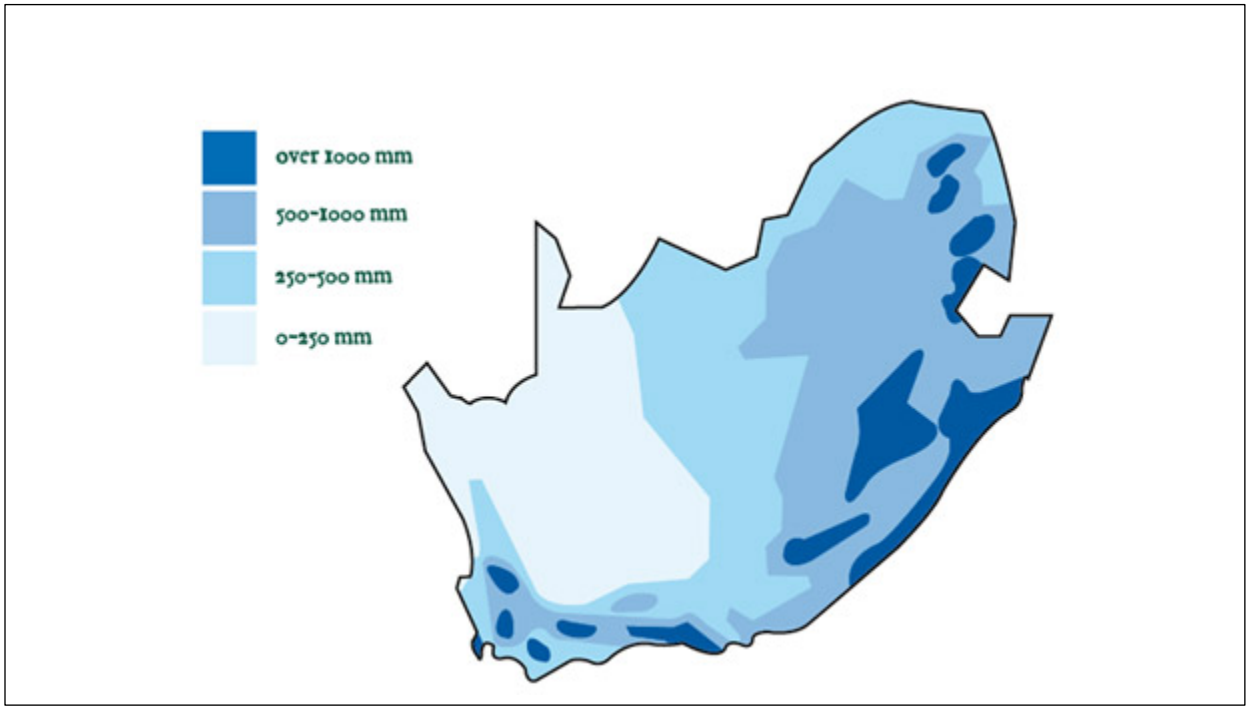

Figure 2: Distribution of the mean annual rainfall in South Africa (Pearson, 2010a).

Increased variability in the weather patterns in South Africa has resulted in irregular drought periods. In December 2015, five of South Africa's nine provinces were declared disaster areas due to water shortages. Mpumalanga, Limpopo, Kwa-Zulu Natal, North West and the Free State provinces were severely affected, and Gauteng was the least affected because of the rainfall experienced (Stelli, 2015). Currently, Gauteng relies on water from the Vaal Dam and supply from the Lesotho Highlands. The Lesotho Highlands Transfer scheme was built to move water

from the Lesotho mountains to the Vaal Dam catchment. 40% of water in the Vaal River is from the Lesotho Highlands Transfer scheme (Webster and Ras, 2016). The Vaal Dam is at present, managed by the Department of Water and Sanitation (DWS) and is the fourth largest dam in South Africa with regards to storage capacity and plays a significant role as the primary supplier of water to the economic heartland of South Africa (Pearson, 2010b). The availability of water in the Vaal Dam makes it possible for households around Gauteng to receive potable water daily. According to statistics, 97.5 % households in Gauteng have access to treated piped water in their dwellings (STATISTICS SOUTH AFRICA, 2018).

The importance of water as a vehicle for the transmission of human pathogenic viruses has been established (Boudaud et al., 2012). Viruses are tiny microorganisms that can cause severe and deadly diseases in humans and animals. Numerous studies have reported that viruses in contaminated soil can survive and migrate long distances where they may contaminate drinking water wells (Attinti & Wei, 2010). Hence, it is important to understand the factors that control the transport of viruses in a natural environment to protect the public health.

The detection of viruses or indicators relevant to the presence of viruses in drinking water continues to be a challenge (Grabow et al., 2004). This challenge is mainly attributed to the fact that some viruses are resistant to some water treatment and disinfection processes (Ribas et al., 1995). The microbial content of water is a primary determinant of whether water is usable. Reports have shown that viruses may remain inside treated water that has met acceptable specifications for treatment, disinfection and indicator organisms (WHO, 2004). Viral pollution of drinking water may depend on source water quality and drinking water treatment efficiency (Boudaud et al., 2012).

The presence of enteric viruses in water poses a significant health risk to consumers (WHO, 2011). Enteric viruses in this context refer to a combined group of viruses that infect the human gastrointestinal tract and are transmittable by the faecal-oral route (Table 1). Well-known members of this cluster include enteroviruses, astroviruses, enteric adenoviruses, rotaviruses, caliciviruses and hepatitis A and E viruses. Enteric viruses have robust capsids that enable them to survive unfavourable conditions in the environment (Lin, 2013). The detection of any enteric viruses in drinking water is an indicator for the potential presence of other enteric viruses (Fong & Lipp, 2005) and evidence of faecal pollution (Fong & Lipp, 2005).

Table 1: Viruses transmitted through drinking water (WHO, 2011) .

VIRUSES	HEALTH SIGNIFICANCE	PERSISTENCE IN WATER SUPPLIES	RESISTANCE TO CHLORINE	RELATIVE INFECTIVITY	IMPORTANT ANIMAL SOURCE
Adenoviruses	Moderate	Long	Moderate	High	No
Astroviruses	Moderate	Long	Moderate	High	No
Enteroviruses	High	Long	Moderate	High	No
Hepatitis A virus	High	Long	Moderate	High	No
Hepatitis E virus	High	Long	Moderate	High	Potentially
Noroviruses	High	Long	Moderate	High	Potentially
Rotaviruses	High	Long	Moderate	High	No
Sapoviruses	High	Long	Moderate	High	Potentially

This study used surrogate viruses instead of enteric viruses to study the effectiveness of the different water treatment process barriers for the removal of viruses. Surrogate viruses were used because they have similar physical properties to enteric viruses but are easier to detect and do not pose a health risk to laboratory workers.

Conventional water treatment consists of the following unit processes: clarification (coagulation, flocculation and sedimentation), filtration, and disinfection. In coagulation, a coagulant is added to raw water and mixed in the rapid mix chamber to form small particles. During flocculation, gentle mixing accelerates the rate of particle collision, and the destabilized particles form larger precipitates called flocs (Jiang, 2015). Sedimentation allows the large flocs to sink to the bottom of a tank or basin. The clear water flows to filter houses where it is filtered through layers of filter media (Ripperger et al., 2012). After filtration, the water is disinfected with various disinfection methods such as chlorine, ultraviolet light or ozone.

Each stage in the water treatment process aims at improving the microbiological, physical and chemical composition of the water. The whole water treatment process is continuously monitored to maintain the quality of the water. If the quality of water deviates from the prescribed limits, then corrective action is taken. Water utilities determine water quality by endpoint monitoring while the WHO emphasizes health-based targets (WHO, 2011).

Bulk drinking water suppliers have adopted the South African National Drinking Water Standard (SANS) 241:2015 drinking water quality standard as a delivery specification (Ekurhuleni Metropolitan Municipality, 2016). Also, bulk drinking water suppliers have internal production specifications, designed to provide a buffer and ensure that the SANS 241:2015 specifications are met. Water utilities benchmark quality water supplied to local authorities against the World Health Organization (WHO, 2011) drinking water quality guidelines.

This study used bacteriophages as surrogate viruses to assess the effectiveness of the water treatment processes in removing viruses. Bacteriophages have been used extensively as surrogates to evaluate water treatment processes for the removal of human enteric viruses (Ribas

et al., 1995). They are readily detected in contaminated surface and groundwater by basic microbiology methods. Bacteriophage reduction by water treatment processes is an easy, rapid, reliable and cost affordable means of evaluating and monitoring the performance of a treatment plant with regards to the removal of viruses (Sobsey et al., 1995).

A spiking test is used to assess efficiency with which a water treatment process can remove viruses. Spiking tests are necessary because the source water for the water treatment plant contains a low number of viruses. This makes the determination of virus removal over a wider range of log steps difficult (Kreißel et al., 2014).

The bacteriophages used in this study as model organisms were MS2 and phi X174. These bacteriophages were used because they are known to be resilient to the different types of removal mechanisms as opposed to other viruses (Abd-Elmaksoud et al., 2014). This study made use of a jar tester and a filtration system to simulate the conventional drinking water treatment process. A specialist will use the information generated through this study as part of a quantitative microbial risk assessment. Also, this information would enable an evaluation of the extent to which the drinking water supplies under consideration conform to a proposed acceptable risk of infection.

1.2 Rationale and Motivation

The presence of viruses in drinking water can cause diseases and, potentially, death. Therefore, it is crucial for water utilities to provide good quality water that is free of microbiological contaminants. It is also important to assess the ability of the treatment processes to achieve water that complies with the WHO guidelines set in 2011 (WHO, 2011). The results from this study provide guidance on the most effective treatment processes for removal of viruses.

1.3 Problem Statement

Viruses may not be efficiently removed during the water treatment process and may affect consumers who drink the water. The presence of viruses in drinking water following treatment could be as a consequence of the virus concentration in the source water exceeding the removal efficiency of the treatment process. This happens mainly during a storm when the source water quality deteriorates. Waterborne enteric viruses present a health risk to consumers. To demonstrate that this risk has been mitigated it is important to have evidence that treatment processes can remove or inactivate viruses effectively.

1.4 Aim and objectives

The aim of this study was to determine the effectiveness of water treatment processes for the removal of viruses in drinking water. The objectives of this study were:

1. To set up jar tests to simulate coagulation, flocculation, and sedimentation using polyelectrolyte coagulants, lime, activated silica and ferric chloride.
2. To set up sand filtration columns to simulate the filtration process using filters of fine sand, fine grid and stone.
3. To set up bench scale tests to simulate disinfection using chlorine as the disinfectant.
4. To determine the log removal efficiency of clarification, sand filtration and disinfection.
5. To compare the log removal efficiencies of viruses among the different processes of water treatment.

1.5 Conclusion

Water plays a crucial role in our everyday lives. It is important that consumers are supplied with good quality water that is safe and free from viruses and other pathogens. Viruses are a major cause of human waterborne diseases. These diseases are caused by water that is contaminated by human and animal urine and faeces that contain pathogenic microorganisms. It was therefore important to assess water treatment process barriers for their effectiveness in removing these pathogens. In order to provide safe water, water utilities need to ensure that the pathogens are effectively removed by the water treatment processes used and that the necessary steps are taken should a failure in the process occur.

CHAPTER 2
2. Literature Review

2.1 Water in South Africa

South Africa is the 30th driest country in the world (Centre for Environmental Rights, 2016). In South Africa, water is used for both social and economic purposes. Approximately 60% of water is used for agriculture, 27% for domestic use, 4.3% for power generation, 3.3% for mining, 3% for industries and 2.5% for livestock watering and nature conservation (DWAF, 2013). Water infrastructure in South Africa is well developed in urban areas in comparison to rural areas. In the rural areas, communities get their water from small water treatment plants, boreholes and springs (Momba et al., 2009) whereas in urban areas consumers get a more constant supply of water from municipal sources (DWAF, 1996).

2.2 Sources of water in South Africa

South Africa is located in a predominantly semi-arid part of the world and relies on surface water resources for most of its urban, industrial and irrigation requirements (DWAF, 2004). However, it is important to note that the natural water resources within South Africa are unevenly distributed (Fig.3). The eastern side of South Africa has more water source areas than the western side (Fig.3). Since South Africa's water resources are scarce and extremely limited (DWAF, 2004), it is therefore essential to implement integrated water management techniques to ensure that water is both protected and used to its full potential (DWAF, 2013). South Africa's inland water resources consist of rivers, dams, wetlands, groundwater (Fig.3) and rainfall.

South Africa has the following major rivers: Orange River that flows through Lesotho, Free State & Northern Cape Provinces; Limpopo River in Limpopo Province; Vaal River that flows through Mpumalanga, Gauteng, Free State & Northern Cape Provinces; Thukela River in Kwa-

Zulu Natal Province; Olifants River that flows through Mpumalanga & Limpopo Provinces; Gamtoos River in Eastern Cape Province; Great Kei River in Western Cape Province; Komati River in Mpumalanga Province; Great Fish River in Eastern Cape Province and Molopo River in the North West Province (Water Wise, 2016). There are 569 dams in South Africa with individual capacities exceeding one million cubic meters. Among these dams, the largest ones capture about 70% of the total mean annual runoff

(Strydom and Oelofse, 2010).

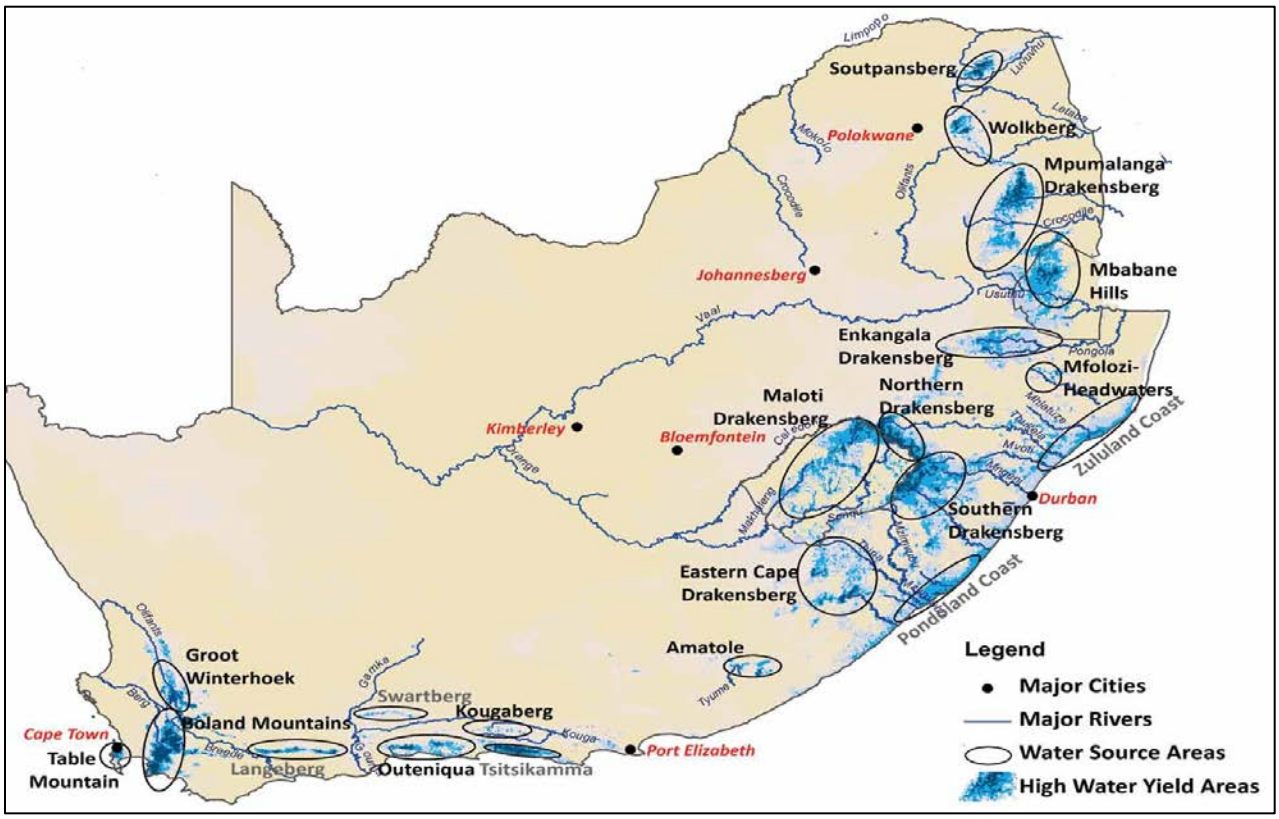

Figure 3: South Africa's Resource Areas (WWF report, 2013).

Rivers and dams are used for a variety of purposes in South Africa. In rural areas rivers are used for irrigation, domestic purposes and livestock watering. Some rivers flow into dams, the water is then transferred to water treatment plants for purification. The treated water is used for industrial, domestic and mining purposes. Groundwater serves a variety of purposes in different

parts of the country. It is mostly used for irrigation in many areas, whereas in the Highveld it is used for mining. Rural areas in KwaZulu-Natal, Western Cape, Eastern Cape, Mpumalanga, and Limpopo use groundwater for domestic purposes (DWA, 2016). Rainfall in South Africa is seasonal and unevenly distributed across the country (WWF report, 2013). The eastern half of the country is much wetter than the western half due to the nature of the weather conditions. The rainfall pattern and subsequent run-off are highly seasonal, and this causes short wet seasons and extended dry seasons in many parts of the country (DWA, 2016).

Wetlands are also a vital source of water in South Africa. Wetlands are needed for flood attenuation, recharging of groundwater, cleaning of water, and maintaining the base flow of streams and rivers during dry periods (Cessford & Burke, 2005). The Klip River wetland is among one of the most economically important wetlands in Africa and was the first reliable water supply to the towns of the Witwatersrand goldfields (Mccarthy et al., 2007). As the communities within the Witwatersrand goldfield grew, the demand for water also rapidly increased and as a result the wetland water source became insufficient. The demand for water was mainly due to a growing population, intensified agriculture and industrialisation.

To meet the increased demand for water, dams such as the Vaal Barrage were built. At this point water in the central and western Witwatersrand was not only used for domestic purposes but also for economic use such as agriculture. However, current estimates show that over 50% of South Africa's wetlands have been destroyed (DWA, 2016). The South African water treatment plants rely on surface water from rivers, dams and reservoirs for domestic water production. Hence, assessing the quality of surface water before its treatment has become increasingly important. Surface water is often contaminated with pathogenic microorganisms due to unsanitary practices,

lack of or insufficient hygienic infrastructure as well as improper agricultural practices (Schijven & Hassanizadeh, 2000).

2.3 Water for domestic usage

South Africa relies on large bulk drinking water suppliers for the production of water for domestic purposes. The largest drinking water supplier in South Africa currently processes and supplies approximately 4 460 megaliters of water daily (Rand Water, 2016). This large bulk water supplier has made remarkable progress regarding supply, considering that in 1965 it was supplying only 1000 megaliters a day (Ml/d), while the current supply has increased to approximately 4 460 Ml/d.

Initially, the company used groundwater from the Zuurbekom Wells on the West Rand. At that time the water from the Zuurbekom Wells was of good quality and required less effort to achieve the required standards (Pearson, 2010b). However, with time water from the Zuurbekom Wells was insufficient to cater for the growing population around Witwatersrand. As a result, the Vaal River to the south of Johannesburg was chosen as a new water source.

In 1923, the Vaal River was dammed to form the Vaal River Barrage Reservoir (Pursell, 2007) and then a few years later in 1938, the Vaal Dam was built upstream of the Vaal River Barrage Reservoir, and which is now the primary source of water for the company. Rivers such as the Vaal and Wilge Rivers naturally flow into the Vaal Dam from agricultural land. To produce safe drinking water from the surface water, all contaminants, both chemical and biological, need to be removed.

2.4 Water quality guidelines

Drinking water standards are important in ensuring that water supply companies provide water that is safe and of good quality (Mamba & Verberk, 2008). The SANS 241:2015 is a SABS standard that specifies the quality of drinking water in numerical limits (Grabow et al., 2004).

Table 2. Microbiological determinants referenced by SANS241:2015.

1	2	3	4
Determinant	**Risk**	**Unit**	**Standard limits**
E.coli [a] or faecal coliforms [b]	Acute health	Count per 100 mL	Not detected
Protozoan parasites [c] *Cryptosporidium* and *Giardia* species	Acute health [g]	Count per 10 L	Not detected
Total coliforms [d]	Operational	Count per 100 mL	≤10
Heterotrophic plate count [e]	Operational	Count per mL	≤1000
Somatic coliphages [f]	Operational	Count per 10 mL	Not detected

[a] Definitive, preferred indicator of faecal pollution.

[b] Indicator of unacceptable microbial water quality could be tested instead of *E.coli* but is not the preferred indicator of faecal pollution. Also provides information on treatment efficiency and after growth in distribution networks.

[c] Confirms a risk of infection and faecal pollution and also provides information on treatment efficiency. The detection of selected protozoan parasites confirms a human health risk.

[d] Provides information on treatment efficiency and after growth.

[e] Process indicator that provides information on treatment efficiency, after growth in distribution networks and adequacy of disinfectant residuals.

[f] Process indicator that provides information on treatment efficiency.

[g] Determinant that is presently not easily quantifiable and lacks information about the viability and human infectivity, which, however, does pose immediate unacceptable health risks if present in drinking water.

Water quality is assessed by microbiological, physical, aesthetic and chemical determinants at the point of delivery of the water (Table 2).

2.5 Water treatment process in South Africa

The conventional water treatment process is the most commonly used method in South Africa. It consists of clarification, filtration and disinfection processes (Momba et al., 2009). The process begins with the abstraction of water from the source via a series of pipelines to the plant where it is purified. Upon arrival at the plant, the raw water undergoes a screening process. During the screening process the large floating materials in the water are removed (Jiménez et al., 2010). Removal of the large matter improves the efficiency of the downstream water treatment procedures. After removal of the large particulate matter, the water flows to the inlet point where the pH, turbidity and conductivity are measured.

The pH of the water determines the quantity of chemicals required during the carbonation and stabilization phase. The turbidity levels of the raw water determine the mode of water purification to be used by the plant (Chiloane, 2010). Conductivity is measured to observe any significant changes that occur in source water from the dams. A significant change in conductivity would indicate that the source has received a discharge or other forms of pollutants. The conductivity measurements assist in combating problems associated with periodic pollution of surface water (Chiloane, 2010). In turn, the pollution levels are kept at a minimum thus reducing the dosage of chemicals required to meet the relevant water quality standards. Once the pH, turbidity and conductivity of the water have been assessed the treatment process commences.

