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Addressing Viral Risk in Drinking Water: Evaluating the Impact of
Treatment Processes in Drinking Water Treatment

School of Water, Energy, and the Environment
MSc by Research in Water

MSc by Research
Academic Year: 2022 - 2023

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Associate Supervisor:
Professor Peter Jarvis; Dr Suniti Singh
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This thesis is submitted in partial fulfilment of the requirements for
the degree of MSc by Research in Water

(NB. Remove if the degree award is based solely on examination of the thesis)

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ABSTRACT

Drinking water treatment is designed to improve water quality, including the removal of potential contaminants like viruses. Pilot-scale studies can provide valuable insights into the effectiveness of full-scale treatment processes in managing viral risks. While traditional water treatment processes are effective in removing many contaminants, the specific removal of viruses remains a significant concern. The World Health Organization (WHO) recommends a 2-log reduction of enteric viruses from source to tap water. However, the effectiveness of different treatment processes in achieving this target, especially under varying conditions, requires further investigation. This study demonstrated that optimized coagulation, followed by subsequent treatment processes, can effectively remove Φ -X174 from water, even at high initial concentrations. The results suggest that conventional drinking water treatment methods can effectively manage the risk of viral contamination, aligning with the WHO's recommended log removal target. This study optimised coagulation and conducted challenge trials using a near-real scale drinking water treatment rig to evaluate the effectiveness of individual treatment processes and log reduction of Φ -x 174. At a final concentration of 10^{10} plaque-forming units of Φ -X 174 the somatic coliphage was not detected in the final water. At a final concentration of 10^{14} plaque-forming units of Φ -X 174, coliphage was detected with no coagulant dose but was removed to below the detection limit for both optimal (10 mg-Fe³⁺/L) and sub-optimal (2 mg-Fe³⁺/L) coagulation conditions.

Keywords:

Virus removal, coagulation, pilot-scale, optimal coagulation, sub-optimal coagulation, Phi-X 174

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr. Franics Hassard and Prof. Peter Jarvis. I would also like to thank Dr. Suniti Singh and Dr. Irene Carra for the invaluable guidance and support they provided through this research journey. I would also like to thank my industry sponsors from WRc for their support and feedback throughout the MRes. I would like to acknowledge the substantial contributions of Dr. Suniti Singh and Mr Matt Andrews for the time spent in the laboratories and Pilot Hall, devoting expertise to ensure the success of the experiments. Additionally, I extend my appreciation to my family and partner for their unwavering support and understanding throughout this time.

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LIST OF ABBREVIATIONS

AOP	Advanced oxidation process
ATCC	American Type Culture Collection
CFU	Colony-forming units
CDC	Centres for Disease Control and Prevention
DAF	Dissolved air floatation
DALY	Disability-adjusted life years
DBP	Disinfection by-product
DPD	diethyl-p-phenylene diamine
DNA	Deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
GAC	Granular activated carbon
HRT	Hydraulic retention time
ICC	Intact cell count
ISO	International Organisation for Standardisation
LRV	Log-reduction value
NER	Nucleotide excision repair
NOM	Natural organic matter
NRF-WWT	National Research Facility in Water and Wastewater Treatment
NTU	Nephelometric turbidity unit
PCR	Polymerase chain reaction
PFU	Plaque-forming units
qPCR	Quantitative polymerase chain reaction
RGF	Rapid-gravity filter
RNA	Ribonucleic acid
SSF	Slow sand filter
TCC	Total cell count
UF	Ultra-filtration
UV	Ultraviolet
WHO	World Health Organisation
Φ-X 174	Phi-X 174 somatic coliphage

1 Introduction

Safe drinking water is a cornerstone of public health. Despite advancements in water treatment technologies, the contamination of drinking water by human and animal pathogens including viruses remains a significant concern worldwide, due to the ingress of faecal material into water sources (Exton et al., 2023) and increased demands placed on drinking water treatment facilities in terms of production volumes and financial pressures (Dupke et al., 2023; Reyes et al., 2013). Viruses in drinking water sources pose a considerable risk to human health due to their low infectious doses and potential for variable resistance to conventional water treatment processes, due to species and strain specific differences. Despite these concerns, viruses represent an understudied aspect of the microbiology of drinking water. Therefore, study on the fate, transport, and removal of viruses in water treatment systems thus represent critical areas of study in the fields of environmental science, public health engineering and microbiology (Chen et al., 2021a; Gibson, 2014).