2.5.1 Clarification

The first step in clarification is coagulation. Coagulation is a physicochemical treatment process that combines small particles into larger aggregates and adsorbs dissolved organic matter onto particulate aggregates (Jiang, 2015). Small particles in the source water, such as viruses, that will not quickly settle from suspension by gravity are destabilized and combined into larger aggregates during the coagulation process, thus enabling their removal through a sedimentation and filtration process (Shirasaki et al., 2009). Coagulation is performed in two stages: the first stage often consists of rapid mixing of the coagulant with the water followed by slow mixing to allow the flocs to aggregate further (Srinivas & Vuppala, 2015).

Langerlier and Ludwig (Jiang, 2015) distinguished the two mechanisms for removal of suspended solids, namely, the double layer compression mechanism and precipitate enmeshment (Jiang, 2015). The double layer compression mechanism allows the particles to overcome repulsive forces resulting in aggregation and precipitation. However, during precipitate enmeshment, small particles are physically enmeshed by precipitates during flocculation and sedimentation.

There are different coagulant chemicals used for drinking water treatment plants. The most widely used coagulants worldwide are aluminium sulphate, lime, polyelectrolyte and iron salts (ferric chloride and ferric sulphate). Several studies have demonstrated the importance of the coagulation process for the removal of enteric viruses and bacteriophages (Shirasaki et al., 2009). Some water treatment plants use hydrated lime for coagulation and flocculation and may add activated silica and ferric chloride as aids to flocculation (Chiloane, 2010). Coagulation can be sufficient with the use of coagulants that are metal ions, salts of iron and polyelectrolytes (Berg, 1973). Several factors such as pH, the nature and dosages of the coagulant used,

temperature and mixing method significantly influence the efficiency of microorganism removal by coagulation (Shirasaki et al., 2013).

Flocculation involves the gentle mixing of flocs formed during coagulation (Fig. 4). Floc sizes increase with additional collisions and interaction with an added coagulant. Macro flocs are then formed, and coagulant aids are added to bind, add weight and strengthen the macro flocs to enable settling. These flocs will then settle out of the water by a process called sedimentation (Fig. 4).

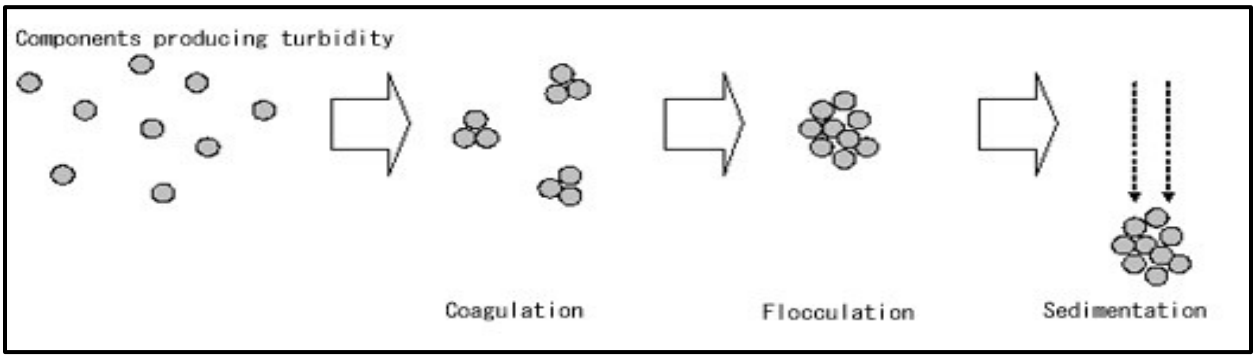

Figure 4: The process of coagulation, flocculation and sedimentation (Safe Drinking Water Foundation, 2007).

Sedimentation is the process where flocs are allowed to settle out of the water by reducing the velocity and turbulence in the water column (Chiloane, 2010). Following sedimentation, water is subjected to carbonation and stabilisation, which is done to protect the inside of the pipelines from corrosion. It is important to note that flocculated sediments should be disposed of appropriately given that they may contain high pathogen loads (Stanfield et al., 2003).

2.5.2 Filtration

The second stage in the conventional water treatment process is filtration. Filtration is the process where almost all of the suspended particles are removed by passing the water through a bed of filter media (Jiménez et al., 2010). After the clarification stage water would still contain a

small amount of matter which would then later be removed through rapid gravity sand filtration yielding a product with a turbidity which is consistently less than one nephelometric turbidity units (NTU) (Pursell, 2007). Filter media consists of different layers of sand packed from bigger to smaller sand particles.

Rapid sand filtration is a physical method that is sufficient for the removal of large suspended particles. This filtering process is determined by two fundamental principles, which are mechanical straining and physical adsorption. Mechanical straining occurs when very large suspended particles adhere to the sand grains as they pass through the filter medium. Physical adsorption takes place when small particles attach to the surface of the sand grains due to van der Waals forces (Marco, 2012).

According to studies on transport through soil matrices, factors such as hydrophobicity, surface charge and isoelectric point influence the adsorption of viruses (Mayer et al., 2015). Other studies suggest that the only factor influencing retention is virus size, with larger viruses being retained more efficiently (Aronino et al., 2009). As more particles are trapped in the filter medium, clogging occurs. Therefore, the need to clean the filter to ensure proper functionality becomes more apparent. Cleaning of the filter medium is often achieved through a series of backwashes. In filter backwashing, air is used to loosen the sand, and then water is flushed upward through the filter (Chiloane, 2010). The sand is re-suspended, and the solid matter is separated in the surface water. Sand filtration is the most widely used filtration process in drinking water treatment. Other filtration processes include microfiltration and ultrafiltration. These processes use membranes of different pore sizes to remove microorganisms.

Higher turbidity removals ranging from 60% to 90% have been demonstrated with the use of roughing filters. The higher the turbidity of the water, the more significant the reduction that can be achieved (WHO, 2004). Despite the excellent progress that has been made in the application of membrane technologies, rapid sand filtration is an old, well-known and reliable water treatment process that is still applied and remains the cheapest and most reliable application for meeting the reuse criteria of secondary effluents (Aronino et al., 2009). It can act as a consistent and efficient barrier for microbial pathogens with proper chemical treatment (Bala & Kondepudi, 2016).

2.5.3 Disinfection

The third stage in the conventional water treatment process is disinfection. Disinfection is a crucial step in the drinking water treatment process because it is during this stage that infectious microorganisms are removed (Cromeans et al., 2010). During this stage, a disinfectant is added into the filtered water to kill any microorganisms to ensure water safety (Asami et al., 2016). Currently, water disinfection is achieved through two main ways, and these are through the use of chemical oxidants such as free chlorine, chloramines, chlorine dioxide and ozone (Stanfield et al., 2003) and or UV photolysis.

Chemical oxidants are likely to cause protein damage to microorganisms. Waterborne viruses exposed to such chemical oxidants often lose the ability to bind to cells, and as a result, their post-binding lifecycle processes are affected (Mayer et al., 2007). UV light can be categorized as UV-A, UV-B, UV-C or vacuum-UV, with wavelengths ranging from about 40nm to 400 nm. UV-B and UV-C are the most effective for inactivating microorganisms in the ranges of the spectrum (200 nm–310 nm), with maximum effectiveness around 265 nm (WHO, 2004).

Factors that influence the efficiency of disinfection are the concentration of the disinfectant used, contact time, temperature and pH. Disinfection can also be affected by the turbidity level of the water. Turbidity can significantly affect the microbial quality of drinking water either by enhancing the growth and survival of microorganisms or by decreasing the efficiency of chemical and/or UV photolysis in water treatment (Obi et al., 2008).

Chlorine gas is the most popular disinfectant in South Africa (Momba et al., 2009). When chlorine is added to water, it reacts with ammonia to form monochloramine, dichloramine and nitrogen trichloride (Yee et al., 2008). These three are termed combined available chlorine which is less reactive but lasts longer in the distribution system. Although free available chlorine is more reactive and can rapidly meet the chlorine demand of the water (Pursell, 2007), it does not last long, hence it is used as a primary disinfectant. Combined available chlorine is used for secondary disinfection because it can last longer in the distribution system. After sedimentation and filtration, all the water is disinfected with chlorine before being introduced into the distribution system (Pursell, 2007).

2.5.4 Laboratory testing of samples and distribution

At the end of the treatment process, samples are taken and tested for any microbial or chemical contaminants. Thereafter, the processed water is pumped from the main purification plants to the main booster pumping station. Each booster pumping station then elevates the water to reservoirs of Johannesburg and its surrounding areas. From these areas, the water flows under gravity to the extreme boundaries of the supply area. The water is supplied through 3056 kilometres of the pipeline into 58 reservoirs, from where it is then delivered to the customers. The customers consist of metropolitan Councils, local Municipalities, mines, industries and direct consumers in Gauteng, Free State, North West and some parts of Mpumalanga (Fig. 5.)

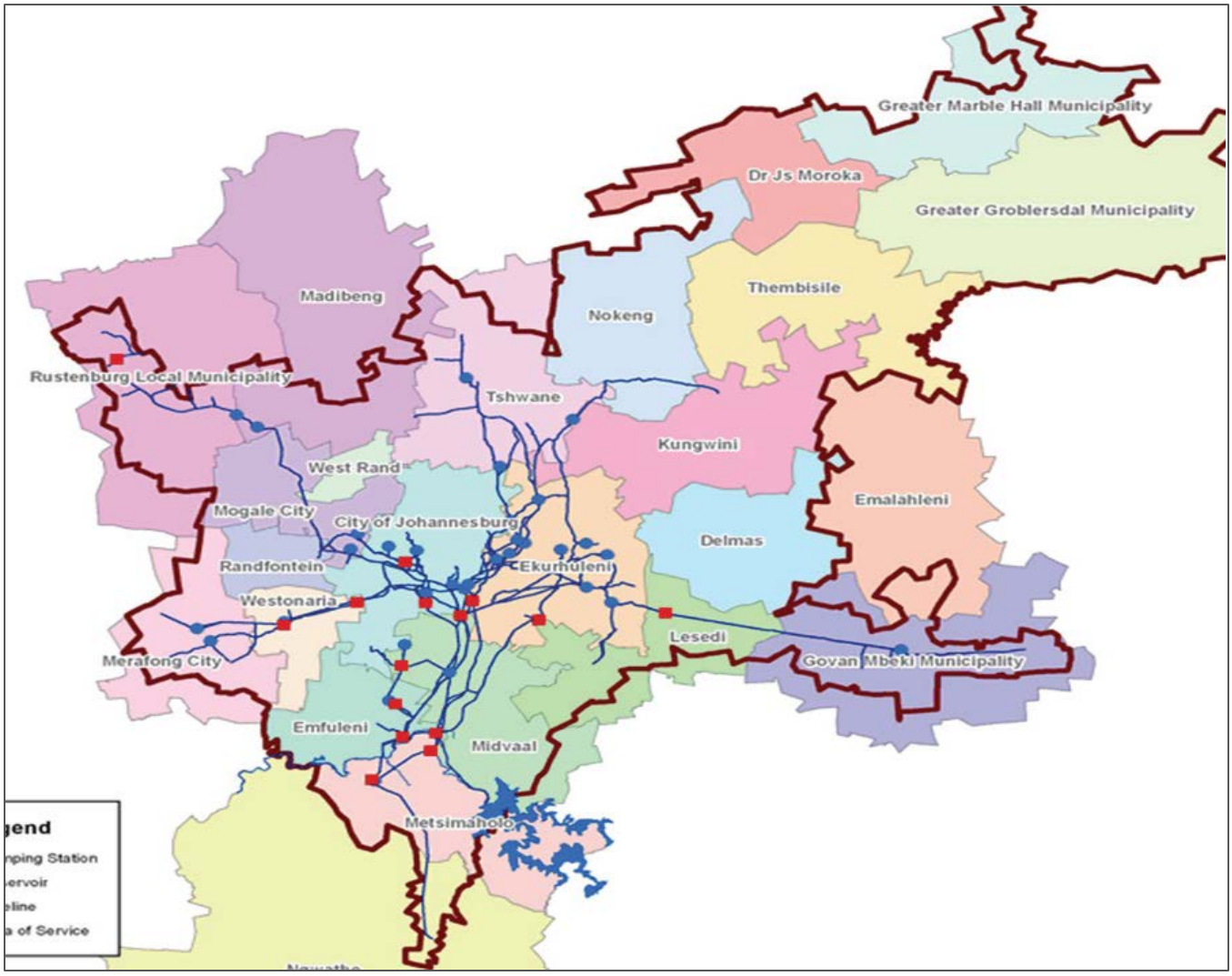

Figure 5 : Rand Water's area of supply in Gauteng Province and surrounding areas

(Ncube et al., 2012)

2.6 Assessing the effectiveness of treatment processes

The main purpose of water treatment is to provide water that is free of any pathogenic microorganisms (Mamba & Verberk, 2008). A wide variety of pathogens can be transmitted by water through four primary routes, namely, water-borne, water-washed, water-based and water-related insect vectors (Macy & Quick, 2011). These pathogens cause diseases such as

gastroenteritis, hepatitis, typhoid fever and cholera and have been associated with contaminated water worldwide.

Despite the significance of these pathogens, the World Health Organization (WHO) does not recommend the establishment of water quality targets that require direct testing of finished waters for pathogens (Kreißel et al., 2014). This is because the direct monitoring of pathogens in finished waters is not considered a reasonable or cost-effective option. This is primarily because pathogen concentrations equivalent to tolerable levels of risk are typically less than one organism per 10^4–10^5 litres of water.

Analytical methods are just not capable of detecting pathogens at such low concentrations. However, the WHO recommends that utilities develop performance targets (WHO, 2011). These targets define the number of pathogens that should be removed by treatment processes. Therefore, utilities have a responsibility to assess their treatment processes to determine if they are capable of removing the required numbers of pathogens. Ultimately the treatment process must meet an overall performance target which will ensure that predefined public health outcome targets are consistently achieved.

There are two main health outcome targets used globally. These are the infection rate metric and the Disability Adjusted Life Year (DALY) (O ' Toole & Sinclair, 2015) (Fig. 6). The United States Environmental Protection Agency (USEPA) has adopted the 'infection rate' metric at a level of 1:10,000 people per year (Gibney et al., 2013). The DALY is a way of measuring the impact of a health problem on a population, or the burden of disease, associated with a specific condition. It is measured by the amount of time that is lost due to imperfect health, taking into account premature death and time lived in a state of ill-health (Water Research, Australia, 2013) .

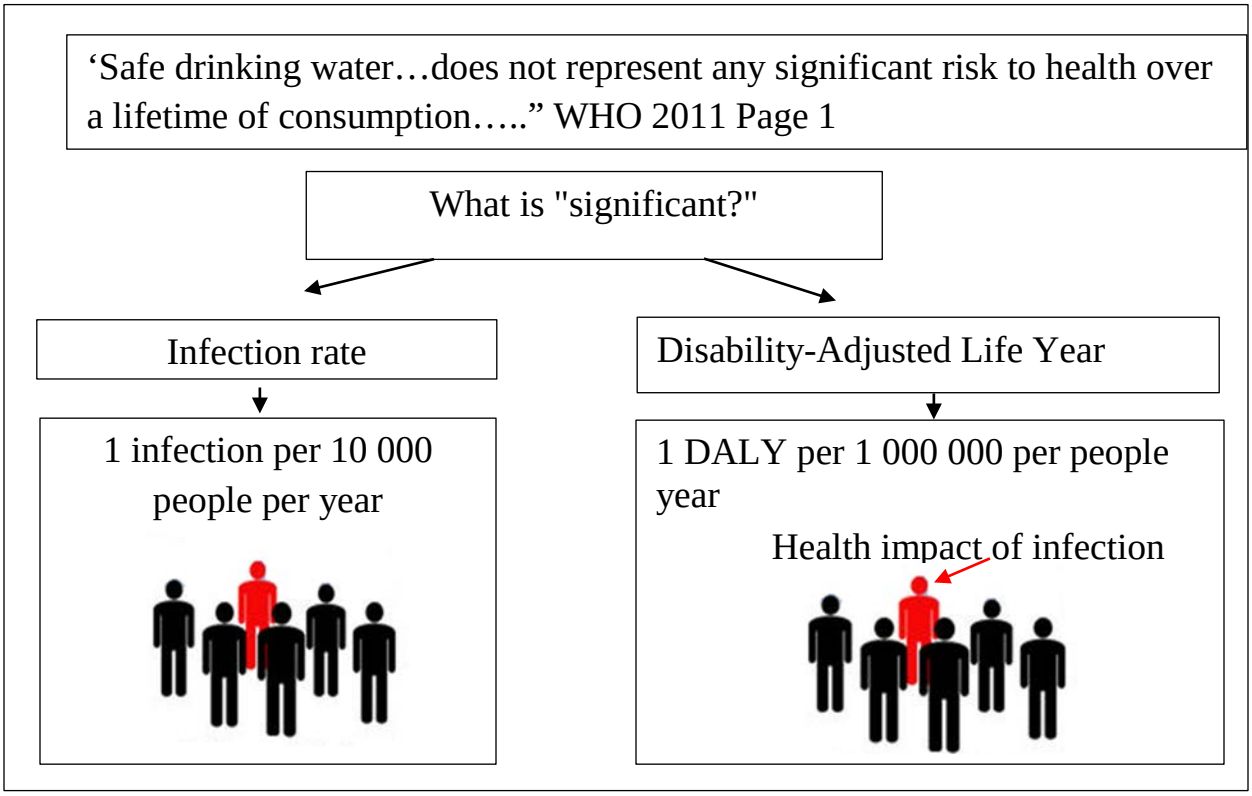

Figure 6: Health-based target metrics. Information on diagram derived from (O ’ Toole & Sinclair, 2015).

Of these two methods, the infection rate metric does not take into account the severity of the infection which could either be relatively minor or severe. In contrast, WHO has adopted the DALY method probably because it puts into consideration the impact of the pathogenic microorganisms on the health of the population (Gibney et al., 2013). Nonetheless, the ultimate aim is to adopt health-based targets (HBTs) for microbial quality monitoring of drinking water so that the public is not exposed to unacceptable levels of pathogenic microorganisms.

Adopting the HBTs does not guarantee that water is entirely free of pathogenic microorganisms. The HBTs ensure that the risk of contracting a waterborne infection is significantly reduced through proper management. An important benefit to the adoption of HBTs is the increased emphasis on the effectiveness of the treatment processes. The effectiveness of a water treatment

process ensures that corrective actions are taken in real-time if a problem is detected. Usually, the corrective measures taken prevent the affected water from being distributed to consumers.

2.7 Effectiveness of water treatment processes for the removal of viruses

The conventional water treatment process has been proven to be useful for the removal of pathogens from water (Asami et al., 2016). The advantage of this treatment method is that it has multiple barrier functions, which ensure proper quality water in case a single treatment step is not working optimally. Each step in the conventional water treatment process plays a role in modifying the physical and chemical properties of the water as it undergoes purification.

This water treatment process has demonstrated efficiency in the removal of various bacteria, viruses and protozoa (Abbaszadegan et al., 2008). For instance, the clarification step in the conventional water treatment process can achieve more than 99% microbial removal and 70-80% turbidity removal whereas gravel pre-filtration can achieve less than 90% microbial removal (Fig. 7) (Jiménez et al., 2010). Studies have shown that clarification combined with rapid sand filtration can remove between 2-3 logs of bacteria; 1-3 logs of viruses and 2-3 logs of *Cryptosporidium* (Stanfield et al., 2003). Rapid sand filtration cannot remove viruses without chemical pre-treatment.

Removal of microbial pathogens by granular filtration does not rely only on the physical processes alone. It involves the transport of particles from suspension to filter medium, followed by the attachment of the particles to the medium (WHO, 2004). The ability of a water treatment process to reduce virus concentration depends on the properties of the virus and properties of the treatment process. For instance, conventional disinfection using chlorine can inactivate 1-3 log

units of viruses, 2 log units of bacteria and 0 – 1.5 log units of protozoan cysts (Jiménez et al. 2010).

A study by Zhang & Farahbakhsh (2007) showed that the overall removal efficiency of total and faecal coliforms by the entire treatment process varied from 4.4 to 5.4 logs and 4.3 to 5.7 logs, respectively. The maintenance of the water treatment facility also plays a vital role in the effectiveness of the treatment process to supply safe drinking water (Bala & Kondepudi, 2016). Management of water supplies is often improved by implementing holistic water safety plans. Within a water safety plan, control measures to reduce potential risks from enteric viruses should focus on prevention of source water contamination by human waste followed by adequate treatment and disinfection.

Many viruses are known to be more resistant to natural inactivation and disinfection than the current bacterial indicators of water quality (Grabow et al., 2004). Thus there is a need to identify a better microbial indicator of water quality with which to monitor the presence of viruses in drinking water.

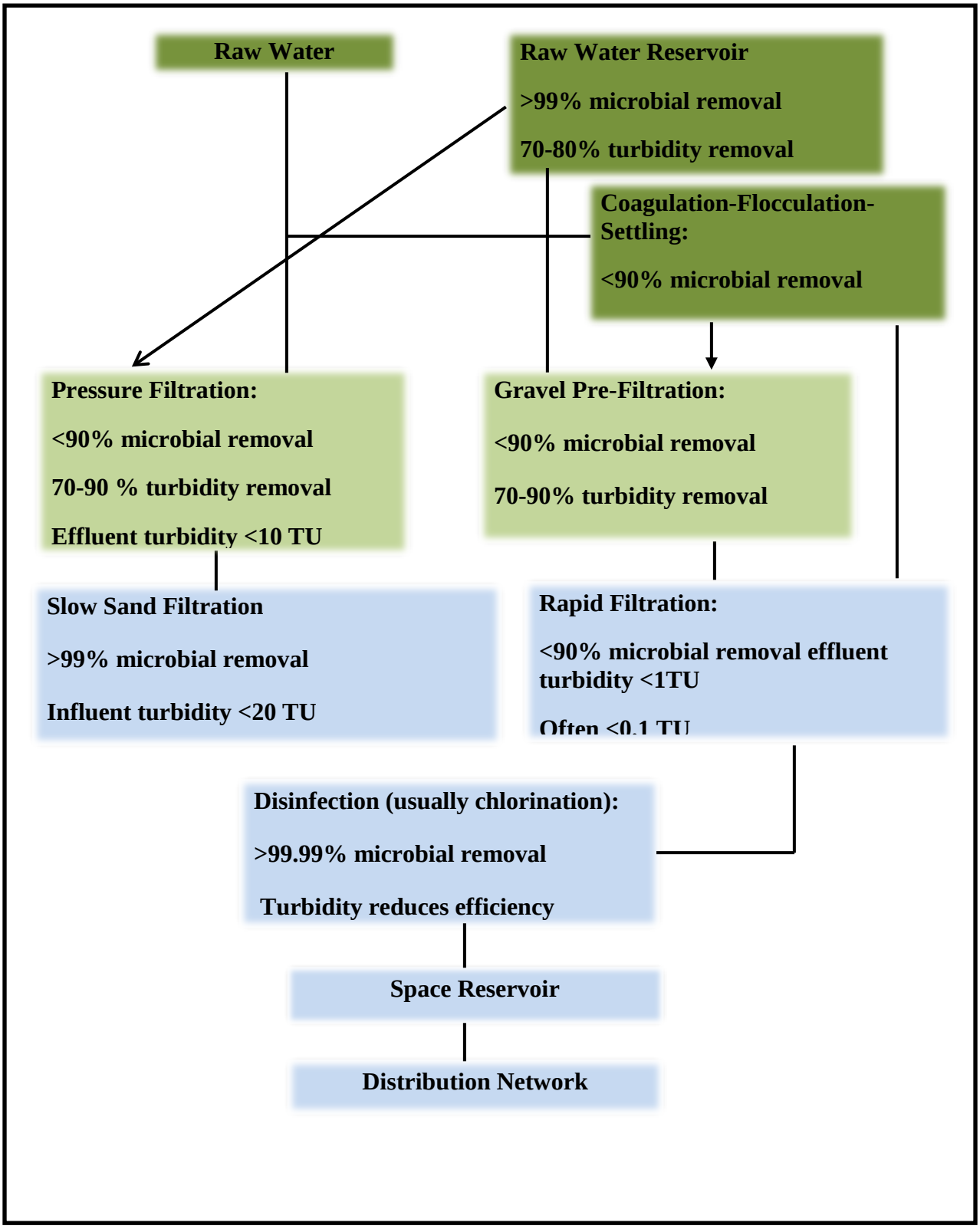

Figure 7: The multiple barrier principle of water treatment (WHO, 2003).