Viruses, unlike most bacterial pathogens, require host cells to replicate and display greater resistance to some environmental stresses. In part, this is due to their smaller size, simple structure and presence in the environment in higher titers (Mbonimpa et al., 2018). Their ubiquity and high abundance, coupled with their resilience makes them challenging to manage in water treatment systems (Ghernaout, 2020). The presence of pathogenic viruses in drinking water sources can lead to outbreaks of diseases such as hepatitis, norovirus gastroenteritis, and poliomyelitis (Gibson, 2014; Teunis et al., 2009). These illnesses can range from mild gastrointestinal discomfort to severe, life-threatening conditions, particularly in vulnerable populations such as the elderly, children, and immunocompromised individuals (Ganesh and Lin, 2013). The significance of viruses to public health is substantial, as they are key contributors to global waterborne diseases. According to the World Health Organization (WHO), approximately 505,000 deaths each year are caused by microbiologically contaminated drinking water, with viral pathogens being a principal causative agent (WHO, 2023). This public health concern is amplified by the emergence of new viral strains or variants and

the re-emergence of established but significant ones, partly driven by factors such as climate change, urbanisation, and contamination from agricultural and wastewater discharge (Dupke et al., 2023; Neiderud, 2015).

Monitoring microbial water quality is an essential component of managing the safety of drinking water (WHO, 2017). Current methods for monitoring include both direct and indirect approaches. Direct methods involve the detection and quantification of specific viruses or their genetic material in water samples. Techniques such as polymerase chain reaction (PCR) and quantitative PCR (qPCR) have become standard tools in environmental virology due to their high sensitivity and specificity (Hassard et al., 2016). However, these methods can be costly and require specialized equipment and trained personnel. Indirect methods for monitoring microbial water quality often involve assessing the presence of indicator organisms. These indicators, typically non-pathogenic bacteria such as *Escherichia coli* and enterococci, are used to infer the potential presence of pathogenic microbes, including viruses (Farrell et al., 2018). While less specific than direct methods, indicator organism testing is the 'gold standard' as it is simpler, faster, and more cost-effective, making it a widely used approach in routine water quality monitoring (McMinn et al., 2017).

1.1 Correlation between bacterial indicators and viral pathogens.

In aquatic environmental monitoring, a critical issue arises from the mismatch between the presence of viruses and the levels of traditional bacterial water quality indicators. This mismatch indicates that even when bacterial levels are low, suggesting water is compliant / safe (from microbiological perspective), viruses may still be present and pose potential health risks. This discrepancy challenges the reliability of current monitoring methods in accurately reflecting the true viral risk in water bodies (Plummer et al., 2014) and led to calls for other indicators of viral presence. This disparity can be attributed to several fundamental differences in the biological and ecological characteristics of viruses and bacteria, as well as the methodologies employed for their detection and analysis. Firstly, the survival and transport mechanisms of viruses and bacteria

in aquatic environments significantly differ (Yates et al., 1987). Viruses, typically smaller and structurally simpler than bacteria, frequently exhibit a high persistence under a diverse range of environmental conditions. These conditions include varying levels of temperature, pH, and salinity, as well as resistance to UV light and various antimicrobial chemicals (heavy metals, reactive oxygen species etc.) in the environment (Fong and Lipp, 2005). For example, viruses are known to be more thermotolerant than bacteria, with somatic coliphages (virus which infect coliform-like bacteria) having a longer persistence from 4°C to 18°C than coliforms in the same water source (Skraber et al., 2004). In the case of bacteriophages (viruses which infect bacteria), they also may continue to replicate in surviving bacterial hosts after being shed in faeces, making them potential source-tracking indicators in place of generic coliform indicators of faecal contamination (Plummer et al., 2014). This resilience enables viruses to remain viable even when the bacterial populations, such as those they might infect, have diminished. In addition, viruses have low molecular weight compared to other pathogens and indicator bacteria, which effectively reduces the size of active sites for interaction between the stressor and the pathogen. This reduced size of active sites limits the effectiveness of some external stressors such as heat when inactivating viruses, thus enhancing their survival in various environmental conditions (Fong and Lipp, 2005). Viruses have more genomic flexibility than bacteria, with double-stranded or single-stranded DNA, or RNA, as their genetic material. This diversity allows them to avoid repair of the host DNA and therefore strongly increasing mutation rates, aiding their survival and adaptability in the environment (Sanjuán and Domingo-Calap, 2021).