2.8 The use of coliphages as surrogates for enteric viruses

This study utilized bacteriophages as surrogates for the removal of viruses from source water. Bacteriophages that infect coliform bacteria are known as coliphages. Their structures consist of the genome which is surrounded by a protein capsid (Jofre et al., 2016). Each group of coliphages has a specific host bacterium that they can infect. For instance, coliphage T7 infects *E.coli* 0157: H7. Bacteriophages are an ideal model that could be used to determine or test the effectiveness of viral removal by a water treatment process.

Bacteriophages offer the following advantages: they are not pathogenic to humans but infect a specific host bacterium, and they can be prepared in large quantities allowing seeding in high numbers. This makes it possible to show a removal efficiency of up to 11 $\log_{10}$. An additional advantage is that samples spiked with bacteriophages can be kept at 4^{o}C for at least two days without any significant change in the concentration of the sample (Jofre et al., 2016).

The assay of bacteriophages is relatively easy, whereas the analysis of pathogenic viruses is much more complex, time-consuming and sometimes not possible at all. The bacteriophages that were used in this study both infect *E. coli* but belong to different groups. Bacteriophage phi X174 is a somatic coliphage and infects *E. coli* through the cell wall. MS2 is an F-specific coliphage and infects *E. coli* through the sex pili. The characteristics of the two bacteriophages are shown in Table 3.

Table 3. Test viruses: sources and characteristics

Virus	Nucleic acid	Isoelectric point	ATCC#	Diameter (nm)	Host- (ATCC#)
MS2	ssRNA	3.9	15597-B1	32	*E.coli*-15597
phi X174	ssDNA	6.6	13706-B1	26	*E.coli*-13706

The bacteriophage phi X 174 (Fig. 8 A), a member of the Microviridin family, is a small, icosahedral bacteriophage of *E. coli* that contains a single-stranded DNA genome (Aronino et al., 2009). The bacteriophage MS2 (Fig. 8 B), which belongs to the Leviridiae family, is an icosahedral, single-stranded RNA virus surrounded by a protein capsid (Shi et al., 2012) .

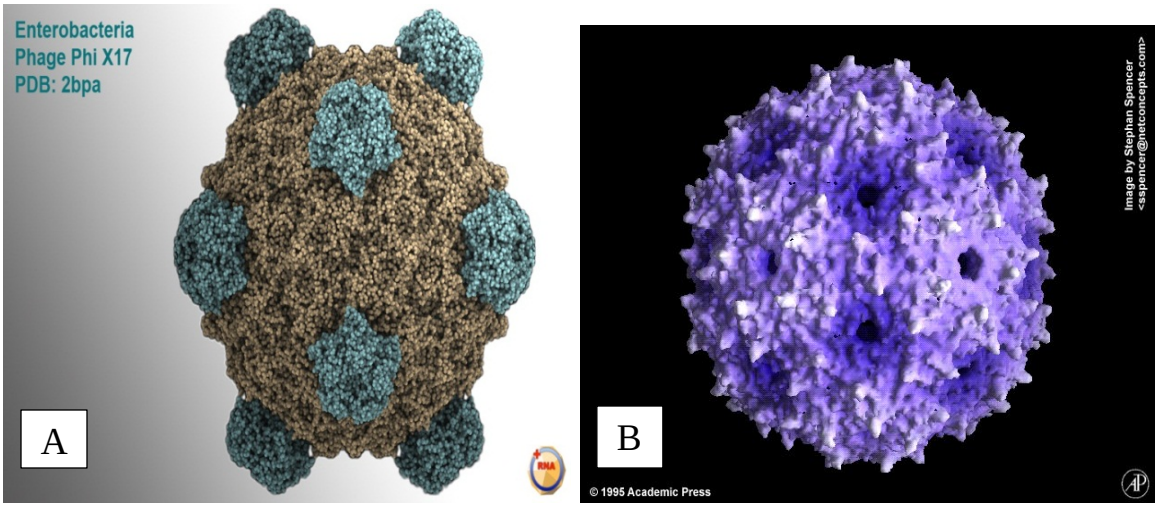

Figure 8: Bacteriophage phi X 174 (A) and MS2 (B) ("Virusworld _ Enterobacteria Phage Phi X174," n.d.).

The methods used for detecting bacteriophages are easy, fast and cost-effective. The ISO, USEPA and Standard Methods have standardized procedures for the enumeration of somatic coliphages (Jofre et al., 2016). For this study, the ISO-10705 standard method was used for the enumeration of both bacteriophages. This method entails mixing the sample with a small volume of semi-solid nutrient medium. A culture of the host strain is added and plated on a solid nutrient medium. After this, the plates are allowed to solidify and then incubated. After incubation the plates are read to check for any visible plaques. The results are expressed as the number of plaque-forming units per unit of sample volume. The usage of bacteriophages as surrogates for enteric viruses in spiking tests has become a state of the art technology for the determination of virus removal by different water treatment technologies (Kreißel et al., 2014).

2.9 Jar testing protocols for evaluating coagulation and flocculation processes

Jar testing is a pilot-scale test that simulates the water treatment process. It is used to determine the proper coagulant dose of the treatment chemicals used in a particular water treatment plant. The coagulant dose is determined by exposing same volume samples of the water to be treated to different doses of the coagulant and then at the same time mixing the samples at a constant rapid mixing time. The flocs formed after coagulation undergoes flocculation and is allowed to settle. Then the turbidity of the samples is measured, and the dose with the lowest turbidity can be said to be at an optimum level. This will enable production scientists to make better-informed decisions regarding the plant's performance.

When simulating a conventional drinking water purification process, a combined water treatment apparatus is used. The combined apparatus consists of a jar tester and a filtration system. Jar testing simulates part of the conventional drinking water treatment process, namely, clarification while the filtration system simulates the rapid sand filtration process. In full-scale water treatment works, jar test studies are often used to determine the optimum performance of a coagulant and its dosage (Jiang , 2015). Another important reason to perform jar testing is to save money because jar tests determine the approximate dosage of chemicals to avoid overdosing or underfeeding (Satterfield, 2005). For this study, the Phipps and Bird jar testers were used. Phipps and Bird Jar testers are designed to perform standard jar tests in a standard lab environment (the principle is explained in section 3.4.2).

CHAPTER 3

3 Materials and methods

3.1 Bacteriophage, bacterial strains and their propagation

Bacteriophages MS2 (ATCC 15597-B1) and phi X174 (ATCC 13706-B1), as well as bacterial strains *E.coli* (ATCC 13706) and *E.coli* (ATCC 15597), were purchased from INDUSTRIAL ANALYTICAL (PTY) LTD (Midrand, SA). The bacteriophages MS2 and phi X 174 were propagated in hosts *E.coli* (ATCC 15597) and *E.coli* (ATCC 13706), respectively, according to ISO 10705-2 standard procedure (ISO 10705-2, 2000). Both strains of *E. coli* were inoculated in nutrient broth and incubated at 36ºC while shaking at 100 revolutions per minute (RPM). After 24 hours 0.25 ml of each *E. coli* strain was inoculated into 25 ml of pre-warmed nutrient broth and incubated at 36ºC while shaking at 100 RPM for 90 minutes. This was followed by the addition of 10 ml of MS2 and phi X174 stock solution to respective host cultures. After five hours the bacteriophage cultures were stored at 4ºC overnight. The following day the aqueous phase was centrifuged at 3000g for 20 minutes and the pellet discarded. The concentration of the bacteriophage in the supernatant was determined by a plaque forming unit (pfu) assay, using the double-layer overlay method described in section 3.2. One ml aliquots of the supernatant were prepared and stored in a freezer below -80ºC until use.

3.2 Bacteriophage enumeration and assay

The bacteriophage enumeration was done using the double agar layer method according to the SABS method 221-1990 (second revision) (Fig. 9). The *E.coli* host was inoculated in nutrient broth medium and incubated at 37ºC overnight in an orbital shaking incubator set at 100 RPM. After incubation, the inoculated broth was placed on crushed ice to stabilize growth. Base agar plates were placed for 2-3 hours in an incubator at 37ºC to dry. This was done to prevent plaques

from swarming into each other and to also prevent the top layer agar from slipping off when inverting the plates during incubation. Top layer agar in test tubes were melted in an autoclave at 110ºC for 15 min and then stored in an oven at 55ºC until it was needed.

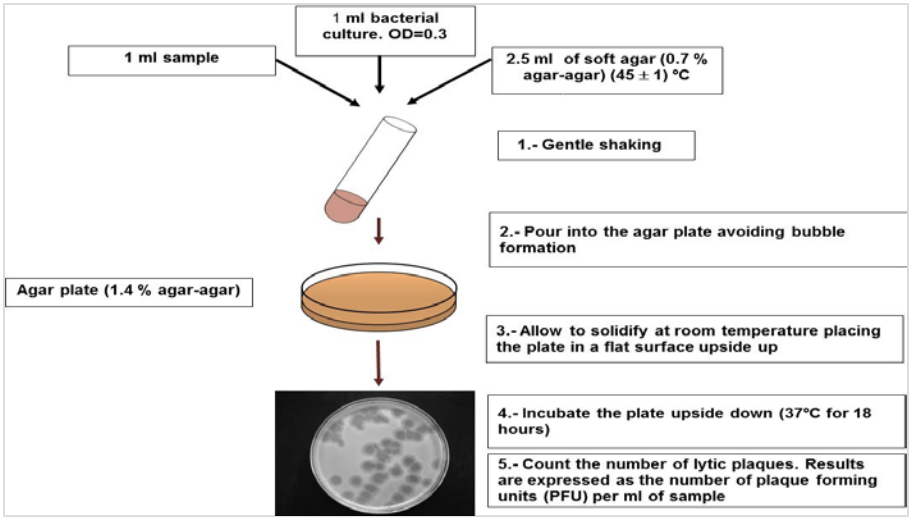

Figure 9: Double agar layer method for phage enumeration (Jofre et al., 2016)

Before the analysis, the top layer agar test tubes were packed onto a heating block at 46ºC, and 0.3 ml of the *E.coli host* was added to each tube. This was followed by the addition of 1 ml of the sample in each tube. The contents were then mixed by rolling the tube between the hands and immediately poured on the base layer agar plates and allowed to solidify on the bench. The solidified plates were incubated in an inverted position at 37ºC overnight, and plaques were counted after 16-18 hours.

3.3 Preparation of water samples for the clarification jar test experiments

Raw water was collected from a water pumping station in Vereeniging and transported to the laboratory where it was stored until it reached a temperature of 23ºC. The pH and turbidity of the sample were measured. After that, a sample of the raw water was taken and analysed for the presence of bacteriophages. This was done to assess if any bacteriophages were present in the sample before spiking. Ten litres of raw water were mixed manually by tilting the container upside down five times after which 2.6 L of water was spiked with bacteriophages MS2 or phi X174 at a concentration of 300 and ± 1000 pfu/ml, respectively. The spiked raw water sample was thoroughly mixed on a magnetic plate stirrer for 20 minutes, thereafter; 10 ml of the spiked water was tested for the presence of bacteriophages. The result was used as a measure of the bacteriophage concentration before treatment. The remaining spiked water was poured into five 500 ml beakers and placed on the jar tester. A beaker containing 500 ml of unspiked water was also placed on the jar tester. The unspiked sample served as the negative control for the experiment. Jar tests were conducted the same day the samples were collected.

3.4 Preparation for the clarification jar test experiment

3.4.1 Coagulants used for the clarification jar tests experiments

The coagulants used for the jar tests were polyelectrolyte Sudfloc 3835 (Blend Tech (Pty) LTD, Kempton Park SA), lime (Thuthukani SNF Chemicals, Randburg SA), sodium silicate (Reba Chemicals, Kempton Park SA), and ferric chloride (Watersol SA, Centurion SA). All the coagulants except for lime were freshly prepared using water.

Jar tests were conducted to simulate three different treatment regimens: (i) treatment with polyelectrolyte Sudfloc 3835, (ii) treatment with a combination of lime and activated silica and (iii) treatment with a combination of lime, activated silica and ferric chloride. For the first

treatment polyelectrolyte was added at a final concentration of 2,3,4,5 and 6 mg/L. For the second treatment, the concentration of the activated silica remained constant at 2 mg/L. However, lime was added at different concentrations of 20, 40, 60, 80 and 100 mg/L. For the third treatment, a combination of lime, activated silica and ferric chloride were used. The final concentration of the activated silica was kept constant at 2 mg/L; the final concentration of the lime and ferric chloride were varied at 20, 40, 60, 80 100 mg/L and 2, 3, 4, 5 and 6 mg/L, respectively. The coagulants were added to all five spiked beakers. The non-spiked beaker served as a negative control, and no chemicals were added to it. The jar test experiments were conducted as described in section 3.4.2.

3.4.2 Jar test protocols

All jar test experiments were performed using a 6-jar apparatus operating at either a low or a high energy regime (Tables 5, 6, 7). All the clarification jar test experiments were done with six 500 ml raw water samples. Before the experiment, five 500 ml samples were spiked with either MS2 or phi X174, and one 500 ml sample was not spiked and did not contain any coagulant. All jar tests were conducted with a Phipps and Bird jar test apparatus (Fig. 10). The jar test protocols for the different chemical treatments are presented in Tables 5, 6 and 7. After the clarification jar test experiments, samples were taken from the centre of each beaker, 20 cm below the surface. The samples were assayed for bacteriophages as described in section 3.2.

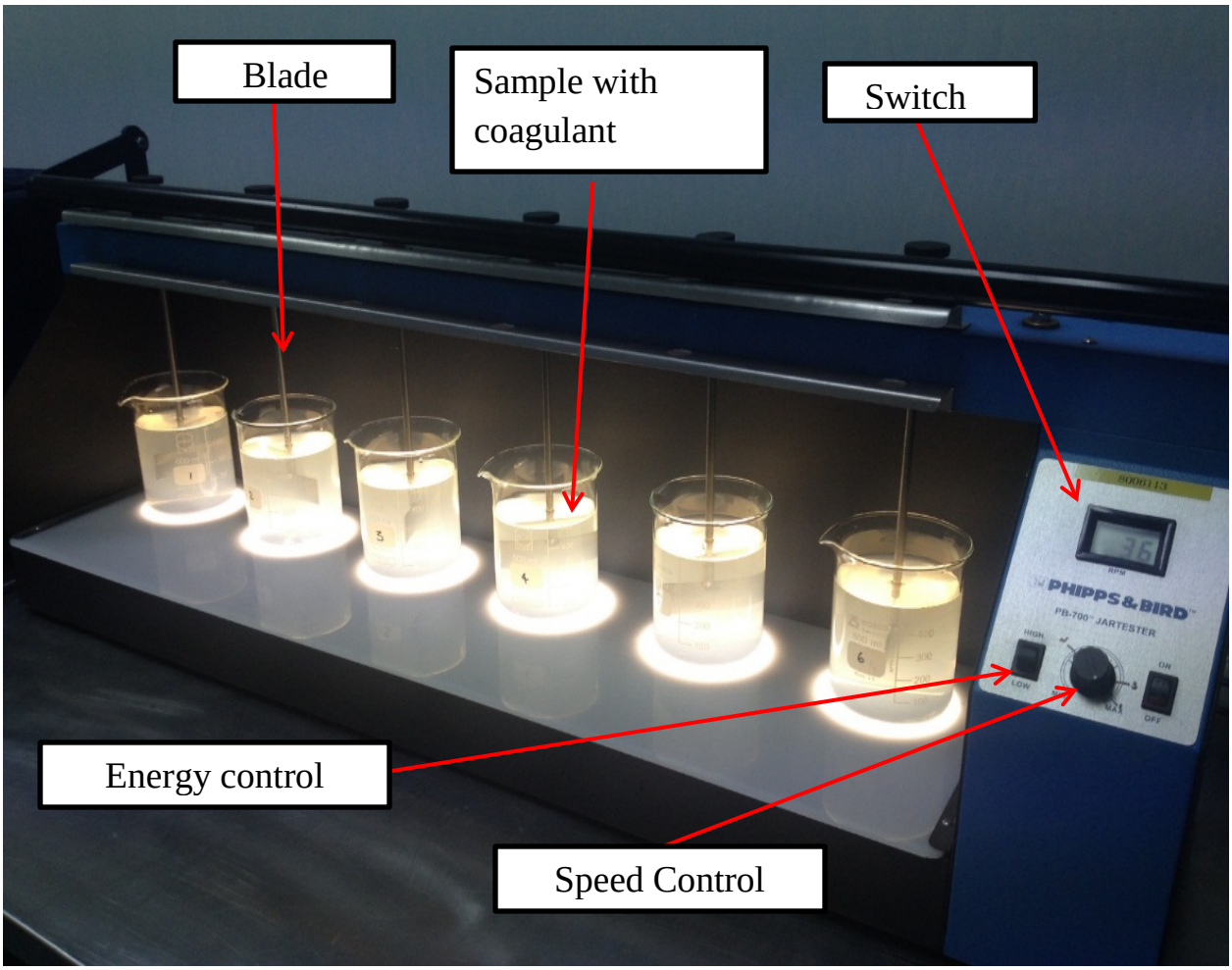

Figure 10: Phipps and Bird Jar test apparatus.

Table 4. Protocol for the low and high energy jar test with 1 chemical.

Low energy jar test with one chemical			**High energy jar test with one chemical**		
Action	**Time**	**RPM**	**Action**	**Time**	**RPM**
a) Mix raw water sample	15 s	120	a) Mix raw water sample	30 s	300
b)Add chemical and stir (Polyelectrolyte)	15 s	120	b) Add chemical and stir (Polyelectrolyte)	15 s	300
c) Turn down energy	8 min	60	c) Turn down energy	30 s	200
d) Switch off stirrer, remove beakers	-	-	d) Turn down energy	8 min	60
e) Allow flocs to settle	15 min	-	e) Turn down energy	90 s	30
			f) Switch off stirrer, remove beakers	-	-
			e) Allow flocs to settle	15 min	-

Table 5. Protocol for the low and high energy jar test with 2 chemicals.

Low energy jar test with two chemicals			**High energy jar test with two chemicals**		
Action	**Time**	**RPM**	**Action**	**Time**	**RPM**
a) Mix raw water sample	15 s	120	a) Mix raw water sample	30 s	300
b) Add first chemical and stir (Activated silica)	15 s	120	b) Add first chemical and stir (Activated silica)	15 s	300
c) Add second chemical and stir (Lime)	15 s	120	c) Add second chemical and stir (Lime)	15 s	300
d) Turn down energy	8 min	60	d) Turn down energy	30 s	200
e) Switch off stirrer, remove beakers	-	-	e) Turn down energy	8 min	60
f) Allow flocs to settle	15 min	-	f) Turn down energy	90 s	30
			g) Switch off stirrer, remove beakers	-	-
			h) Allow flocs to settle	15 min	-

Table 6. Protocol for the low and high energy jar test with 3 chemicals.

Low energy jar test with three chemicals			**High energy jar test with three chemicals**		
Action	**Time**	**RPM**	**Action**	**Time**	**RPM**
a) Mix raw water sample	15 s	120	a) Mix raw water sample	30 s	300
b) Add first chemical and stir (Activated silica)	15 s	120	b) Add chemical and stir (Activated silica)	15 s	300
c) Add second chemical and stir (Lime)	15 s	120	c) Add second chemical and stir (Lime)	15 s	300
d) Add third chemical and stir (Ferric chloride)	15 s	120	d) Add third chemical and stir (Ferric chloride)	15 s	300
e) Turn down energy	8 min	60	e) Turn down energy	30 s	200
f) Switch off stirrer, remove beakers	-	-	f) Turn down energy	8 min	60
g) Allow flocs to settle	15 min	-	g) Turn down energy	90 sec	30
			h) Switch off stirrer, remove beakers	-	-
			i) Allow flocs to settle	15 min	-

3.5 Preparation for sand filtration experiments.

3.5.1 Preparation of water samples for sand filtration.

Clarified water samples for sand filtration were taken after settling from sedimentation tanks at the Vereeniging Water Treatment plant. The sample was transported to the laboratory and allowed to reach room temperature. The turbidity and the temperature of the samples were measured. A 20 L sample was thoroughly mixed by inverting the container 4-5 times. From the 20 L sample, six beakers were each filled with 2 L of the sample. Before experiments, 10 ml of the sample was taken from each beaker to test for the presence of any bacteriophages in the raw water. Five beakers were spiked with phi X174 at 100 pfu/ml or MS2 at 500 pfu/ml. A sixth beaker served as a negative control, and the sample was not spiked. Samples were taken from the beakers to determine the initial bacteriophage counts.

3.5.2 Sand filtration procedure.

Sand filtration was simulated using the Phipps & Bird sand filtration unit (Fig.11). Before filtration, the upper-end caps from filter columns were removed. The filter screens were correctly placed at the bottom of the filter columns to prevent fine sand from escaping. Each filter column was loaded with granular media in the following order: (i) rounded stone (bottom layer), (ii) fine grit (middle layer) and (iii) fine sand (top layer). Each column was packed at a depth of 90 cm with rounded stone, 60 cm with fine grit and 350 cm with fine sand. The sizes of the granular media were 6-12 mm (rounded stone); 2-5 mm (fine grit) and 0.7 mm (fine sand). After initial packing of media, the columns were backwashed 4-6 times to remove the fine sand which may have influenced the turbidity. Once the effluent was clear and the granular media clean the experiments were initiated. The jar tester was placed on top of the sand filtration unit. Six 2 L

beakers containing non-spiked samples were connected to the column filters. The filter columns were conditioned with the non-spiked 2L sample three times before the experiment.