Moreover, the relationship between bacterial indicators and viruses can be compared through the distinct sources and pathways through which viruses and bacteria enter aquatic environments. Viruses, particularly those of concern to human health, are frequently introduced via specific routes such as faecal contamination from humans and animals such as wastewater treatment or runoff (Semenza and Paz, 2021). In contrast, bacteria may originate from a wider array of sources, including both anthropogenic activities and *in situ* growth in the natural environment. For example, high rainfall combined with high-trafficked

impermeable urban surfaces has led to increased surface run-off and total coliform levels similar to that dilute sewage concentrations (Ellis, 2004). Marzouk et al (1980) found no significant statistical correlation between the occurrence of bacterial indicators and the presence of viruses from 155 water sources of which only 29% yielded a virus population.

The methodologies for detecting these microorganisms further exacerbate the disparity between bacterial indicators and viruses in water. Traditional bacterial indicators, like *Escherichia coli* and *Enterococci*, are typically identified through cultivation-based techniques (Haramoto et al., 2018). On the other hand, viruses normally require more sophisticated detection methods, such as PCR, owing to some taxa being hard to culture using standard microbiological techniques (i.e., requiring human or animal cell culture and specialist facilities and resistance is more associated with antibiotics). This fundamental difference in detection methods could contribute to the observed lack of correlation between the levels of viruses and bacterial indicators in aquatic systems (Masachessi et al., 2021).

Viruses and bacteria engage in complex interactions with other microorganisms, as well as with organic and inorganic materials present in the water. These interactions can distinctly influence their survival rates and proliferation capabilities. For instance, certain viruses may adapt to survive in high organic matter environments, a condition that might not be favourable for some bacteria. An example is the enteric pathogenic virus Hepatitis A which is a non-enveloped virus. The sturdy protein capsid of the Hepatitis A virus allows it to remain viable in water contaminated with elevated levels of organic matter, proving unfavourable conditions for some bacteria to survive (Sobsey et al., 1985). From a public health perspective, the significance of these bacteria and viruses diverge. While bacterial indicators are commonly associated with gastrointestinal illnesses, viruses can be responsible for a broader spectrum of diseases, including those affecting the respiratory and neurological systems alongside gastrointestinal illness (Gall et al., 2015). Thus, the presence of bacterial indicators in water does not necessarily imply a similar risk level for viral infections. Additionally, the dynamics of virus-bacteria interactions, such as viral

infection and subsequent lysis of bacterial cells, can lead to a reduction in bacterial populations while allowing for the maintenance or potentially an increase of bacteriophage populations (Fujioka, Loh and Lau, 1980). This interaction further complicates the relationship between virus and bacterial levels in aquatic environments.

Lastly, the variability inherent in the sampling and analytical techniques used for studying viruses and bacteria can lead to inconsistencies and, consequently, a lack of correlation in their observed concentrations. A report from the Centres for Disease Control and Prevention (CDC) in 2004 revealed that among 17 waterborne disease outbreaks linked to infectious causes, only 10 showed associations with positive total or faecal coliform counts (Blackburn et al., 2004). In conclusion, the disparity in correlating viruses with bacterial water quality indicators stems from a complex interplay of biological characteristics, environmental interactions, detection methodologies, and public health implications. This underlines the necessity for a comprehensive and multifaceted approach in assessing water quality, particularly in safeguarding public health from other pathogens.

1.2 Coliphages as aquatic indicators of viral quality

Somatic coliphages, a subset of bacteriophages, are known for their ability to infect coliforms including *Escherichia coli* bacteria. They are characterized by their diverse capsid structures, which can house either single- or double-stranded DNA as genetic material. These capsids vary in complexity, ranging from simple cubic symmetry to more elaborate formations with distinct components such as heads, tails, and tail-fibres (Singh, Pitchers and Hassard, 2022). Morphologically, somatic coliphages are categorized into groups A to D, and they are classified into families like Myoviridae, Siphoviridae, Podoviridae, and Microviridae. Each family is distinguished by specific features including DNA type, tail length, and capsid size (Singh, Pitchers and Hassard, 2022). In water quality testing, somatic coliphages serve as important viral indicators. Their presence in drinking water is commonly used to infer potential contamination from human or animal faeces. Detection of somatic coliphages in water samples is thus a critical measure in

assessing the microbiological safety of drinking water (Singh, Pitchers and Hassard, 2022). Consequently, their presence offers a relatively swift and straightforward means of identifying faecal pollution, representing possible risks from viruses. As their behaviour in water and food resembles that of human enteric viruses more closely than faecal indicator bacteria, making them valuable indicators for water quality assessment (British Standards Institution., 2000).