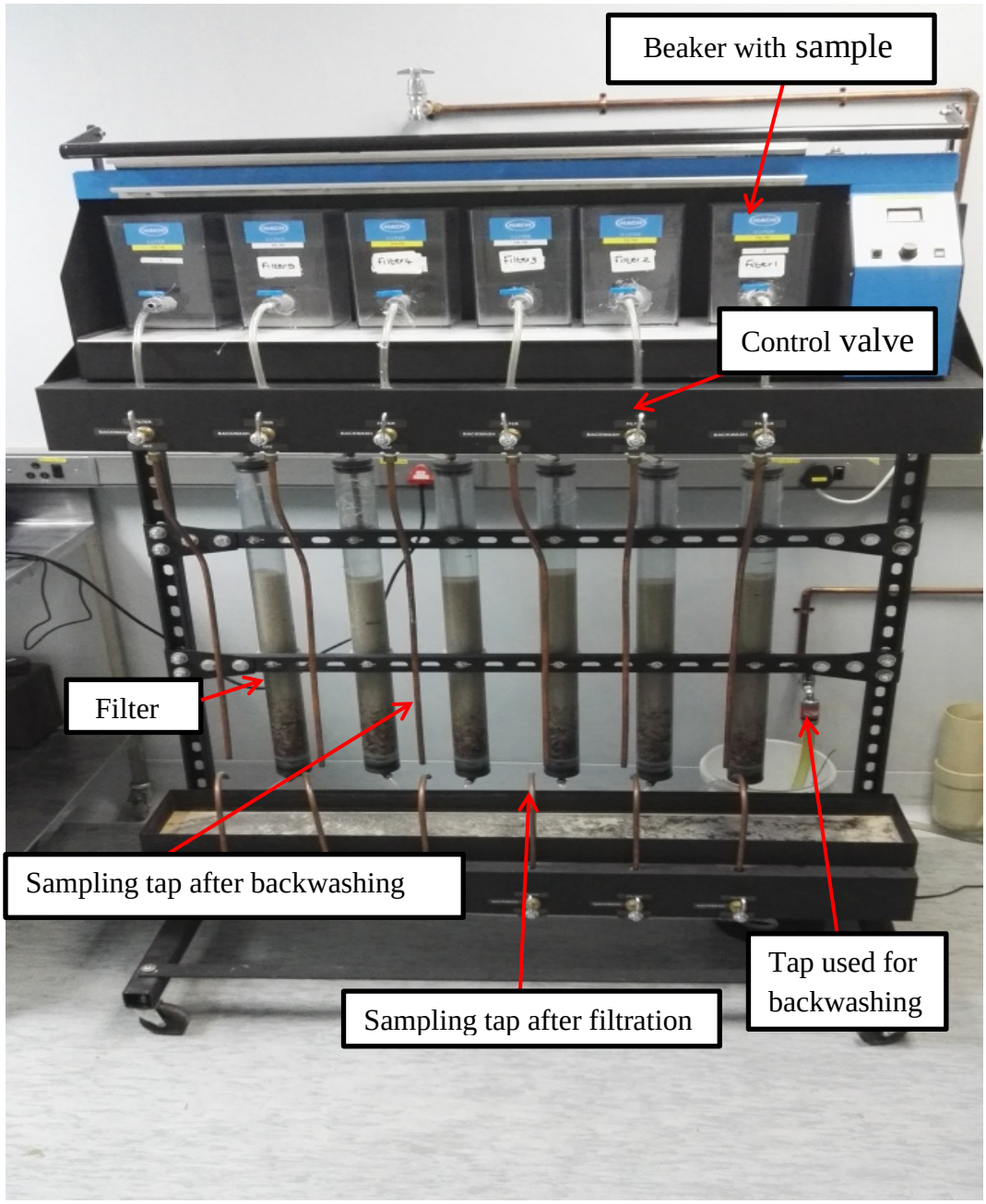

Figure 11: Phipps & Bird sand filtration unit.

After that, five 2 L beakers with sample were spiked with a concentration of ± 100 pfu /ml phi X174 or ± 500 pfu /ml MS2 and stirred for 15-20 minutes. The 6th beaker served as a negative

control and was not spiked. All the samples were filtered through the sand filtration columns, and 1 L glass beakers were placed at the end of the hook-shaped copper tubes to allow a filtered water sample to be collected from each filter column. Samples were collected with 50 ml syringes approximately 20 cm below the surface. After filtration, filter columns were backwashed by pumping water from the tap up through the sand filter. At the top of the filter column, the dirty water flowed out through the inlet pipe and into the drain. A backwashing cycle was done for a minimum of five minutes with tap water and rinsed with distilled water after each test procedure. This was done to clean the filter columns. Samples were taken after backwashing and later analysed to ensure that no viruses were left in the columns after backwashing that might affect the next filtration cycle. The columns were filled with sterile distilled water and re-packed by gently tapping the side of the columns. All the samples collected were assayed for bacteriophages as described in section 3.2

3.6 Preparation for disinfection experiments

3.6.1 Preparation of water samples for disinfection

Glass sample bottles were treated with a solution of water and 5 mls of 3.5% m/v sodium hypochlorite overnight. This was done to make the sample bottles chlorine demand free. Before sampling, the sample bottles were rinsed three times with distilled water and tested for the presence of chlorine using the DPD method, which is a colorimetric methodology using a DR/890 colorimeter. One hundred milliliters' of chlorinated water samples were collected from a water pumping station in Vereeniging and transported to the laboratory where it was stored until it reached the appropriate temperature (13ºC or 23ºC) to be tested. Before the experiments, the temperature of the samples was measured**.**

3.6.2 Disinfection experiments

Three 100 ml samples were placed in a water bath set at minimum (13ºC) or maximum (23ºC) temperatures for the study area. One sample was for spiking, the second sample served as a negative control and was not spiked, and the third sample was used to monitor the temperature. Before simulation of disinfection, a 10^{-3} dilution of phi X174 or MS2 was prepared. Three 1 ml samples were then withdrawn for assessment of the bacteriophage concentration before disinfection. Once the desired temperature was reached, the sample was spiked with 1 ml of the 10^{-3} dilution of phi X174 or MS2. Samples were taken at predetermined contact times (2, 5 and 10 minutes) for measurement of the residual chlorine and bacteriophages as described in section 3.2. Samples were collected in sterile 50 ml tubes containing 8 µl sodium thiosulfate to neutralize the chlorine and ensure the integrity of the bacteriophages.

3.7 Statistical analysis of data

The statistical technique or model used for this study was the t-test. A t-test is used to determine whether there is a significant difference between the means of two groups. Differences were considered significant if the *p* value was less than 0.05 at a 95% confidence level. All assays for this study were performed in triplicate. The average plague counts of triplicate plates prepared from one sample were regarded as the bacteriophage concentration.

CHAPTER 4

4. Results

4.1 Introduction

This study aimed to determine the effectiveness of clarification, sand filtration and disinfection for the removal of viruses in drinking water. Jar tests were performed to simulate clarification while sand filtration was simulated using a column filtration system. Disinfection was also simulated at a bench scale. The chemicals that were used for clarification were polyelectrolyte (Sudfloc 3835), lime, activated silica and ferric chloride. Jar tests were performed using a high and low energy regime. Sand filtration tests were performed using a combined water treatment apparatus, and disinfection was performed using free chlorine. This study also compared the removal of two bacteriophages, namely, phi X174 and MS2.

4.2 Log removal efficiency for the water treatment process barriers

Before considering treatment efficiency, it is first necessary to review the format for expressing pathogen removal efficiency (i.e., log reduction). Essentially each log reduction represents the removal of 90% of the pathogens (phi X174 and MS2) present. The relationship between log reduction efficiencies, the percentage removal and the absolute number of pathogens removed is assessed below:

- 1-log reduction = removal of 9000 out of 10000 microbes = 90% reduction
- 2-log reduction = removal of 9900 out of 10000 microbes = 99% reduction
- 3-log reduction = removal of 9990 out of 10000 microbes = 99.9% reduction
- 4-log reduction = removal of 9999 out of 10000 microbes = 99.99% reduction

4.3 Bacteriophage count in raw water before spiking

Before spiking the raw water for jar test experiments, each sample was tested for the presence of bacteriophages prior to spiking (Tables 7 and 8). The results for the initial bacteriophage count before spiking with phi X174 are shown in Table 7.

Table 7. Bacteriophage results of the raw water before spiking with phi X174

Date(s) on which sample was collected	Dates on which sample was used in experiments		Initial bacteriophage count prior to spiking (pfu/ml)
19/10/2016	19/10/2016	19/10/2016	0
21/11/2016	21/11/2016	21/11/2016	0
17/11/2016; 23/11/2016	17/11/2016	23/11/2016	0

Table 8 shows similar results for MS2.

Table 8. Bacteriophage results of the raw water before spiking with MS2

Date (s) on which sample was collected	Dates on which sample was used in experiments		Initial bacteriophage count before spiking (pfu/ml)
14/12/2016; 15/12/2016	14/12/2016	15/12/2016	0
11/01/2017; 16/01/2017	11/01/2017	16/01/2017	0
17/01/2017; 18/01/2017	17/01/2017	18/01/2017	0

4.4. Jar tests using polyelectrolyte (sudfloc 3835)

The raw water samples used in the study had turbidities that ranged between 76.5 NTU and 139.0 NTU and a temperature range between 22º C and 24º C. The samples were spiked with phi X174 and MS2, respectively, and jar tests were performed at either high or low energy regimes to determine the turbidity reduction and log removal of the bacteriophages. The high energy regime involves initial rapid mixing of the sample and coagulant at 300 rpm (Tables 4, 5 and 6).

The low energy regime involves initial slow mixing of the sample and coagulant at 120 rpm (Tables 4, 5 and 6).

4.4.1 Turbidity reduction using polyelectrolyte (sudfloc 3835)

The jar test simulations showed that an increase in polyelectrolyte concentration resulted in a decrease in turbidity of the raw water spiked with either phi X174 or MS2.

Table 9. Turbidity reduction using polyelectrolyte (sudfloc 3835) for high and low energy regimes

Jar number	Concentration of Polyelectrolyte (mg/L)	Turbidity reduction in phi X174 spiked samples		Turbidity reduction in MS2 spiked samples	
		Low energy	High energy	Low energy	High energy
		% Mean [a]	% Mean [a]	% Mean [a]	% Mean [a]
1	2	69.81 ± 0.07***	74.55 ± 0.13***	79.57 ± 0.00***	75.36± 0.05***
2	3	81.40 ± 0.00	86.41 ± 0.07	82.88 ± 0.04	85.03 ± 0.05
3	4	86.18 ± 0.00	89.32 ± 0.02	92.29± 0.04	90.91 ± 0.05
4	5	91.72 ± 0.01	91.77 ± 0.03	94.75 ± 0.00	93.93 ± 0.02
5	6	93.23 ± 0.03***	93.34 ± 0.01***	96.53 ± 0.01***	94.11 ± 0.00***
6	0	0	0	0	0

***Represents significance level at p < 0.05 (comparison between tests done at a low and high energy regimen)**

**** Represents significance level at p < 0.001 (comparison between tests done at a low and high energy regimen)**

***** Represents significance level at p < 0.0001 (comparison between tests done at a low and high energy regimen)**

a = represents percentage reduction in turbidity, where 0% means no reduction and 100% mean total reduction in turbidity. The mean values are derived from one experiment performed in triplicate.

When the polyelectrolyte was used at a concentration of 2 mg/L the turbidity of the water spiked with phi X174 decreased by 69.81% ± 0.07 and 74.55% ± 0.13 in the low and high energy regimes, respectively. The observed difference in the percentage mean turbidity reduction between the low and high energy regimes at 2 mg/L was highly significant ($p<0.0001$ level).

The results also showed that when the polyelectrolyte concentration was increased to 3, 4, 5 and 6 mg/L, respectively, further decreases in the turbidity in phi X174 spiked raw water samples were observed (Table 9). There was a highly significant difference ($p<0.0001$ level) in the mean percentage turbidity reduction at 2 mg/L and 6 mg/L polyelectrolyte concentration in the phi X174 spiked samples. A similar observation was made in samples spiked with MS2 (Table 9). When polyelectrolyte was used at a concentration of 2 mg/L the turbidity decreased by 79.57% ± 0.00 and 75.36% ± 0.05 in the low and high energy regimes, respectively. The observed difference in the percentage mean turbidity reduction in water spiked with MS2 between the low and high energy regimes at 2 mg/L was highly significant ($p<0.0001$). Similarly, for the phi X174 spiked samples the turbidity decreased as the polyelectrolyte concentration increased.

4.4.2 Percentage and log removal of phi X174 and MS2 using polyelectrolyte

This study assessed the ability of a polyelectrolyte to remove either phi X174 or MS2 from a raw water sample. The results showed that the two phages were removed with varying efficiencies. When polyelectrolyte was used at a concentration of 2 mg/L (Jar 1) there was an 85.33% ± 0.70 and 79.06 % ± 0.48 reduction in MS2 particles in the jar tests at a low and high energy regime, respectively (Fig. 12 A and B). When the concentration of polyelectrolyte was increased to 3, 4, 5 and 6 mg/L a further decrease in MS2 particles was observed (Fig. 12 A and B). The most significant decrease in MS2 was observed at a concentration of 6 mg/L (Jar 5). The efficiency with which MS2 was removed in Jar 5 was similar for both the low and high energy regimes.

When the polyelectrolyte was used at a concentration of 2 mg/L (Jar 1), there was a 15.63 % ± 4.94 and 28.38 % ± 4.62 reduction in phi X174 particles in jar tests operated at the low and high energy regimes, respectively (Fig. 12 C and D). The same figures showed that when the concentration of polyelectrolyte was increased to 3, 4, 5 and 6 mg/L a further decrease in phi X174 particles was observed.

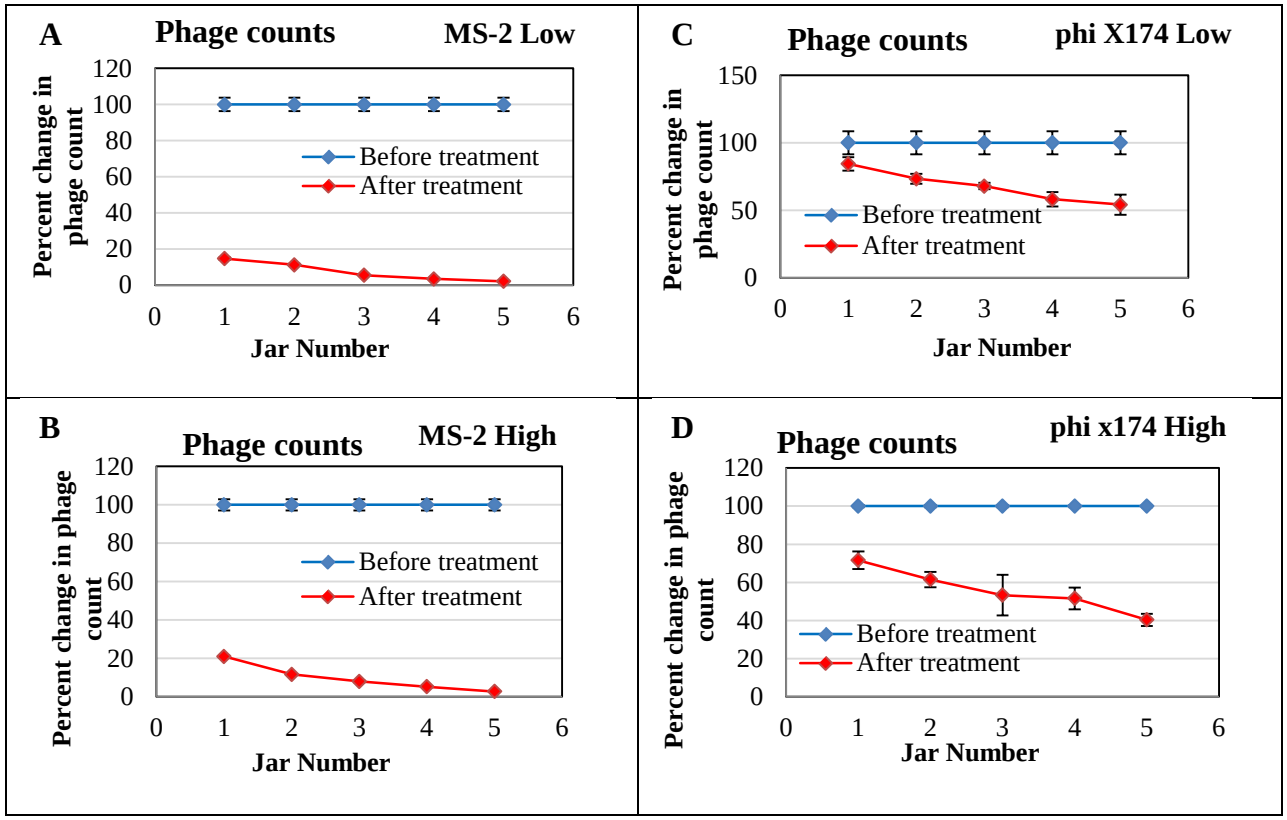

Figure 12. Jar test experiments for the removal of bacteriophages (%) using polyelectrolyte. A and B represents MS2 at low and high energy regimes. C and D represent phi X174 at low and high energy regimes. Measurements were done in triplicate. Where error bars are not visible, they are hidden by the markers.

Unlike phi X174, a contrast in the efficiency of removal of MS2 particles was observed. MS2 was removed effectively by polyelectrolyte from the lowest dose of 2 mg/ L. However phi X174 showed resistance at this dose. When the polyelectrolyte concentrations were increased, phi

X174 removal efficiency was still exceeded by MS2. The most significant decrease in phi X174 and MS2 was observed when the polyelectrolyte was used at a concentration of 6 mg/L (Jar 5). MS2 was reduced by 1.67 (low regimen) and 1.56 (high regimen) log units whereas phi X174 was reduced by 0.27 (low regimen) and 0.39 (high regimen) log units (Fig.13).

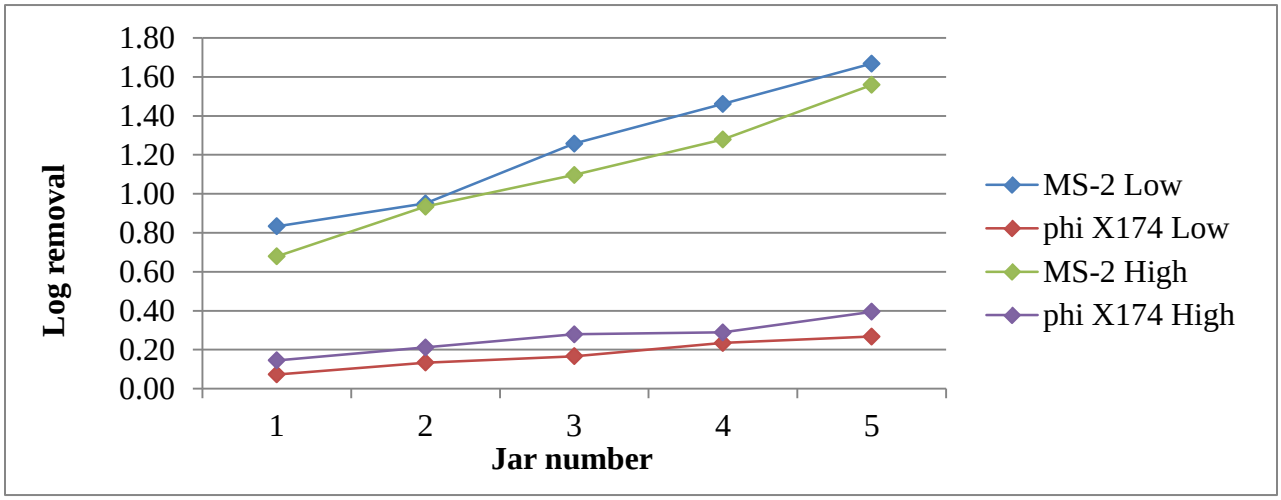

Figure 13. Log removal efficiency of MS2 and phi X174 using polyelectrolyte.

The results showed that the efficiency with which MS2 was removed by polyelectrolyte at all the different concentrations was significantly ($p<0.05$) higher to that observed for phi X174 under the same conditions (Fig. 12 and 13).

4.5 Jar tests using lime and activated silica

Combinations of lime and activated silica were used to determine the turbidity reduction and log removal of phi X174 and MS2 spiked water. During this study, the activated silica concentration was kept constant at 2 mg/L while the concentration of lime was varied between 20 to 100 mg/L.

4.5.1 Turbidity reduction using lime and activated silica

The jar test simulations showed that lime and activated silica efficiently decreased the turbidity of the raw water. When lime was used at a concentration of 20 mg/L the turbidity in phi X174

spiked samples decreased by 94.74 % ± 0.01 and 97.34 % ± 0.00 in the low and high energy regimes, respectively. The observed difference in the percentage mean turbidity reduction between the low and high energy regimes at 20 mg/L was highly significant (*p<0.0001*).

Table 10. Turbidity removal using lime and activated silica for high and low energy regimes

Jar number	Concentration of lime (mg/L)	Turbidity reduction in phi X174 spiked samples	Turbidity reduction in phi X174 spiked samples	Turbidity reduction in MS2 spiked samples	Turbidity reduction in MS2 spiked samples
		Low energy	High energy	Low energy	High energy
		% Mean [a]	% Mean [a]	% Mean [a]	% Mean [a]
1	20	94.74± 0.00***	97.34± 0.00***	86.07± 0.00***	87.77± 0.12***
2	40	98.22± 0.04	98.79± 0.00	94.22± 0.01	96.52± 0.04
3	60	98.24± 0.04	98.73± 0.01	94.57± 0.00	96.83± 0.05
4	80	98.66± 0.00	97.62± 0.01	94.51± 0.00	97.66± 0.02
5	100	98.27± 0.01	98.01± 0.01	96.07± 0.00***	97.98± 0.02***
6	0	0	0	0	0

***Represents significance level at p < 0.05 (comparison between tests done at a low and high energy regimen)**

**** Represents significance level at p < 0.001 (comparison between tests done at a low and high energy regimen)**

***** Represents significance level at p < 0.0001 (comparison between tests done at a low and high energy regimen)**

a= Value represent % amount by which the turbidity was reduced. Where 0% means no reduction and 100% mean total reduction in turbidity. The mean values are derived from one experiment performed in triplicate.

The results also showed that when the lime concentration was increased to 40, 60, 80 and 100 mg/L a further but minimal decrease in turbidity was observed (Table 10).

A similar observation was made in samples spiked with MS2. When lime was used at a concentration of 20 mg/L the turbidity due to MS2 decreased by 86.07% ± 0.00 and 87.77 % ± 0.12 in the low and high energy regimes, respectively. Also when the lime concentration was increased to 40, 60, 80 and 100 mg/L a further but minimal decrease in turbidity was observed (Table 10). The observed difference in the percentage mean turbidity reduction when lime was used at a low concentration of 20 mg/L (Jar 1) and a high concentration of 100 mg/L (Jar 5) in MS2 spiked samples was significant ($p<0.0001$) at either a low or high energy regime.

4.5.2 Percentage and log removal of phi X174 and MS2 using lime and activated silica

This study assessed the ability for a combination of lime and activated silica to remove either phi X174 or MS2 from the water. The results showed that both phages were removed with varying efficiencies. When lime was used at a concentration of 20 mg/L (Jar 1), there was a 63.01 % ± 2.47 and 85.95 % ± 0.63 reduction in MS2 particles in jar tests done at either a low or high energy regime respectively (Fig. 14 A and B). The reduction in MS2 particles translates into a 0.43 (low regimen) and 0.85 (high regimen) log removal (Fig. 15). The difference in mean removal of MS2 was highly significant (at a $p<0.0001$ level) in jar 1 with either a high or low regime. When the concentration of lime was increased to 40, 60, 80 and 100 mg/L a further decrease in MS2 particles was observed (Fig. 14 A and B).

When lime was used at a concentration of 20 mg/L (Jar 1), there was a 3.91% ± 1.03 and 11.21% ± 1.90 reduction in phi X174 particles in jar tests done at either a low or high energy regime, respectively (Fig. 14 C and D). The decrease in phi X174 translates in a 0.02 (low regimen) and

0.05 (high regimen) log removal (Fig. 15). The difference in mean removal of phi X174 was significant (at a $p < 0.05$ level) in jar 1 at either a low or high energy regime.

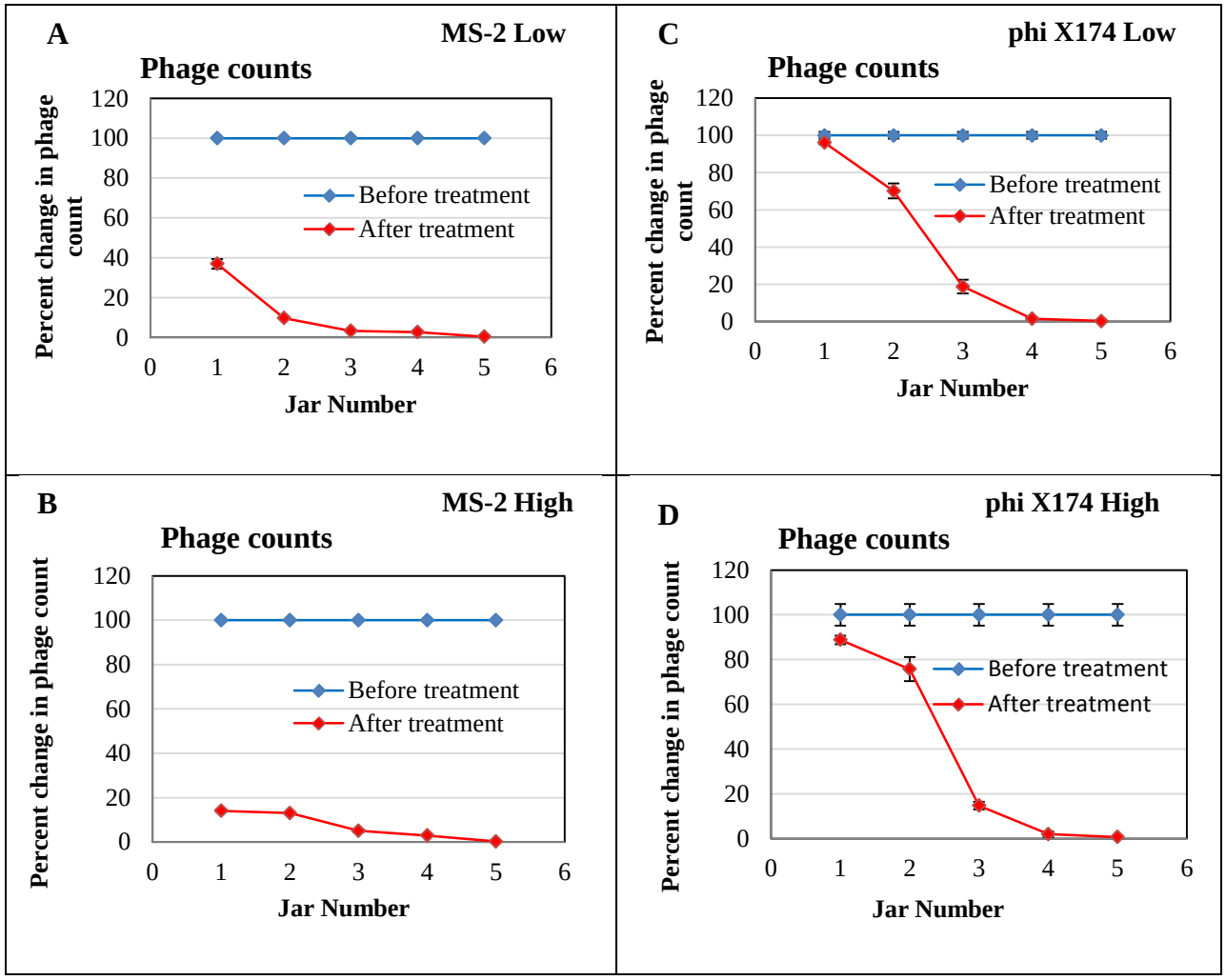

Figure 14. Jar test experiments for the removal of bacteriophages (%) using lime and activated silica. A and B represents MS2 at low and high energy regimes, and C and D represent phi X174 at low and high energy regimes. Measurements were conducted in triplicate. Where error bars are not visible, they are hidden by the markers.

When the concentration of lime was increased to 40, 60, 80 and 100 mg/L a further decrease in phi X174 particles was observed. The greatest decrease in phi X174 and MS2 was observed when lime was used at a concentration of 100 mg/L (Jar 5).

Jar 4 (80 mg/L) and Jar 5 (100 mg/L) had the highest lime concentrations, and both bacteriophages were removed at a similar rate (Fig. 15). Figure 13 shows that at lime concentrations below 80 mg/L phi X174 was removed less efficiently than MS2. At the lowest concentration (Jar 1), MS2 was reduced by 0.43 (low regimen) and 0.85 (high regimen) log units whereas phi X174 was reduced by 0.02 (low regimen) and 0.05 (high regimen). The observed difference in the log removals of the two phages at 20 mg/L lime concentration was highly significant (at p *<0.0001* level). Beyond a lime concentration of 80 mg/L a similar log removal was observed for both phages (Fig. 15).

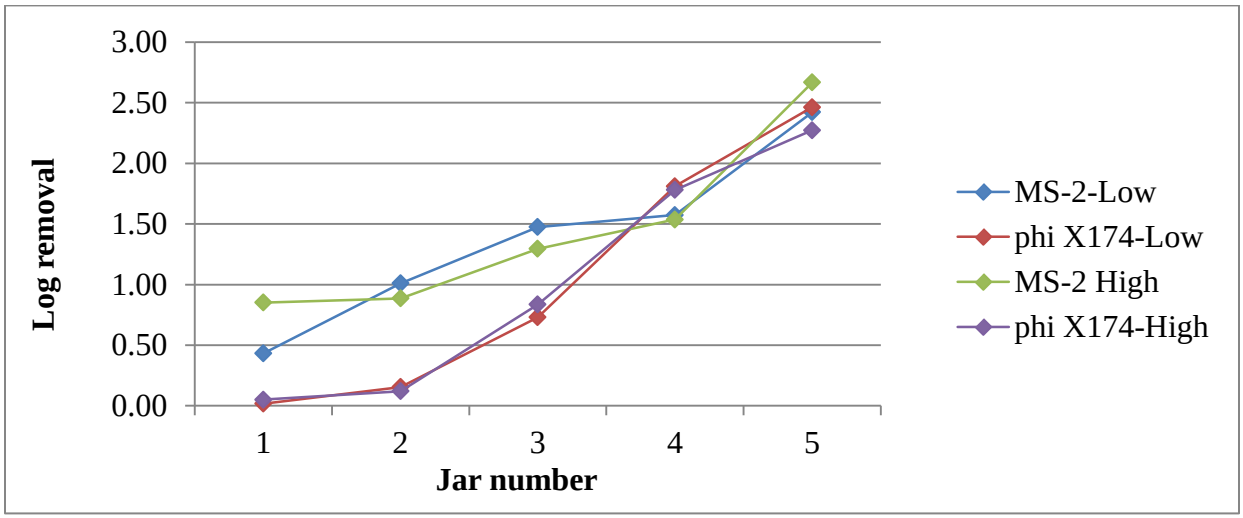

Figure 15. Log removal efficiency of MS2 and phi X174 using lime and activated silica.

4.6 Jar tests using lime, activated silica and ferric chloride

Combinations of lime, activated silica and ferric chloride were used to determine the turbidity reduction and log removal of phi X174 and MS2 spiked water. During this part of the study, the concentration of activated silica was kept constant at 2 mg/L while the concentration of lime was varied between 20 to 100 mg/L whereas that of ferric chloride was varied between 2 to 6 mg/L (Table 11).

4.6.1 Turbidity reduction using lime, activated silica and ferric chloride

The jar test simulations showed that a combination of lime, activated silica and ferric chloride efficiently decreased the turbidity of the raw water spiked with either phi X174 or MS2. When lime and ferric chloride were used at concentrations of 20 mg/L and 2 mg/L, respectively, the turbidity in phi X174 spiked samples decreased by 96.68 % ± 0.02 and 98.86 % ± 0.01 in the low and high energy regimes, respectively (Table 11).

The observed differences in the percentage mean turbidity reduction in phi X174 spiked samples between the low and high energy regimes when lime and ferric chloride were used at concentrations 20 mg/L and 2mg/L, respectively, was highly significant ($p<0.0001$). The results also showed that when the concentrations of both lime and ferric chloride were increased towards the maximum a further but minimal decrease in turbidity was observed (Table 11). A similar observation was made in samples spiked with MS2. When lime (20 mg/L) and ferric chloride (2 mg/L) were used, the turbidity decreased by 80.67% ± 0.13 and 93.91 % ± 0.01 in the low and high energy regimes, respectively.

Table 11. Turbidity removal using lime activated silica and ferric chloride

Jar number	Concentration		Turbidity reduction in phi X174 spiked samples		Turbidity reduction in MS2 spiked samples	
			Low energy	High energy	Low energy	High energy
	Lime(mg/L)	Ferric chloride (mg/ L)	% Mean [a]	% Mean [a]	% Mean [a]	% Mean [a]
1	20	2	96.68± 0.02***	98.86± 0.01***	80.67± 0.13***	93.91± 0.01***
2	40	3	96.87± 0.00	98.55± 0.01	95.24± 0.04	98.44± 0.03
3	60	4	98.50± 0.05	99.47± 0.01	95.99± 0.01	97.74± 0.09
4	80	5	98.02± 0.02	97.60± 0.00	96.68± 0.02	97.63± 0.02
5	100	6	97.87± 0.01	99.86± 0.00	95.18± 0.02***	97.82± 0.04***
6	0	0	0	0	0	0

***Represents significance level at p < 0.05 (comparison between tests done at a low and high energy regimen)**

**** Represents significance level at p < 0.001 (comparison between tests done at a low and high energy regimen)**

***** Represents significance level at p < 0.0001 (comparison between tests done at a low and high energy regimen)**

a= Value represent % amount by which the turbidity was reduced. Where 0% means no reduction and 100% mean total reduction in turbidity. The mean values are derived from one experiment performed in triplicate.

The observed difference in the percentage mean turbidity reduction when lime and ferric chloride were used at the lowest concentrations (Jar 1) and the highest concentrations (Jar 5) in MS2 spiked samples was highly significant ($p<0.0001$) at both low and high energy regimes. Similarly, with the phi X174 containing samples an increase in the concentrations of both lime and ferric chloride produced a further decrease in turbidity (Table 12).

4.6.2 Percentage and log removal of phi X174 and MS2 using lime, activated silica and ferric chloride

Different patterns of removal were observed for MS2 and phi X174 when the combination of lime, activated silica and ferric chloride were used. When lime (20 mg/L) and ferric chloride (2 mg/ml) were used, 89.63 % ± 0.51 and 70.62 % ± 3.33 MS2 particles were removed in the low and high energy regimes, respectively (Fig. 16 A and B). The reduction in MS2 particles translated to a 0.98 (low regimen) and 0.53 (high regimen) log removal (Fig. 17). The observed difference in the mean reduction of the two phages was highly significant ($p < 0.0001$) when lime (20 mg/L) and ferric chloride (2 mg/L) were used.

When lime (20 mg/L) and ferric chloride (2 mg/L) were used, 12.9 % ± 0.41 and 9.22 % ± 0.84 phi X174 particles were removed at low and high energy regimes, respectively (Fig. 16 C & D). .The decrease in phi X174 translated to a 0.06 (low regimen) and 0.04 (high regimen) log removal (Fig. 17). The difference in mean removal of phi X174 was significant (at a $p < 0.05$ level) in jar 1 at either a low or high energy regime. Despite the difference in phage removal efficiencies at low doses, it was found that above the 60 mg/L lime and 4 mg/L ferric chloride dose (Jar 3) almost complete removal was observed for both phages (Fig.16).

The energy regime appeared to have little impact on removal efficiencies with the exception of MS2 at low lime doses, where the low energy regime appeared to have facilitated a more effective removal (Fig. 16 A). To further assess the relationship between lime and ferric chloride concentration and phage removal, the data presented in Figure 16 was converted from percentage values to log removal values (Fig. 17).

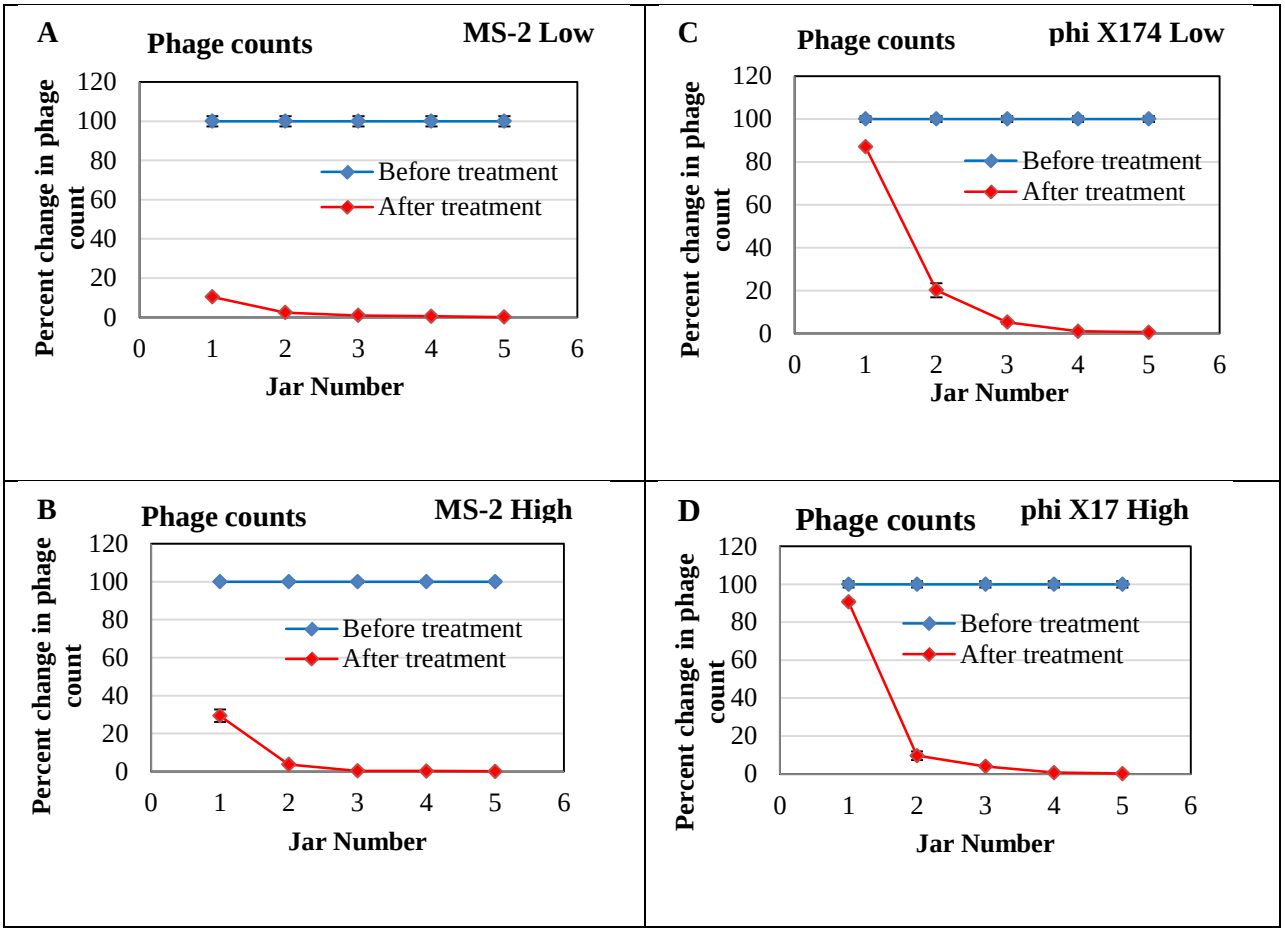

Figure 16. Jar test experiments for the removal of bacteriophages (%) using lime, activated silica and ferric chloride. A and B represents MS2 at a low and high energy regime, and C and D represent phi X174 at a low and high energy regime. Measurements were conducted in triplicate. Where error bars are not visible, they are hidden by the markers.

The use of lime, activated silica and ferric chloride at a dose of 100 mg/L lime (Jar 5) and 6 mg/L ferric chloride in the low and high energy regimes resulted in a log removal of 3.03 and 3.04 log units of MS2. On the other hand, phi X174 reduced by 2.27 and 2.56 log units at a concentration of 100 mg/L lime and 6 mg/L ferric chloride (Jar 5) at low and high energy regimes, respectively.

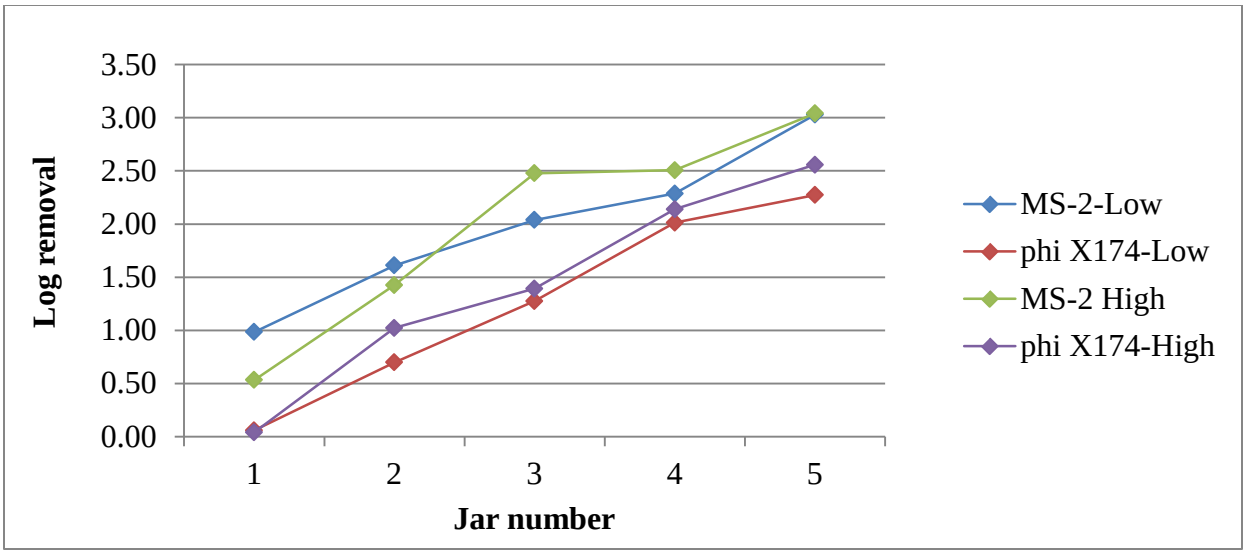

Figure 17. Log removal efficiency of MS2 and phi X174 using lime, ferric chloride and activated silica.

The observed difference in the log removal of MS2 and phi X174 when lime and ferric chloride were used at the highest concentrations was significant ($p<0.05$) at either a low or high energy regime (Fig. 17). A similarity in the log removal of both bacteriophages was observed only in jar 4 with a concentration of 80 mg/L lime and 5 mg/L ferric chloride (Fig. 16). The removal of both bacteriophages was between 2 and 2.5 log units.

4.7 Sand filtration simulation

This study aimed to determine the effectiveness of sand filtration for the removal of viruses in drinking water. The sand filtration simulation experiments were conducted using a Phipps and Bird combined water treatment systems. The sand filter columns were packed with different layers of sand (see section 3.5.2) and used to filter spiked samples. The temperature of the samples was kept between 22ºC –24ºC. The pH of the samples ranged from 7.6 to 8.6.

4.7.1 Percentage and log removal of phi X174 and MS2 using sand filtration

This study assessed the ability of sand filtration to remove either phi X174 or MS2 from the water after the clarification process. The results showed that the two phages were removed with varying efficiencies (Fig. 18).

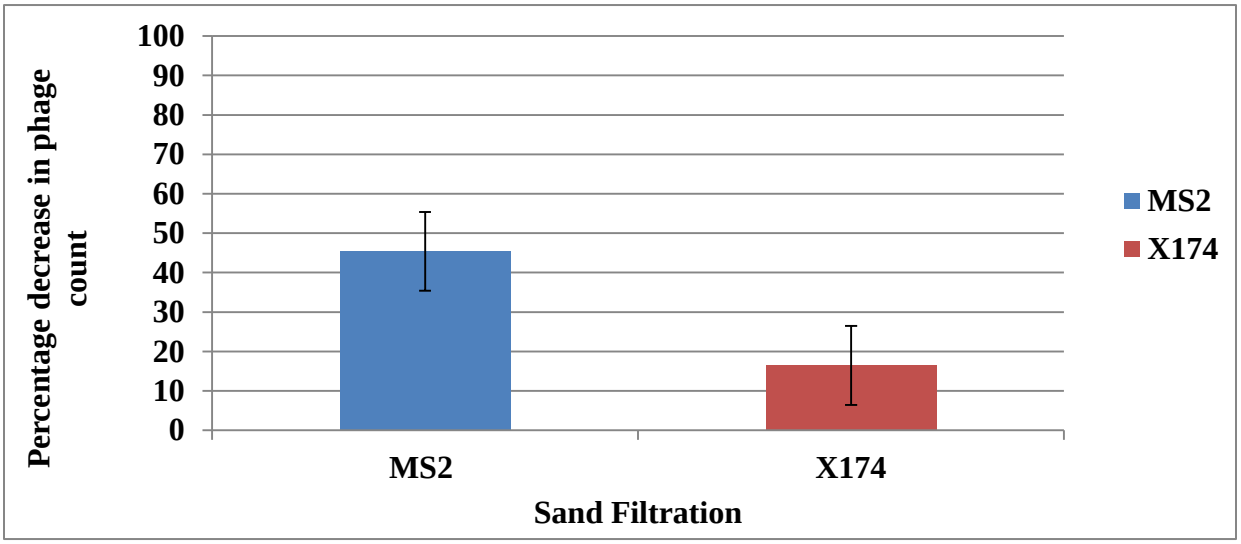

Figure 18. Bacteriophage removal (%) by sand filtration. Measurements were conducted in triplicate.