1.3 Pathogenic viruses in drinking water sources

Viruses are a category of non-cellular, intracellular parasites lacking metabolic capabilities. Their structure comprises a protein capsid enclosing nucleic acid. They replicate by hijacking a host cell's machinery for genomic replication, transcription, and release, often causing cellular damage and potentially triggering disease and release of more virions into water (Kong et al., 2021; Taylor, 2014). Pathogenic viral contamination of drinking water commonly occurs via two main pathways: (1) contamination from faecal material (Bhowmick et al., 2020), and (2) inadequate treatment processes in systems preparing water for consumption (Gibson, 2014). The latter has been a cause for concern recently, with heightened public awareness regarding the risks posed by pathogens in the domestic water supply. This has been further exacerbated by the COVID-19 pandemic, which brought viral health risks in water supply to the forefront of public consciousness (Bhowmick et al., 2020). The pandemic emphasised the broader risks associated with pathogens in various environments, including water sources. This increased public awareness may prompt more attention to ensure consumers that water treatment processes are robust against viral contamination. Partly, this is because illness occurrences contribute to the viruses' transient presence in water sources as shedding rates reflect community infections (Pinon and Vialette, 2018). Environmental reservoirs and transport mechanisms also contribute significantly to viral contamination. Viruses can travel extensive distances through water systems and may find temporary refuge in sediments and organic matter, offering partial protection from degrading environmental factors and leading to periodic reappearances in the water due to catchment processes such as entrainment and resuspension (Gibson, 2014) or

draining down reservoirs during periods of water stress. Lodder et al, (2010) detected 75%, 83%, 100% and 97% of enteroviruses, reoviruses, somatic phages, and F-specific phages respectively from 75 source water samples over a four-year period. It is thought that pathogenic viruses are normally effectively removed by conventional drinking water treatment; however, failure of one or more of these treatment assets can lead to exposure of the virus to consumers leading to a potential public health risk (Asami et al., 2016).

Finally, limitations in monitoring pathogenic viruses in water sources pose challenges. The complexity and vast number of viruses make continuous and comprehensive surveillance difficult, potentially overlooking transient spikes or variations in viral concentrations. High mutation rates, especially in RNA viruses, further contribute to variability in water sources. These rapid genetic changes result in the emergence of new viral strains, complicating detection and posing challenges for water safety. Such genetic variability causes quick and unpredictable changes in the viral landscape of water sources.

In essence, the transient and variable presence of pathogenic viruses in drinking water sources stems from contamination methods, environmental factors affecting viral stability, variability in sources and concentrations, rapid viral evolution, and complexities in environmental transport and monitoring. These factors highlight the importance of adaptable water management strategies and a thorough understanding of viral removal in drinking water treatment processes to protect public health and safety.

1.4 Pathogenic viruses' fate, transport, and removal in drinking water treatment processes

The development and application of advanced water treatment technologies are considered for new innovative water reuse schemes to provide sufficient protection against waterborne viruses and removal in such systems has been extensively studied (Gerba and Betancourt, 2019; Pype et al., 2016). Most water undergoes conventional drinking water treatment processes, yet there remains limited data on their effectiveness, especially for viral removal through coagulation-flocculation, clarification, and filtration (Aronino et al., 2009; Chen et

al., 2021b; Pivokonsky et al., 2024; Zhu, Clifford and Chellam, 2005). Disinfection processes in contrast have been well studied (Hurst, 1991; Kong et al., 2021; Wigginton et al., 2012; Wigginton and Kohn, 2012), but most studies have prioritised bench scale experiments of single unit processes with surrogate bacteriophages (Hijnen, Beerendonk and Medema, 2006) highlighting a potential knowledge gap for studies into the interaction between different treatment processes within flowsheets (this study) and the behaviour of pathogens (beyond scope).