When samples spiked with MS2 were filtered through the sand columns a 45.41 % ± 7.30 reduction or a 0.26 log removal in MS2 particles was achieved (Fig. 19). However, a different efficiency in the removal is phi X174 was observed. When samples spiked with phi X174 were filtered through the sand columns, there was a 16.48 % ± 11.98 reduction or a 0.08 log removal of phi X174 particles. The difference in the reduction of the two phages by sand filtration was highly significant ($p < 0.0004$).

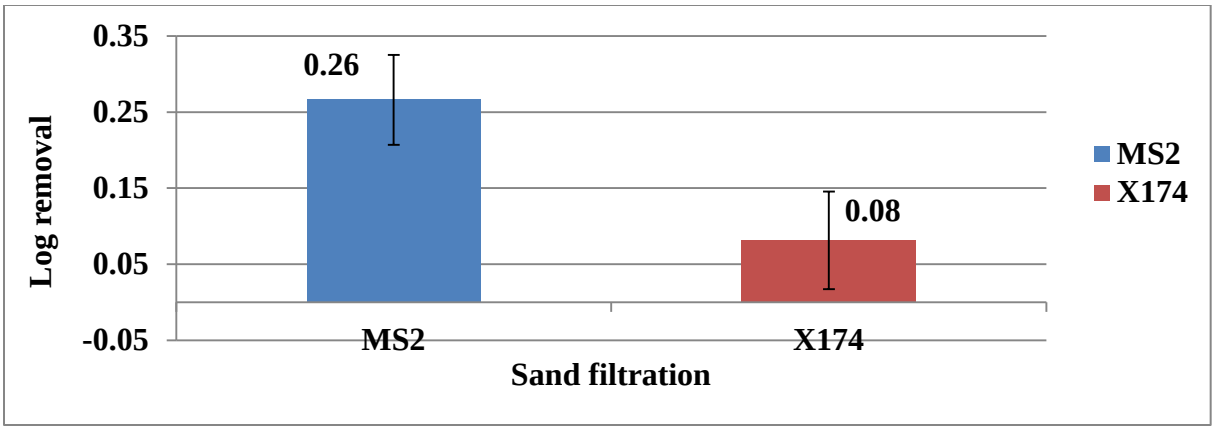

Figure 19. Bacteriophage removal (log removal) by sand filtration. Red represents phi X174, and blue represents MS2. Measurements were conducted in triplicate.

4.8 Disinfection simulation experiments

Bench-scale disinfection simulation tests were performed to determine the effectiveness of free chlorine to remove MS2 and phi X174. The experiments were conducted within a temperature range of 13ºC and 23ºC to also assess the effect of temperature on chlorine disinfection. The bacteriophage removal and the free chlorine concentration were tested after 2, 5 and 10 minutes of exposure to free chlorine.

4.8.1 Percentage and log removal of MS2 and phi X174 by disinfection

The results obtained from the disinfection experiments showed that phi X174 was no longer detectable after 2 minutes (Fig. 20) of exposure to chlorine at 13ºC. Furthermore, phi X174 was no longer detectable in exposures to chlorine that lasted longer than 2 minutes at 13ºC within the time range. However, the removal of MS2 was drastically different from that observed in disinfection experiments based on phi X174. The results showed that the removal of MS2 improved with time at 13ºC (Fig. 20).

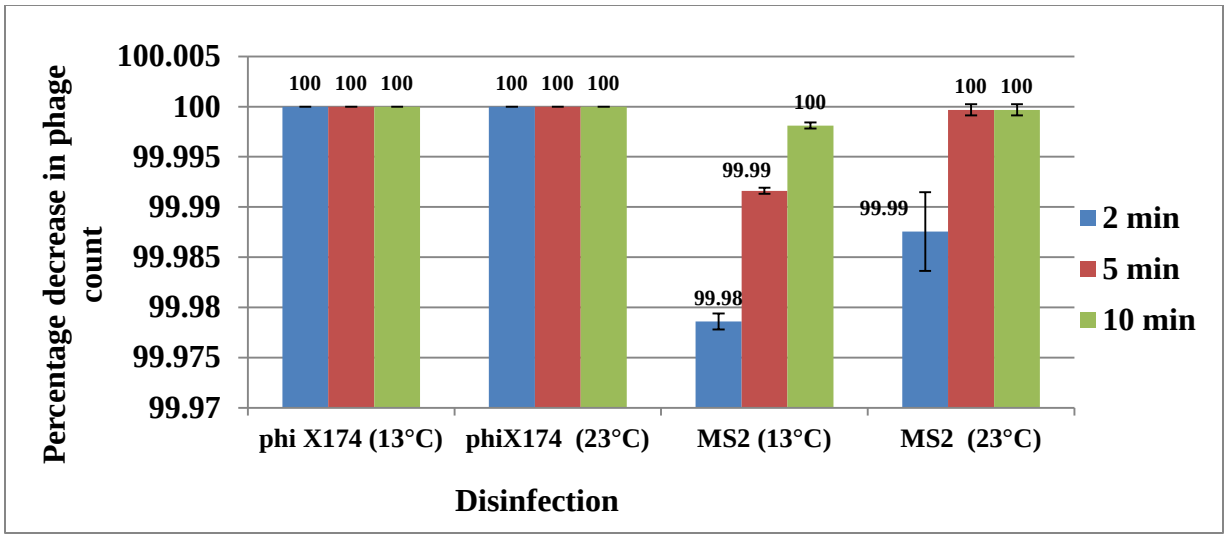

Figure 20. Bacteriophage removal (%) of MS2 and phi X174 by disinfection at 13ºC and 23 ºC. Measurements were conducted in triplicate. Where error bars are not visible, the standard deviation is very small.

The difference in reduction of the two phages at 13ºC after 2 minutes of disinfection was highly significant (*p >0.0001*). The removal (100% or 5.34 and 5.31 log reduction) of both phi X174 and MS2 at 23ºC was similar when disinfection was allowed to proceed for 5 and 10 min (Fig. 21). Although a disinfection period of 2 min at 23ºC resulted in the total reduction of phi X174, it was insufficient to cause a 100% reduction in MS2.

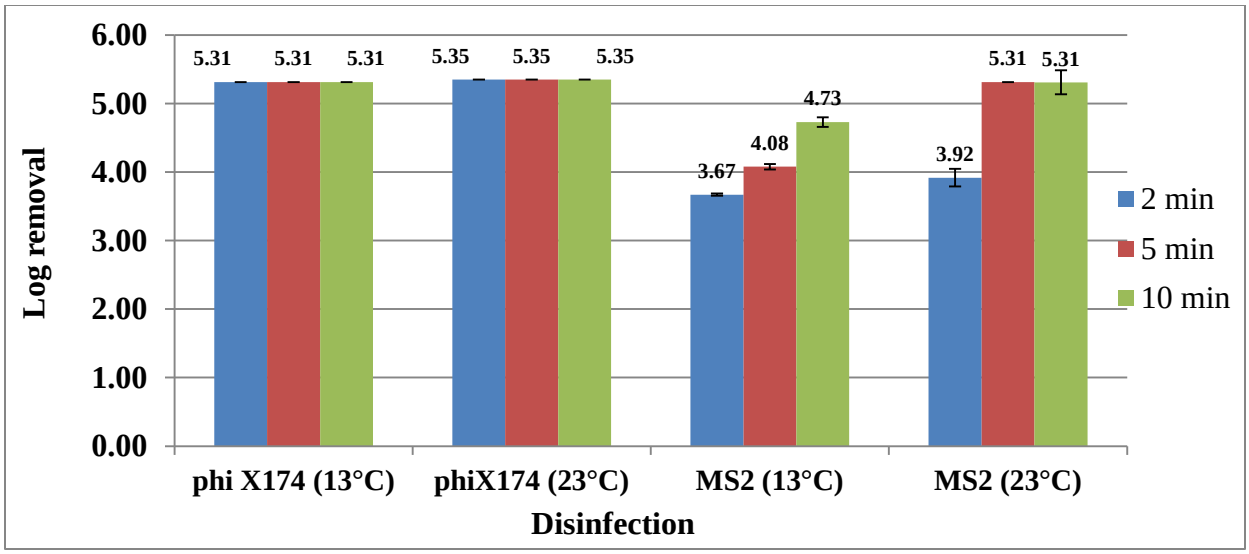

Figure 21. Bacteriophage removal (log removal) of MS2 and phi X174 by disinfection at 13ºC and 23 ºC. Blue represents 2 minutes, red represents 5 minutes and green reperesents 10 minutes.

Due to the inability for the disinfection process to remove MS2 with the same efficiency observed in experiments consisting of phi X174, the duration of disinfection was increased to 20 and 30 minutes. The results showed complete removal of MS2 after 20 and 30 minutes of disinfection (Fig. 22).

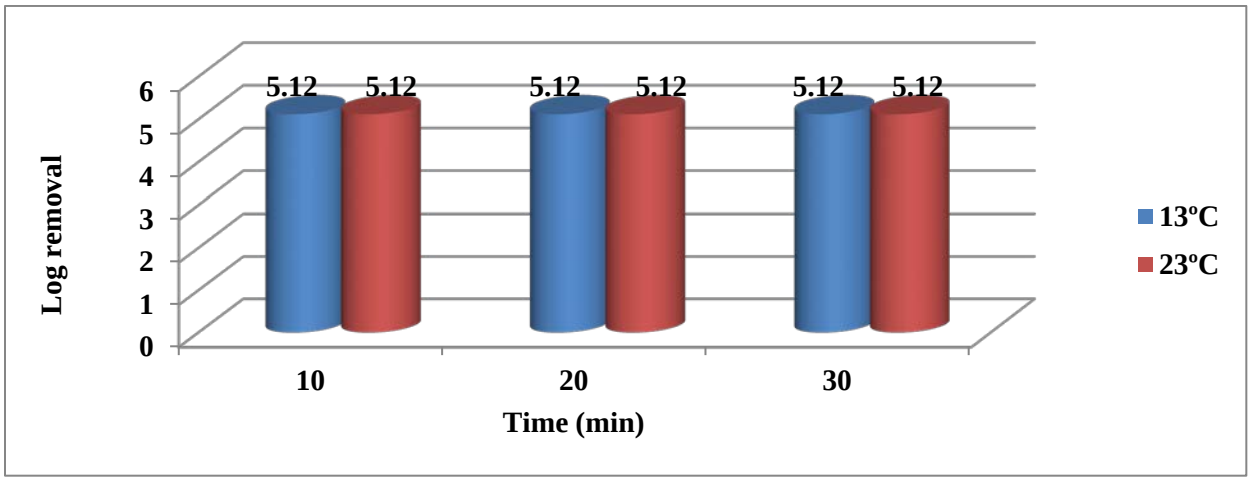

Figure 22. Log removal of MS2 at 13 ºC and 23ºC.

4.9 An overview of the different treatment process barriers in removing viruses during water treatment

The results obtained from this study showed that each barrier in the water treatment process removed or inactivated viruses differently. Clarification is the first barrier in the water treatment process. During clarification, the most effective virus removal or inactivation was achieved using the combination of lime activated silica ferric chloride. This combination removed or inactivated both phages by approximately 3 log units (Fig. 23). Polyelectrolyte treatment was the least effective in removing or inactivating viruses.

Sand filtration is the second barrier in the water treatment process. The results showed that this barrier was ineffective for the removal of viruses. A log removal of > 1 log unit was achieved for both phages (Fig. 23) during sand filtration.

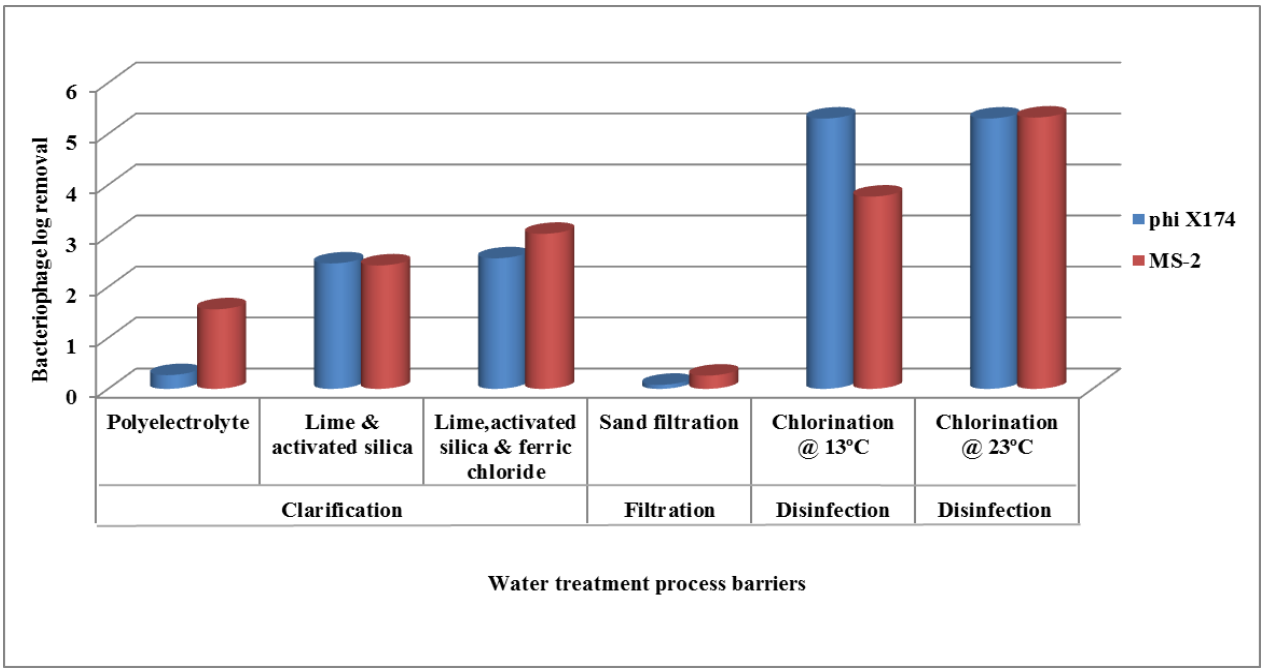

Figure 23. Bacteriophage log removal of phi X174 and MS-2 during the water treatment process. Bacteriophage log removal efficiencies result for clarification at optimum doses.

The final water treatment barrier studied was disinfection. Disinfection removed or inactivated both phages by 3, 5- 5 log units (Fig. 22) at a low and high temperature.

4.10 Conclusion

The results showed that each barrier of the water treatment process was able to remove viruses. The extent to which each barrier removed viruses varied and this due to the difference in the effectiveness of each barrier to remove viruses. The results also showed that sand filtration was the least effective barrier and disinfection was the most effective barrier for the removal of viruses in drinking water.

CHAPTER 5

5. Discussion

5.1 Introduction

The presence of pathogenic enteric viruses in water remains a point of significant concern, and therefore it is essential to monitor the efficiency with which these viruses are removed during the different water treatment steps. This study assessed the effectiveness as well as the efficiency with which the water treatment process barriers can remove viruses from source water on a bench scale. In this study, bacteriophages phi X174 and MS2 were used as surrogates for enteric viruses.

5.2 The effectiveness of clarification for the removal of viruses

Clarification, the first barrier of the water treatment process involves coagulation. This study assessed the ability of three different chemicals: (i) Polyelectrolyte (SUDFLOC 3835), (ii) a combination of lime and activated silica and, (iii) a combination of lime, activated silica and ferric chloride to reduce turbidity as well as remove viruses. Jar tests were used to simulate clarification. The jar tests were operated at low (slow mixing speed) and high (rapid mixing speed) energy regimes.

5.2.1 Polyelectrolyte treatment efficiency to reduce turbidity and remove viruses

The initial clarification experiments were conducted using a polyelectrolyte. The polyelectrolyte has been shown to form hydrogen bonds with particulate matter suspended in water thereby forming flocs during coagulation (Pongchalermporn, 2002). Coagulation, especially with polyelectrolytes, is more effective at high energy in the initial stages of mixing in comparison to low energy. High energy enables colloidal particles to come into contact with the coagulant in a

short time period. This results in the formation of flocs between the polyelectrolyte and the particulate matter in the raw water. Once flocs are formed they settle down through a process called sedimentation thus reducing the turbidity of raw water.

In this study, the polyelectrolyte (SUDFLOC 3835) was added at different concentrations to raw water spiked by either phi X174 or MS2. The results showed that turbidity decreased as the concentration of the polyelectrolyte increased (Table 9). The highest reduction in turbidity was achieved at a concentration of 6 mg/L. At this concentration, the reduction in turbidity for samples spiked with X174 and MS2 was 93.23 ± 0.01% and 96.53 ± 0.01%, respectively.

The turbidity reduction observed for the high and low energy regimes at a coagulant dose of 6 mg/L was not equivalent. The low energy regimen was able to significantly ($p<0.0001$) reduce the turbidity of raw water spiked by either phi X174 or MS2 when compared to the high energy regimen (Table 9). The lower mixing speed may have enhanced the removal of particles due to reduced shearing of flocs as they were being formed.

The extent to which the polyelectrolyte removed phi X174 and MS2 was also assessed. The results showed that increasing concentrations of polyelectrolyte removed a higher proportion of the phages from the test samples (Fig. 11). As was the case with turbidity, the maximum removal of phages was achieved with a polyelectrolyte concentration of 6 mg/L. At this concentration, a 1.67 and 0.39 log reduction was observed for MS2 and phi X174, respectively (Fig. 12).

The results indicated that MS2 was more effectively removed than phi X174 (Fig. 11). These results are similar to the findings of reported studies that showed that male-specific bacteriophages such as MS2 are removed with higher efficiency in comparison to somatic bacteriophages such as phi X174 (Mayer et al., 2007). One of the characteristics that may have

contributed to this phenomenon is the hydrophobicity of the virus particles. Dika et al. (2015) reported that MS2 is more hydrophobic than phi X174. Previous studies on NOM (natural organic matter) have shown that hydrophobic fractions are more effectively removed than hydrophilic fractions during coagulation (Matilainen et al., 2010). Thus, virus particles that have a more hydrophobic surface can be expected to be removed with higher efficiency than viruses having a less hydrophobic surface (Shirasaki et al., 2016).

The results from this study also suggest that MS2 may not be an appropriate surrogate to use for the assessment of the removal of viruses by coagulation. This is because MS2 was removed more effectively than phi X174 and may therefore provide overestimates of the effectiveness of coagulation for virus removal. Since phi X174 appears to be more resilient to removal, it may be a more appropriate surrogate to use where conservative estimates of virus removal are required.

It was also noted that there was a difference in the extent to which turbidity and phages were removed by the polyelectrolyte (Table 9 & Fig. 11). While this study showed that the polyelectrolyte could effectively reduce the turbidity, it inactivated or removed the phages to a lesser extent. This shows that even though treatment with the polyelectrolyte may appear to be effective in decreasing the turbidity, it does not necessarily mean that viruses have been removed. Shirasaki et al. (2016) also reported that a study by Rao et al. (1988) found that turbidity did not influence virus removal. Furthermore, studies in the literature have shown that various polyelectrolytes perform poorly in the presence of colour, turbidity, and COD (chemical oxygen demand) (IWA, n.d.). This may be a reason for the low bacteriophage removal relative to turbidity observed in this study.

Overall the amount by which phages were removed during this study was broadly consistent with the values reported in previous studies that showed that clarification could remove up to 74% of viruses (Obi et al., 2008).

5.2.2 The combination of lime and activated silica efficiency to reduce turbidity and remove viruses

In this study, clarification was conducted using lime and activated silica. Lime was used as the coagulant, and the activated silica was the coagulant aid. Activated silica is added first followed by the addition of lime. The role of activated silica is to increase the weight of the flocs and to strengthen them to avoid breaking during sedimentation (Leopold & Freese, 2009).

For this study the combination of lime and activated silica was added to raw water spiked with either MS2 or phi X174. The lime concentrations ranged from 20 mg/L to 100 mg/L. The activated silica concentration was kept constant at 2 mg/L. The turbidity reduction and phage removal or inactivation increased with an increase in lime concentration. This was most likely due to an increase in the number of flocs formed at higher lime concentrations. As the flocs formed and settled out they trapped and removed phages from the water column and decreased its turbidity.

The findings from this study showed that even low doses of lime (i.e., 20 mg/L) achieved over 86% reduction in turbidity in the raw water spiked with either of the surrogate phages. Lime doses of 80 to 100 mg/L achieved turbidity reductions of over 97% samples with both phages (Table 10). Turbidity reduction was less effective at lime doses exceeding 100 mg/L (Table 10). At lime doses of 100 mg/L, the increased turbidity may have resulted from residual lime particles remaining in suspension.

The relationship between the energy regime and turbidity removal was also assessed. It was found that at low lime concentrations the high energy regime was slightly more effective for turbidity removal than the low energy regime. While this was found to be statistically significant the difference in performance was relatively small and may have no practical relevance. At high lime doses, the high-energy regime was slightly more effective for the removal of turbidity than the low energy regime (Table 10). The difference that was observed between the high and low energy regimes was also relatively small.

The lime and activated silica was also assessed for the removal of viruses from the raw water spiked with either phi X 174 or MS2. The results showed a highly significant ($p<0.0001$) removal of MS2 at low lime doses of 20 mg/L (Fig.13). These results are similar to the observations made in the study with the polyelectrolyte coagulant (Section 5.2.1). The differences in hydrophobicity between MS2 and phi X174 may have contributed to their removal efficiency in this study. At the highest lime concentration (100 mg/L) an equivalent removal of over 2 log units was observed for both phages (Fig.14).

The energy regime used did not appear to influence virus removal. At a lime concentration of 100 mg/L, there were a 2.42 and 2.67 log unit removal of MS2 at the low and high energy regimes, respectively (Fig.14). In addition, at this concentration, the removal of phi X174 was 2.46 and 2.27 log units at the low and high energy regimes, respectively. Therefore, when lime is used as a coagulant for virus removal, there is a need to use the high energy regime.