Disinfection is generally quite effective against most microbial contaminants but may not always be sufficient for the removal of viruses in all cases. For example, adenoviruses are resistant to UV radiation primarily due to their double-stranded DNA, which is more resilient to UV-induced damage compared to single-stranded RNA or DNA viruses. This resilience allows many strains of adenoviruses to withstand the DNA damage typically caused by UV radiation (Eischeid, Thurston and Linden, 2011; Oguma, Rattanakul and Bolton, 2016). Coxsackieviruses, a type of enterovirus, tend to be more resistant to chlorine compared to other viruses. This resistance is attributed to their protein capsid, which protects the virus's genetic material from the oxidative effects of chlorine. The robust nature of the capsid in coxsackieviruses provides a strong barrier to inactivation of these viruses (Gerba, Gramos and Nwachuku, 2002; Hill et al., 1971).

Emerging technologies such as membrane filtration, advanced oxidation processes (AOP), and UV irradiation are being increasingly employed to enhance virus removal as part of a multibarrier treatment approach (Al-Hazmi et al., 2022). These technologies offer the potential for higher removal efficiencies but also come with challenges such as elevated operational costs and the need for more sophisticated operational management systems (Al-Hazmi et al., 2022). Understanding the behaviour of viruses in water systems and developing effective strategies for their removal are crucial for ensuring the safety of drinking water (Payment, Armon and Gerba, 1989; Springthorpe and Sattar, 2007) as global population grows and environmental pressures increase enabling decision makers to adapt to the evolving challenges posed by viral pathogens (Perveen

and Amar-UI-Haque, 2023; WHO, 2017). In conclusion, the presence of human viral pathogens, both emerging and existing, poses a challenge for water utilities in the provision of safe, wholesome, and compliant drinking water (Favere et al., 2020). Subsequent sections will provide context to viral fate and transport through different treatment processes.

1.4.1 Physical and chemical removal processes

Conventional drinking water treatment generally consists of coagulation, flocculation, sedimentation, filtration, adsorption, and disinfection steps (Garfí et al., 2016; Hurst, 1991; Piri, Homayoonnezhad and Amirian, 2010). Generally, two types of processes govern the removal of viruses in water treatment: physico-chemical processes which primarily focus on the separation of colloidal particles in processes such as coagulation and chemical disinfection processes which act as the final barrier against viral loading into distribution (Asami et al., 2016). Each stage plays a role in removing both natural organic matter (NOM) and microorganisms, yet the interaction between viruses and these treatment processes determines the efficacy of their removal. Also, source water factors such as the composition of NOM could determine how viruses adhere to particles (Ren et al., 2023). Virus-particle interactions in water vary with diverse types of NOM, catchment characteristics, and water chemistry factors like pH and ionic strength.

Steps such as coagulation and flocculation and subsequent clarification primarily focus on the removal of NOM including humic substances, fulvic acids and other organic compounds (Matilainen, Vepsäläinen and Sillanpää, 2010).. For instance, Ren et al., (2023) determined that the presence of NOM improved the removal rate of MS2 in an ultrafiltration (UF) system, with the main removal mechanisms being size exclusion with the number of viruses retained on the membrane surface being influenced by raw water NOM composition.

Chemical additions lead to charge neutralisation by diminishing repulsive forces between colloidal particles due to the reduction of the electrical double layers which results in the formation of micro-particles. These micro-particles collide and aggregate during the flocculation process, forming larger particles known as

flocs. These flocs are then removed in subsequent clarification steps, which include sedimentation or dissolved air flotation (Matilainen, Vepsäläinen and Sillanpää, 2010). The surface charge of viruses influences the removal by particle adhesion, with the addition of chemical coagulants aiding in the adhesion of the virus to particulate matter to form the floc. This can then be removed by clarification steps, ultimately reducing the viral load in the post-treated water (Zhu, Clifford and Chellam, 2005). The effectiveness of virus removal in water treatment through coagulation can be markedly different depending on whether the process is dominated by charge neutralization or sweep flocculation, each having distinct mechanisms (Li et al., 2006).