This study showed that the combination of lime and activated silica is effective for the reduction of turbidity and removal or inactivation of viruses. The ability of lime to reduce turbidity in this study is supported a by previous studies that have shown that lime treatment can aid in the

clarification of turbid waters (Freese & Hodgson, 2004). The substantial log removal of both phages in this study may be attributed to the hydroxide alkalinity of the lime that has an antimicrobial effect and causes microbial inactivation. Additionally, previous studies have reported that lime treatment also reduces the number of microorganisms by flocculation in sedimentation (Grabow et al., 2000). Overall the log removals achieved for MS2 and phi X174 in this study are similar to studies that have shown coagulation, flocculation and sedimentation to result in a 0.1 to 3.4 log removal of viruses depending on coagulation conditions (WHO, 2011).

5.2.3 The combination of lime, activated silica and ferric chloride efficiency for the removal of turbidity and viruses

The third set of clarification experiments used a combination of lime, activated silica and ferric chloride. This treatment was assessed for the reduction of turbidity and removal of viruses. The properties of lime and activated silica were discussed above (Section 5.2.2). This section will focus on the addition of ferric chloride to the lime and silica combination. Ferric chloride is a flocculent aid and plays a role in removing turbidity and other organic contaminants (Leopold & Freese, 2009). When added to raw water, ferric chloride undergoes a complex hydrolysis reaction, and the reaction products cause destabilization of contaminants in the water (Pongchalermporn, 2002). The destabilized contaminants aggregate and are physically separated from the surrounding medium by gravity sedimentation.

The lime, activated silica and ferric chloride were added to raw water spiked with either phi X174 or MS2 in the following order: activated silica, lime, and ferric chloride. The lime concentrations ranged from 20 mg/L to 100 mg/L, and the ferric chloride concentrations ranged from 2-6 mg/L. The activated silica concentration was kept constant at 2 mg/L. The results from this study showed that the turbidity reduction was substantial even with the lowest doses of lime

and ferric chloride (Table 11). The lowest dose regime involved 20 mg/L lime, 2 mg/L activated silica and 2 mg/L of ferric chloride. This regime achieved a turbidity reduction of over 90%. Increasing the doses of lime and ferric chloride beyond 80 mg/L and 5 mg/L respectively (Fig.16) did not reduce turbidity but instead raised it. The residual lime particles remaining in suspension may have caused the observed increase in turbidity.

This study also assessed the effectiveness of the combination of lime, activated silica and ferric chloride for the removal or inactivation of phages. The results showed that an increase in the concentration of the lime and ferric chloride increased the phage removal or inactivation. Although an increase in phage removal was observed, each phage was removed with a different efficiency, especially at the lowest and highest concentrations of lime and ferric chloride (Fig.16). At the highest concentrations of lime and ferric chloride, a 3.03 and 3.04 log removal was observed for MS2 for the low and high energy regimes (Fig. 17). At these concentrations, a 2.27 and 2.56 log removal was observed for phi X174 for the low and high energy regimes. This finding showed that MS2 was removed or inactivated more effectively than phi X174, irrespective of the energy regime.

The findings from this study showed that the combination consisting of lime, activated silica, and ferric chloride is sufficient for turbidity reduction. This combination also showed that the concentrations of lime and ferric chloride required for substantial turbidity reduction were relatively low. The results showed that MS2 was removed more effectively than phi X174 (Fig.16). This is similar to results reported for the polyelectrolyte (Section 5.2.1) and the combination consisting of lime and activated silica alone (Section 5.2.2). The hydrophobicity of MS2 may have played a role in its differential removal relative to phi X174.

5.3 The effectiveness of sand filtration for the removal of viruses

Rapid sand filtration was the second treatment barrier studied. This treatment involves water passing through a filter consisting of a packed bed of granular materials. Before filtration the water still contained approximately 5% of the suspended matter in the raw water, and this is removed by rapid sand filtration yielding a product with turbidity which is less than 1 NTU.

During filtration suspended material in the water are trapped on the grains of filter media. Virus removal by sand filtration is probably due to retention of solid particles or flocs onto which the virus is already associated. The retention and transport behavior of viruses in porous media have been extensively studied in columns (Attinti & Wei, 2010). However, these studies do not allow detailed investigation on the interactions that occur between viruses and porous materials (Attinti and Wei, 2010).

In this study, samples spiked with either phi X174 or MS2 were filtered through a column layered with rounded stones with a diameter of 6-12 mm, fine grit (2-5 mm) and fine sand (0.7 mm). The findings from this study showed that sand filtration removed phi X174 particles by 16.97% whereas MS2 was removed by 45.47% (Fig.18). The log removals of phi X174 and MS2 were 0.07 and 0.26 log units, respectively (Fig.19). This study showed that the two phages were removed with different efficiencies. Bacteriophage MS2 showed better retention than X174. This finding is consistent with a study that showed that F-specific phages have a higher tendency to adsorb to solid surfaces such as membrane surfaces and particulate matter, than somatic phages resulting in a higher removal of F-specific phages (Zhang & Farahbakhsh, 2007).

Even though the phages were removed with varies efficiencies, it is important to note that in both cases the removals were very low and this is consistent with other studies. Sobsey et al.

(1995) showed a log removal of about 0.5 for F-specific coliphages. The 0.26 log unit removal of MS2 observed in this study is lower than the findings in the study of Sobsey et al. (1995). Also, according to Jiménez et al. (2010), rapid sand filters were able to remove approximately 1 log unit of enteroviruses from a coagulated primary effluent. The low log removals of both phages in this study may also be attributed to the fact that viruses are small and difficult to remove by filtration methods

This study has shown that rapid sand filtration is ineffective for the removal of viruses. Additionally, previous studies have described the elimination of some particular coliphages by sand filtration to be low and inconsistent, as it has also been described for human enteric viruses (Ribas et al., 1995). Although this water treatment barrier may be ineffective for the removal of viruses, it plays a significant role in the multiple barrier systems by reducing turbidity to a level that allows effective chlorine disinfection.

5.4 The effectiveness of disinfection for the removal of viruses

The final treatment barrier studied for the removal of viruses was disinfection. Disinfection involves the removal, inactivation or killing of pathogenic microorganisms using disinfectants. Of the drinking water disinfectants, free chlorine is the most affordable, easily and widely used. The protein damage of strong oxidizers such as free chlorine is suggested to be the dominant mechanism causing viral inactivation. Free chlorine degrades the N-terminal end of amino acids as well as the free amine, aromatic and organosulfur side chains of C, H, K, M, W or Y amino acids. This inhibits the ability of viruses to bind to cells or affects post-binding life cycle processes (Mayer et al., 2015). Chlorine disinfection is highly effective against nearly all waterborne pathogens, with notable exceptions being *Cryptosporidium parvum* oocysts and *Mycobacteria* species (WHO, 2004b).

The effectiveness of disinfection can be influenced by factors such as turbidity and temperature. Disinfection is more effective where turbidity is less than 1 NTU (Momba et al., 2009). Higher turbidity levels are associated with increased chlorine demand and suspended particles may shield microorganisms from disinfection. High turbidity levels may also support microbial growth. Together these factors contribute to the decreased effectiveness of chlorine disinfection with increasing turbidity levels (Obi et al., 2008).

The effectiveness of chlorine disinfection is also related to temperature and pH. Chlorine is a more effective disinfectant at elevated temperatures (20-25°C) than low temperatures (5-10°C). Disinfection with free chlorine is also more effective when pH values range between 5 and 7 than when pH values lie outside of this range. In this study, the free chlorine concentration range tested was from 0.51 to 1.05 mg/ml. The contact times were 2, 5 and 10 minutes. The experiments were conducted at a seasonal low temperature of 13ºC and a high temperature of 23ºC. The results from this study showed that free chlorine was not equally effective at inactivating the two surrogate viruses. Bacteriophage phi X174 was removed by >5 log units, within two minutes, at a low (13ºC) and high (23ºC) temperature (Fig.20). On the other hand, MS2 behaved differently and showed less sensitivity to disinfection. Bacteriophage MS2 was removed by 3.6 and 3.92 log units after two minutes at a low (13ºC) and high (23ºC) temperature, respectively (Fig.21). There was a significant ($p < 0.0001$) difference between the log removals of the two bacteriophages at 13ºC (Fig. 21). This study also showed that extending the contact time to 10 minutes resulted in the complete removal of both and phi X174 and MS2 (Fig. 20).

The difference in the pattern of removal of the two phages during disinfection may be attributed to the physical structure of the phages and the temperature. MS2 was detected in chlorinated

water at 13ºC after two minutes (Fig.20). This is consistent with the fact that chlorine is less effective at lower temperatures. Also, many experiments confirmed that the resistance of F-RNA coliphages to unfavorable conditions and disinfection processes resembles or exceeds that of most human enteric viruses (Grabow, 2001). This study also showed that extending the contact time to 20 and 30 minutes resulted in the complete removal of MS2 (Fig. 22). On the other hand, phi X174 was readily removed by disinfection (Fig. 20 & Fig. 21), and this could be attributed to the fact that somatic coliphages are less resistant to disinfection than F-specific coliphages (Jofre et al., 2016). These results are consistent with previous data indicating that F-specific coliphages are more resistant to chlorination than somatic coliphages (Ribas et al., 1995).

The findings from this study showed that disinfection could remove > 4 log units of both phages after 10 minutes (Fig. 20) which is consistent with previous studies that have shown that at doses of a few mg/l and contact times of about 30 minutes, free chlorine generally inactivated >4 $\log_{10}$ (>99.99%) of enteric bacteria and viruses (WHO, 2007). Considering that the most critical factor in phage removal is chlorination, it is important to note that a failure in this barrier will pose a considerable risk to consumers. Therefore, it is crucial to have the necessary control measures in place should a failure occur.

5.5 A comparison of the log removal efficiency of the different process barriers in removing viruses during water treatment

In this study, the log removal efficiencies of the different process barriers in removing viruses were determined. The first water treatment barrier was clarification, and it was conducted using (i) polyelectrolyte, (ii) lime and activated silica and (iii) the combination of lime, activated silica and ferric chloride. Of these the lime, activated silica and ferric chloride treatment was most effective for the removal or inactivation of the phage surrogates (Fig. 23).

The second stage in the water treatment process was sand filtration. Sand filtration was the least effective barrier for the removal of phages in this study (Fig. 23). This was consistent with previous studies which showed that sand filtration is not an effective barrier for viruses (Marco, 2012).

The final water treatment barrier studied was disinfection. The results from this study showed this treatment barrier effectively removed both phages with the removal of phi X174 exceeding that of MS2. Of all the barriers studied, disinfection was the most effective at removing or inactivating the phage surrogates (Fig. 23).

5.6 Conclusion

This study assessed the effectiveness of the water treatment processes for removing viruses. It used bench scale simulations of treatment processes and two bacteriophage surrogates (i.e., MS2 and phi X174). The water treatment processes involved the following stages: clarification, filtration and disinfection. The combination of these processes is often referred to as a multi-barrier system. One of the benefits of such a system is that a failure in one barrier may be compensated for by effective operation of the next barrier.

When considering multi-barrier systems, it is essential to consider the role of each stage's effectiveness in two ways. Firstly, the ability of each stage to directly remove or inactivate viruses should be considered. Secondly, the extent to which each stage adjusts the properties of water making it suitable for downstream processes should be considered. For example, a given treatment step may have almost no capacity to remove viruses. However, it may still play a critical role in virus removal if it enables subsequent treatment steps to function effectively.

For this study, clarification was shown to remove viruses and prepare water for the downstream processes. During the study, three different chemical regimes were assessed. Of these, the combination consisting of lime, activated silica and ferric chloride was found to be the most effective at simultaneously removing bacteriophages and turbidity. Irrespective of the treatment regime used a failure of clarification would cause contaminants to clog the sand filters in the next treatment step, and contaminated water would most likely pass through to disinfection. The water reaching the disinfection stage would then have a high chlorine demand, and this would diminish the effectiveness of this critical barrier.

Sand filtration is the second barrier in the multiple barrier systems. This barrier was found to be relatively ineffective at removing viruses. However, it was considered to be significant due to its impact on downstream processes. Sand filtration decreases the turbidity of water before disinfection. A failure of this barrier would mean that the water reaching the disinfection stage would have a high chlorine demand reducing the effectiveness of chlorination. It follows that while sand filtration may not be able to remove viruses as shown in this study, it still plays a vital role in preparing the water for disinfection.

The final barrier in the multiple barrier systems is disinfection. Disinfection was shown to be very effective for the removal or inactivation of viruses in this study. As a final barrier, failure means that the health of the public will be at risk since there is no remaining barrier to compensate for the failure. It is therefore essential to have measures in place to take corrective action before the water reaches consumers.

A failure of any one of the three barriers presents a risk from enteric viruses. This is because each barrier either removes viruses directly or ensures that subsequent processes can function

effectively or both. Overall the treatment stages should not be considered as discrete barriers but rather an integrated system that must function throughout to avoid a risk to customers.

Another factor considered was the suitability of the two surrogate phages to assess the effectiveness of the treatment barriers. In this regard, it was noted that when selecting a surrogate it is in the best interests of consumer health to choose one that is as resistant to treatment as possible. This will result in more conservative estimates of treatment efficiency highlighting risks to consumers from enteric viruses that may be resistant to treatment.

This study has shown that bacteriophage phi X174 was more resistant to clarification and sand filtration than MS2. Therefore phi X174 is potentially a better surrogate than MS-2 for clarification and sand filtration. On the other hand, MS2 was shown to be more resistant to disinfection. This makes M-S2 a potentially better surrogate than phi X174 for disinfection studies. In conclusion, both phages deserve further attention as model organisms for the evaluation of removal of viruses in water treatment plants.

5.7 Recommendations

- The combination of lime and activated silica or the combination of lime, activated silica and ferric chloride can significantly reduce trubidity. However, they should be applied in highly contaminated source waters. The use of these chemicals requires skilled workers and prior sludge removal. The costs involved are too high for this treatment to be used as the sole clarification treatment process.
- Polyelectrolyte is an effective coagulant for the removal of turbidity and should be used in plants where the source water to be treated is less contaminated. The advantages of this

coagulant are the savings in capital cost associated with the single sedimentation stage and a considerable reduction in the volume of sludge to be disposed of.

- Sand filtration is not an adequate barrier for the removal of viruses. However, it should be considered to play a significant role in turbidity removal in multiple barrier systems.
- Further research on disinfection is necessary to investigate how extremely low temperatures and longer contact times may influence the disinfection process.
- The results of the study showed the efficiency of the plant in removing viruses only; the information cannot be used for other organisms.

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APPENDICES

Appendix 1 Jar tests results for phi X174 using polyelectrolyte at a low energy regime

Experiment date	19/10/2016
Coagulant	Poly
Energy regime	Low
Temperature of raw water °C	22.6

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	77.4	77.4	77.4	77.40	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	77.4	23.4	23.3	23.4	23.37	0.06
2	77.4	14.4	14.4	14.4	14.40	0.00
3	77.4	10.7	10.7	10.7	10.70	0.00
4	77.4	6.4	6.41	6.41	6.41	0.01
5	77.4	5.21	5.24	5.26	5.24	0.03

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
1	100.00	30.23	30.10	30.23	30.19	0.07
2	100.00	18.60	18.60	18.60	18.60	0.00
3	100.00	13.82	13.82	13.82	13.82	0.00
4	100.00	8.27	8.28	8.28	8.28	0.01
5	100.00	6.73	6.77	6.80	6.77	0.03

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	124	134	113	123.67	10.50

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	123.67	111	99	103	104.33	6.11
2	123.67	95	86	91	90.67	4.51
3	123.67	81	84	87	84.00	3.00
4	123.67	66	79	71	72.00	6.56
5	123.67	75	57	69	67.00	9.17

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment
1	100.00	8.49	89.76	80.05	83.29	84.37
2	100.00	8.49	76.82	69.54	73.58	73.32
3	100.00	8.49	65.50	67.92	70.35	67.92
4	100.00	8.49	53.37	63.88	57.41	58.22
5	100.00	8.49	60.65	46.09	55.80	54.18

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	2.0934	2.1271	2.0531	2.0912	0.04

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	2.0912	2.0453	1.9956	2.0128	2.02	0.03
2	2.0912	1.9777	1.9345	1.9590	1.96	0.02
3	2.0912	1.9085	1.9243	1.9395	1.92	0.02
4	2.0912	1.8195	1.8976	1.8513	1.86	0.04
5	2.0912	1.8751	1.7559	1.8388	1.82	0.06

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.046	0.096	0.078	0.07	0.03
2	N/A	0.113	0.157	0.132	0.13	0.02
3	N/A	0.183	0.167	0.152	0.17	0.02
4	N/A	0.272	0.194	0.240	0.24	0.04
5	N/A	0.216	0.335	0.252	0.27	0.06

Appendix 2 Jar tests results for phi X174 using polyelectrolyte at a high energy regime

Experiment date	19/10/2016
Coagulant	Poly
Energy regime	High
Temperature of raw water °C	23

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	77	77	77	77.00	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	77	19.7	19.6	19.5	19.60	0.10
2	77	10.5	10.4	10.5	10.47	0.06
3	77	8.21	8.24	8.23	8.23	0.02
4	77	6.36	6.32	6.33	6.34	0.02
5	77	5.14	5.12	5.13	5.13	0.01

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
1	100.00	25.58	25.45	25.32	25.45	0.13
2	100.00	13.64	13.51	13.64	13.59	0.07
3	100.00	10.66	10.70	10.69	10.68	0.02
4	100.00	8.26	8.21	8.22	8.23	0.03
5	100.00	6.68	6.65	6.66	6.66	0.01

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	176	175	174	175.00	1.00

Expressed directly

Concentration of chemical used	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
2mg	175.00	116	130	130	125.33	8.08
3mg	175.00	107	115	101	107.67	7.02
4mg	175.00	74	111	95	93.33	18.56
5mg	175.00	81	89	101	90.33	10.07
6mg	175.00	71	76	65	70.67	5.51

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
2mg	100.00	0.57	66.29	74.29	74.29	71.62	4.62
3mg	100.00	0.57	61.14	65.71	57.71	61.52	4.01
4mg	100.00	0.57	42.29	63.43	54.29	53.33	10.60
5mg	100.00	0.57	46.29	50.86	57.71	51.62	5.75
6mg	100.00	0.57	40.57	43.43	37.14	40.38	3.15

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	2.2455	2.2430	2.2405	2.2430	0.00

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	2.2430	2.0645	2.1139	2.1139	2.10	0.03
2	2.2430	2.0294	2.0607	2.0043	2.03	0.03
3	2.2430	1.8692	2.0453	1.9777	1.96	0.09
4	2.2430	1.9085	1.9494	2.0043	1.95	0.05
5	2.2430	1.8513	1.8808	1.8129	1.85	0.03

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.179	0.129	0.129	0.15	0.03
2	N/A	0.214	0.182	0.239	0.21	0.03
3	N/A	0.374	0.198	0.265	0.28	0.09
4	N/A	0.335	0.294	0.239	0.29	0.05
5	N/A	0.392	0.362	0.430	0.39	0.03

Appendix 3 Jar tests results for phi X174 using the combination of lime and activated silica at a low energy regime

Experiment date	22/11/2016
Coagulant	lime+silica
Energy regime	low
Temperature of raw water °C	24.1

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	131	131	131	131.00	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	131	6.9	6.89	6.88	6.89	0.01
2	131	2.34	2.34	2.33	2.34	0.01
3	131	2.33	2.31	2.26	2.30	0.04
4	131	1.76	1.75	1.76	1.76	0.01
5	131	2.28	2.26	2.26	2.27	0.01

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
1	100.00	5.27	5.26	5.25	5.26	0.01
2	100.00	1.79	1.79	1.78	1.78	0.00
3	100.00	1.78	1.76	1.73	1.76	0.03
4	100.00	1.34	1.34	1.34	1.34	0.00
5	100.00	1.74	1.73	1.73	1.73	0.01

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	289	286	296	290.33	5.13

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
20mg	290.33	276	282	279	279.00	3.00
40mg	290.33	198	217	196	203.67	11.59
60mg	290.33	66	53	45	54.67	10.60
80mg	290.33	5	3	6	4.67	1.53
100mg	290.33	1	1	1	1.00	0.00

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
20mg	100.00	1.77	95.06	97.13	96.10	96.10	1.03
40mg	100.00	1.77	68.20	74.74	67.51	70.15	3.99
60mg	100.00	1.77	22.73	18.25	15.50	18.83	3.65
80mg	100.00	1.77	1.72	1.03	2.07	1.61	0.53
100mg	100.00	1.77	0.34	0.34	0.34	0.34	0.00

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	2.4609	2.4564	2.4713	2.4629	0.01

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	2.4629	2.4409	2.4502	2.4456	2.45	0.00
2	2.4629	2.2967	2.3365	2.2923	2.31	0.02
3	2.4629	1.8195	1.7243	1.6532	1.73	0.08
4	2.4629	0.6990	0.4771	0.7782	0.65	0.16
5	2.4629	0.0000	0.0000	0.0000	0.00	0.00

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.022	0.013	0.017	0.02	0.00
2	N/A	0.166	0.126	0.171	0.15	0.02
3	N/A	0.643	0.739	0.810	0.73	0.08
4	N/A	1.764	1.986	1.685	1.81	0.16
5	N/A	2.463	2.463	2.463	2.46	0.00

Appendix 4 Jar tests results for phi X174 using the combination of lime and activated silica at a high energy regime

Experiment date	21/11/2016
Coagulant	lime+silica
Energy regime	high
Temperature of raw water °C	23.4

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	131	130	131	130.67	0.58

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	130.666667	3.47	3.47	3.47	3.47	0.00
2	130.666667	1.58	1.58	1.57	1.58	0.01
3	130.666667	1.65	1.67	1.65	1.66	0.01
4	130.666667	3.12	3.12	3.1	3.11	0.01
5	130.666667	2.61	2.59	2.6	2.60	0.01

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
1	100.00	2.66	2.66	2.66	2.66	0.00
2	100.00	1.21	1.21	1.20	1.21	0.00
3	100.00	1.26	1.28	1.26	1.27	0.01
4	100.00	2.39	2.39	2.37	2.38	0.01
5	100.00	2.00	1.98	1.99	1.99	0.01

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	275	256	281	270.67	13.05

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	270.67	236	246	239	240.33	5.13
2	270.67	209	217	189	205.00	14.42
3	270.67	45	37	37	39.67	4.62
4	270.67	5	9	2	5.33	3.51
5	270.67	1	3	1	1.67	1.15

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
1	100.00	4.82	87.19	90.89	88.30	88.79	1.90
2	100.00	4.82	77.22	80.17	69.83	75.74	5.33
3	100.00	4.82	16.63	13.67	13.67	14.66	1.71
4	100.00	4.82	1.85	3.33	0.74	1.97	1.30
5	100.00	4.82	0.37	1.11	0.37	0.62	0.43

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	2.4393	2.4082	2.4487	2.4321	0.02

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	2.4321	2.3729	2.3909	2.3784	2.38	0.01
2	2.4321	2.3201	2.3365	2.2765	2.31	0.03
3	2.4321	1.6532	1.5682	1.5682	1.60	0.05
4	2.4321	0.6990	0.9542	0.3010	0.65	0.33
5	2.4321	0.0000	0.4771	0.0000	0.16	0.28

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.059	0.041	0.054	0.05	0.01
2	N/A	0.112	0.096	0.156	0.12	0.03
3	N/A	0.779	0.864	0.864	0.84	0.05
4	N/A	1.733	1.478	2.131	1.78	0.33
5	N/A	2.432	1.955	2.432	2.27	0.28

Appendix 5 Jar tests results for phi X174 using the combination of lime, activated silica and ferric chloride at a low energy regime

Experiment date	23/11/2016
Coagulant	lime,silica,ferric
Energy regime	low
Temperature of raw water °C	23

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	133.5	133.5	133.5	133.50	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	133.5	4.41	4.44	4.45	4.43	0.02
2	133.5	4.18	4.18	4.17	4.18	0.01
3	133.5	2.07	1.99	1.95	2.00	0.06
4	133.5	2.67	2.64	2.63	2.65	0.02
5	133.5	2.83	2.85	2.85	2.84	0.01

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
20;2mg	100.00	3.30	3.33	3.33	3.32	0.02
40;3mg	100.00	3.13	3.13	3.12	3.13	0.00
60;4mg	100.00	1.55	1.49	1.46	1.50	0.05
80;5mg	100.00	2.00	1.98	1.97	1.98	0.02
100;6mg	100.00	2.12	2.13	2.13	2.13	0.01

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	370	380	374	374.67	5.03

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
20mg;2mg	374.67	328	326	325	326.33	1.53
40mg;3mg	374.67	67	90	70	75.67	12.50
60mg;4mg	374.67	18	19	23	20.00	2.65
80mg;5mg	374.67	4	2	6	4.00	2.00
100mg;6mg	374.67	4	2	1	2.33	1.53

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
20;2mg	100.00	1.34	87.54	87.01	86.74	87.10	0.41
40;3mg	100.00	1.34	17.88	24.02	18.68	20.20	3.34
60;4mg	100.00	1.34	4.80	5.07	6.14	5.34	0.71
80;5mg	100.00	1.34	1.07	0.53	1.60	1.07	0.53
100;6mg	100.00	1.34	1.07	0.53	0.27	0.62	0.41

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	2.5682	2.5798	2.5729	2.5736	0.01

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	2.5736	2.5159	2.5132	2.5119	2.51	0.00
2	2.5736	1.8261	1.9542	1.8451	1.88	0.07
3	2.5736	1.2553	1.2788	1.3617	1.30	0.06
4	2.5736	0.6021	0.3010	0.7782	0.56	0.24
5	2.5736	0.6021	0.3010	0.0000	0.30	0.30

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.058	0.060	0.062	0.06	0.00
2	N/A	0.748	0.619	0.729	0.70	0.07
3	N/A	1.318	1.295	1.212	1.28	0.06
4	N/A	1.972	2.273	1.795	2.01	0.24
5	N/A	1.972	2.273	2.574	2.27	0.30

Appendix 6 Jar tests results for phi X174 using the combination of lime, activated silica and ferric chloride at a high energy regime

Experiment date	17/11/2016
Coagulant	Lime,Silica, Ferric
Energy regime	High
Temperature of raw water °C	23

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	139.1	139.1	139.1	139.10	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	139.1	1.58	1.59	1.6	1.59	0.01
2	139.1	2	2.03	2.04	2.02	0.02
3	139.1	0.728	0.742	0.749	0.74	0.01
4	139.1	3.34	3.34	3.33	3.34	0.01
5	139.1	0.196	0.199	0.198	0.20	0.00

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
1	100.00	1.14	1.14	1.15	1.14	0.01
2	100.00	1.44	1.46	1.47	1.45	0.01
3	100.00	0.52	0.53	0.54	0.53	0.01
4	100.00	2.40	2.40	2.39	2.40	0.00
5	100.00	0.14	0.14	0.14	0.14	0.00

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	355	367	363	361.67	6.11

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	361.67	329	331	325	328.33	3.06
2	361.67	26	42	37	35.00	8.19
3	361.67	13	15	16	14.67	1.53
4	361.67	3	2	3	2.67	0.58
5	361.67	1	1	1	1.00	0.00

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
1	100.00	1.69	90.97	91.52	89.86	90.78	0.84
2	100.00	1.69	7.19	11.61	10.23	9.68	2.26
3	100.00	1.69	3.59	4.15	4.42	4.06	0.42
4	100.00	1.69	0.83	0.55	0.83	0.74	0.16
5	100.00	1.69	0.28	0.28	0.28	0.28	0.00

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	2.5502	2.5647	2.5599	2.5583	0.01

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	2.5583	2.5172	2.5198	2.5119	2.52	0.00
2	2.5583	1.4150	1.6232	1.5682	1.54	0.11
3	2.5583	1.1139	1.1761	1.2041	1.16	0.05
4	2.5583	0.4771	0.3010	0.4771	0.42	0.10
5	2.5583	0.0000	0.0000	0.0000	0.00	0.00

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.041	0.038	0.046	0.04	0.00
2	N/A	1.143	0.935	0.990	1.02	0.11
3	N/A	1.444	1.382	1.354	1.39	0.05
4	N/A	2.081	2.257	2.081	2.14	0.10
5	N/A	2.558	2.558	2.558	2.56	0.00

Appendix 7 Jar tests results for MS2 using polyelectrolyte at a low energy regime

Experiment date	15/12/16
Coagulant	Poly
Energy regime	Low
Temperature of raw water °C	24.2

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	141	141	141	141.00	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	141	28.8	28.8	28.8	28.80	0.00
2	141	24.2	24.1	24.1	24.13	0.06
3	141	10.9	10.9	10.8	10.87	0.06
4	141	7.4	7.4	7.39	7.40	0.01
5	141	4.91	4.9	4.88	4.90	0.02

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
2mg	100.00	20.43	20.43	20.43	20.43	0.00
3mg	100.00	17.16	17.09	17.09	17.12	0.04
4mg	100.00	7.73	7.73	7.66	7.71	0.04
5mg	100.00	5.25	5.25	5.24	5.25	0.00
6mg	100.00	3.48	3.48	3.46	3.47	0.01

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	1670	1800	1750	1740.00	65.57

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
2mg	1740.00	269	246	251	255.33	12.10
3mg	1740.00	183	196	206	195.00	11.53
4mg	1740.00	96	97	95	96.00	1.00
5mg	1740.00	70	62	50	60.67	10.07
6mg	1740.00	37	36	39	37.33	1.53

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
2mg	100.00	3.77	15.46	14.14	14.43	14.67	0.70
3mg	100.00	3.77	10.52	11.26	11.84	11.21	0.66
4mg	100.00	3.77	5.52	5.57	5.46	5.52	0.06
5mg	100.00	3.77	4.02	3.56	2.87	3.49	0.58
6mg	100.00	3.77	2.13	2.07	2.24	2.15	0.09

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	3.2227	3.2553	3.2430	3.2403	0.02

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	3.2403	2.4298	2.3909	2.3997	2.41	0.02
2	3.2403	2.2625	2.2923	2.3139	2.29	0.03
3	3.2403	1.9823	1.9868	1.9777	1.98	0.00
4	3.2403	1.8451	1.7924	1.6990	1.78	0.07
5	3.2403	1.5682	1.5563	1.5911	1.57	0.02

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.811	0.849	0.841	0.83	0.02
2	N/A	0.978	0.948	0.926	0.95	0.03
3	N/A	1.258	1.254	1.263	1.26	0.00
4	N/A	1.395	1.448	1.541	1.46	0.07
5	N/A	1.672	1.684	1.649	1.67	0.02

Appendix 8 Jar tests results for MS2 using polyelectrolyte at a high energy regime

Experiment date	14.12.2016
Coagulant	Poly
Energy regime	high
Temperature of raw water °C	24.1

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	128	128	128	128.00	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	128	31.6	31.5	31.5	31.53	0.06
2	128	19.1	19.2	19.2	19.17	0.06
3	128	11.6	11.6	11.7	11.63	0.06
4	128	7.75	7.75	7.79	7.76	0.02
5	128	7.54	7.54	7.54	7.54	0.00

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
2mg	100.00	24.69	24.61	24.61	24.64	0.05
3mg	100.00	14.92	15.00	15.00	14.97	0.05
4mg	100.00	9.06	9.06	9.14	9.09	0.05
5mg	100.00	6.05	6.05	6.09	6.07	0.02
6mg	100.00	5.89	5.89	5.89	5.89	0.00

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	1710	1720	1630	1686.67	49.33

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	1686.67	361	345	354	353.33	8.02
2	1686.67	194	192	202	196.00	5.29
3	1686.67	132	144	129	135.00	7.94
4	1686.67	88	93	85	88.67	4.04
5	1686.67	44	45	51	46.67	3.79

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
2mg	100.00	2.92	21.40	20.45	20.99	20.95	0.48
3mg	100.00	2.92	11.50	11.38	11.98	11.62	0.31
4mg	100.00	2.92	7.83	8.54	7.65	8.00	0.47
5mg	100.00	2.92	5.22	5.51	5.04	5.26	0.24
6mg	100.00	2.92	2.61	2.67	3.02	2.77	0.22

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	3.2330	3.2355	3.2122	3.2269	0.01

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	3.2269	2.5575	2.5378	2.5490	2.55	0.01
2	3.2269	2.2878	2.2833	2.3054	2.29	0.01
3	3.2269	2.1206	2.1584	2.1106	2.13	0.03
4	3.2269	1.9445	1.9685	1.9294	1.95	0.02
5	3.2269	1.6435	1.6532	1.7076	1.67	0.03

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.669	0.689	0.678	0.68	0.01
2	N/A	0.939	0.944	0.922	0.93	0.01
3	N/A	1.106	1.069	1.116	1.10	0.03
4	N/A	1.282	1.258	1.297	1.28	0.02
5	N/A	1.583	1.574	1.519	1.56	0.03

Appendix 9 Jar tests results for MS2 using the combination of lime and activated silica at a low energy regime

Experiment date	16/01/17
Coagulant	lime+silica
Energy regime	Low
Temperature of raw water °C	24.4

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	122	122	122	122.00	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	122	17	17	17	17.00	0.00
2	122	7.05	7.07	7.05	7.06	0.01
3	122	6.63	6.63	6.62	6.63	0.01
4	122	6.69	6.7	6.7	6.70	0.01
5	122	4.79	4.79	4.79	4.79	0.00

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
20mg	100.00	13.93	13.93	13.93	13.93	0.00
40mg	100.00	5.78	5.80	5.78	5.78	0.01
60mg	100.00	5.43	5.43	5.43	5.43	0.00
80mg	100.00	5.48	5.49	5.49	5.49	0.00
100mg	100.00	3.93	3.93	3.93	3.93	0.00

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	1460	1460	1460	1460.00	0.00

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	1460.00	530	580	510	540.00	36.06
2	1460.00	150	142	136	142.67	7.02
3	1460.00	50	45	52	49.00	3.61
4	1460.00	35	36	47	39.33	6.66
5	1460.00	6	4	7	5.67	1.53

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
20mg	100.00	0.00	36.30	39.73	34.93	36.99	2.47
40mg	100.00	0.00	10.27	9.73	9.32	9.77	0.48
60mg	100.00	0.00	3.42	3.08	3.56	3.36	0.25
80mg	100.00	0.00	2.40	2.47	3.22	2.69	0.46
100mg	100.00	0.00	0.41	0.27	0.48	0.39	0.10

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	3.1644	3.1644	3.1644	3.1644	0.00

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	3.1644	2.7243	2.7634	2.7076	2.73	0.03
2	3.1644	2.1761	2.1523	2.1335	2.15	0.02
3	3.1644	1.6990	1.6532	1.7160	1.69	0.03
4	3.1644	1.5441	1.5563	1.6721	1.59	0.07
5	3.1644	0.7782	0.6021	0.8451	0.74	0.13

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.440	0.401	0.457	0.43	0.03
2	N/A	0.988	1.012	1.031	1.01	0.02
3	N/A	1.465	1.511	1.448	1.47	0.03
4	N/A	1.620	1.608	1.492	1.57	0.07
5	N/A	2.386	2.562	2.319	2.42	0.13

Appendix 10 Jar tests results for phi MS2 using the combination of lime and activated silica at a high energy regime

Experiment date	11.01.17
Coagulant	lime+silica
Energy regime	High
Temperature of raw water °C	23.8

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	124	124	124	124.00	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	124	15.3	15.2	15	15.17	0.15
2	124	4.27	4.36	4.33	4.32	0.05
3	124	3.88	3.91	4	3.93	0.06
4	124	2.92	2.9	2.87	2.90	0.03
5	124	2.49	2.53	2.5	2.51	0.02

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
20mg	100.00	12.34	12.26	12.10	12.23	0.12
40mg	100.00	3.44	3.52	3.49	3.48	0.04
60mg	100.00	3.13	3.15	3.23	3.17	0.05
80mg	100.00	2.35	2.34	2.31	2.34	0.02
100mg	100.00	2.01	2.04	2.02	2.02	0.02

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	930	910	950	930.00	20.00

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	930.00	135	133	124	130.67	5.86
2	930.00	117	126	120	121.00	4.58
3	930.00	52	42	48	47.33	5.03
4	930.00	26	27	28	27.00	1.00
5	930.00	2	1	4	2.33	1.53

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
20mg	100.00	2.15	14.52	14.30	13.33	14.05	0.63
40mg	100.00	2.15	12.58	13.55	12.90	13.01	0.49
60mg	100.00	2.15	5.59	4.52	5.16	5.09	0.54
80mg	100.00	2.15	2.80	2.90	3.01	2.90	0.11
100mg	100.00	2.15	0.22	0.11	0.43	0.25	0.16

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	2.9685	2.9590	2.9777	2.9684	0.01

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	2.9684	2.1303	2.1239	2.0934	2.12	0.02
2	2.9684	2.0682	2.1004	2.0792	2.08	0.02
3	2.9684	1.7160	1.6232	1.6812	1.67	0.05
4	2.9684	1.4150	1.4314	1.4472	1.43	0.02
5	2.9684	0.3010	0.0000	0.6021	0.30	0.30

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.838	0.845	0.875	0.85	0.02
2	N/A	0.900	0.868	0.889	0.89	0.02
3	N/A	1.252	1.345	1.287	1.29	0.05
4	N/A	1.553	1.537	1.521	1.54	0.02

Appendix 11 Jar tests results for MS2 using the combination of lime, activated silica and ferric chloride at a low energy regime

Experiment date	17.01.17
Coagulant	lime,silica,ferric
Energy regime	Low
Temperature of raw water °C	24.4

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	75	75	75	75.00	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	75	14.5	14.4	14.6	14.50	0.10
2	75	3.6	3.54	3.58	3.57	0.03
3	75	3	3.01	3.02	3.01	0.01
4	75	2.5	2.5	2.48	2.49	0.01
5	75	3.6	3.63	3.62	3.62	0.02

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
20mg	100.00	19.33	19.20	19.47	19.33	0.13
40mg	100.00	4.80	4.72	4.77	4.76	0.04
60mg	100.00	4.00	4.01	4.03	4.01	0.01
80mg	100.00	3.33	3.33	3.31	3.32	0.02
100mg	100.00	4.80	4.84	4.83	4.82	0.02

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	1380	1350	1310	1346.67	35.12

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	1346.67	132	142	145	139.67	6.81
2	1346.67	31	36	32	33.00	2.65
3	1346.67	12	13	12	12.33	0.58
4	1346.67	7	8	6	7.00	1.00
5	1346.67	2	1	1	1.33	0.58

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
20mg	100.00	2.61	9.80	10.54	10.77	10.37	0.51
40mg	100.00	2.61	2.30	2.67	2.38	2.45	0.20
60mg	100.00	2.61	0.89	0.97	0.89	0.92	0.04
80mg	100.00	2.61	0.52	0.59	0.45	0.52	0.07
100mg	100.00	2.61	0.15	0.07	0.07	0.10	0.04

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	3.1399	3.1303	3.1173	3.1292	0.01

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	3.1292	2.1206	2.1523	2.1614	2.14	0.02
2	3.1292	1.4914	1.5563	1.5051	1.52	0.03
3	3.1292	1.0792	1.1139	1.0792	1.09	0.02
4	3.1292	0.8451	0.9031	0.7782	0.84	0.06
5	3.1292	0.3010	0.0000	0.0000	0.10	0.17

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	1.009	0.977	0.968	0.98	0.02
2	N/A	1.638	1.573	1.624	1.61	0.03
3	N/A	2.050	2.015	2.050	2.04	0.02
4	N/A	2.284	2.226	2.351	2.29	0.06
5	N/A	2.828	3.129	3.129	3.03	0.17

Appendix 12 Jar tests results for MS2 using the combination of lime, activated silica and ferric chloride at a high energy regime

Experiment date	18/01/2017
Coagulant	lime+silica+ferric
Energy regime	High
Temperature of raw water °C	23.9

Expressed directly

Turbidity of raw water NTU (Before)	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	136	136	136	136.00	0.00

Expressed directly

Turbidity in each jar after testing NTU	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	136	8.3	8.28	8.27	8.28	0.02
2	136	2.17	2.1	2.09	2.12	0.04
3	136	3.21	3.07	2.96	3.08	0.13
4	136	3.25	3.23	3.2	3.23	0.03
5	136	3.03	2.95	2.92	2.97	0.06

Expressed as percentage

Turbidity after testing NTU	Before treatment	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
1	100.00	6.10	6.09	6.08	6.09	0.01
2	100.00	1.60	1.54	1.54	1.56	0.03
3	100.00	2.36	2.26	2.18	2.26	0.09
4	100.00	2.39	2.38	2.35	2.37	0.02
5	100.00	2.23	2.17	2.15	2.18	0.04

Expressed directly

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	2010	2000	1980	1996.67	15.28

Expressed directly

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	1996.67	660	530	570	586.67	66.58
2	1996.67	77	78	70	75.00	4.36
3	1996.67	7	6	7	6.67	0.58
4	1996.67	5	6	8	6.33	1.53
5	1996.67	1	2	3	2.00	1.00

Expressed as percentage

Phage count in raw water after spiking pfu/mL	Before treatment	Std Dev	Replicate 1	Replicte 2	Replicate 3	After treatment	Std Dev
1	100.00	0.77	33.06	26.54	28.55	29.38	3.33
2	100.00	0.77	3.86	3.91	3.51	3.76	0.22
3	100.00	0.77	0.35	0.30	0.35	0.33	0.03
4	100.00	0.77	0.25	0.30	0.40	0.32	0.08
5	100.00	0.77	0.05	0.10	0.15	0.10	0.05

Expressed as log value

Phage count in raw water after spiking pfu/mL	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
	3.3032	3.3010	3.2967	3.3003	0.00

Expressed as a log value

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	3.3003	2.8195	2.7243	2.7559	2.77	0.05
2	3.3003	1.8865	1.8921	1.8451	1.87	0.03
3	3.3003	0.8451	0.7782	0.8451	0.82	0.04
4	3.3003	0.6990	0.7782	0.9031	0.79	0.10
5	3.3003	0.0000	0.3010	0.4771	0.26	0.24

Expressed as a log removal

Phage count in raw water after spiking pfu/mL	Initial value	Replicate 1	Replicte 2	Replicate 3	Average	Std Dev
1	N/A	0.481	0.576	0.544	0.53	0.05
2	N/A	1.414	1.408	1.455	1.43	0.03
3	N/A	2.455	2.522	2.455	2.48	0.04
4	N/A	2.601	2.522	2.397	2.51	0.10
5	N/A	3.300	2.999	2.823	3.04	0.24

Appendix 13 Sand filtration results for phi X174

Phage counts before sand filtraiton	Phage counts after sand filtration (pfu/ml)				
	Filter 1	**Filter 2**	**Filter 3**	**Filter 4**	**Filter 5**
86	84	81	63	83	65
95	54	69	91	87	83
81	69	69	72	84	66
98					
87					
		After sand filtration			
Average	89.40	74.67			
Standard Deviation	6.95	10.71			
Average percentage removal %	16.48				
Log before	1.9513				
Log after	1.8731				
Log removal	0.0782				
	X174				
Average percentage removal %	16.48				
Standard deviation	11.99				

Appendix 14 Sand filtration results for MS2

Phage counts before sand filtraiton	Phage counts after sand filtration				
	Filter 1	**Filter 2**	**Filter 3**	**Filter 4**	**Filter 5**
621	388	249	384	393	326
600	323	320	417	362	317
683	313	314	400	311	319
623					
609					

	Before sand filtration	**After sand filtration**
Average	627.20	342.40
Standard Deviation	32.56	45.79

Average percentage removal %	45.41
Log before	2.7974
Log after	2.5345
Log removal	0.2629

	MS2
Average percentage removal %	45.41
Standard deviation	7.30

Appendix 15 Disinfection results for phi X174

Date	**Temperature (º C)**	**Free Chlorine (mg/L)**	**Time (minutes)**	**CT (mg/l/min)**	**Phage count before (pfu/ml)**	**Phage count after (pfu/ml)**	**Log removal**
04/07/2017	13	0.84	2 mins	1.68	206000	0	<5.31
		0.80	5 mins	4.0		0	<5.31
		0.78	10 mins	7.8		0	<5.31
22/06/2017	23	0.87	2 mins	1.74	221000	0	<5.34
		0.84	5 mins	4.2		0	<5.34
		0.82	10 mins	8.2		0	<5.34

Appendix 16 Disinfection results for MS2

DATE	Temperature (º C)	Free Chlorine (mg/L)	Time (minutes)	CT (mg/l/min)	Phage count before (pfu/ml)	Phage count after (pfu/ml)	Log removal
04/07/2017	13	0.93	2 mins	1.86	189000	32	3.77
		0.85	5 mins	4.25		19	4
		0.84	10 mins	8.4		3	4.8
10/08/2017	23						
		1.05	2 mins	2.1	206000	26	3.92
		0.87	5 mins	4.35		1	5.31
		0.85	10 mins	8.5		1	5.31

Appendix 17 Disinfection results for MS2 at extended contact times

DATE	Temperature (º C)	Free Chlorine (mg/L)	Time (minutes)	Phage count before (PFU)	Phage count after (PFU)	Log removal
24/08/2017	13	0.78	10mins	132000	0	5.12
	13	0.68	20 mins		0	5.12
	13	0.65	30 mins		0	5.12
24/08/2017	23	0.33	10 mins	132000	0	5.12
	23	0.28	20 mins		0	5.12
	23	0.27	30 mins		0	5.12