In charge neutralization, coagulants with a positive charge are introduced into the water. These coagulants work by neutralizing the charges on suspended particles, including viruses, which typically carry a net negative charge in water environments. As the electrostatic repulsion between these particles diminishes, they come closer together, forming microflocs (Oriekhova and Stoll, 2014). However, the limitation of this method lies in its efficiency, particularly for viruses. Due to their small size compared to other suspended particles, simply neutralizing a virus's charge does not guarantee its aggregation into larger particles or flocs for effective removal (Matsushita, Matsui and Shirasaki, 2006). Viruses may not bind effectively to larger flocs in charge neutralisation due to numerous factors beyond size disparity. Firstly, the unique surface properties of viruses, particularly their protein coats, can impede interaction and incorporation into flocs. Additionally, the balance between hydrophobic and hydrophilic properties on virus surfaces might not align with those of floc particles, leading to poor binding. The stability and mobility of viruses in water, even after charge neutralization, can also prevent them from settling or being trapped within the flocs. Moreover, the irregular shapes and structures of viruses compared to other particles can hinder physical entrapment or adherence to the flocs.

On the other hand, sweep flocculation operates through a different principle. In this process, the addition of coagulants leads to the formation of a precipitate or 'sweep floc.' These flocs physically sweep through the water,

ensnaring and adsorbing suspended particles, including viruses (Zhu, Clifford and Chellam, 2005). The mechanism here is less reliant on charge interactions and more on the physical entrapment of viruses to/within the larger flocs. This physical entrapment allows for the removal of a broader range of particle sizes, including the smaller virus particles, and is thought to be less influenced by viral properties as a mechanism. Possibly, making sweep flocculation in general terms more effective for virus removal compared to charge neutralisation.

The choice between charge neutralization and sweep flocculation in water treatment primarily depends on various factors of the water source, such as the type and concentration of suspended particles, the specific nature, and the overall chemical composition of the water but rarely the concentration of microbial contaminants such as viruses present (Tanneru, Rimer and Chellam, 2013). However, charge neutralisation efficiency decreases in low-turbidity waters where floc formation is limited, which is essential for virus capture. In high dissolved organic carbon waters, alternative treatments are needed for removal of particulate and dissolved contaminants (Cruz et al., 2020). A major downside of sweep flocculation is higher coagulant use, increasing costs and sludge volume, which requires additional treatment and disposal. Excess coagulant can also impact water quality, potentially breaching drinking water standards for certain metals. Therefore, while effective for virus removal, sweep flocculation's applicability and cost should be assessed based on water source characteristics (Matilainen, Vepsäläinen and Sillanpää, 2010).

In water treatment, the filtration mechanisms employed in Rapid Gravity Filters (RGFs), Slow Sand Filters (SSFs), and Granular Activated Carbon (GAC) filters influence virus removal, each with distinct approaches and effectiveness. In the context of RGFs, collision refers to the physical contact between virus particles and the filter media. As water passes through the filter, the flow dynamics and the movement of the water bring the virus particles into contact with the grains of the filter media, such as sand or gravel. This collision is influenced by various factors, including the flow rate of the water, the size and shape of the filter media, and the size and characteristics of the virus particles themselves (Asami et al.,

2016). Once a collision occurs, the virus particles adheres to the surface of the filter media after colliding with it during interception. This adhesion can be influenced by a range of factors, such as the electrostatic charges between the virus and the filter media, the hydrophobicity of the virus surface, and the presence of coagulants in the water reducing repulsion between virus and media which both have a net negative charge thus promoting the aggregation of virus particles, thereby increasing the likelihood filter media-viral interaction and thus successful filtration (Diamadopoulos and Vlachos, 1996). The effectiveness of RGFs in virus removal through collision and adhesion is notable, especially when the filter media is carefully chosen and the operational parameters, like flow rate and coagulant use, are optimised. This makes RGFs a reliable choice in water treatment processes for reducing many microbial contaminants including virus (Zhu, Clifford and Chellam, 2005).

SSFs, operating at slower rates with finer media, are highly effective in virus removal. Like RGFs, they employ physical mechanisms of collision and adherence. However, a key feature of SSFs is the development of a biological layer, or *schmutzdecke*, on the filter surface (Weber-Shirk and Dick, 1997). This layer hosts a diverse microbial community that contributes to virus degradation or inactivation, combining physical straining with biological degradation to effectively remove viruses.

In contrast, GAC filters, traditionally known for their adsorption capabilities, are generally less effective for virus removal compared to RGFs and SSFs (Hijnen et al., 2010). The primary reason is that virus removal in GAC systems is lesser, is that the technology is designed for micropollutant adsorption whereas viral capture is thought to be more reliant on filtration mechanisms. Due to the structure and function of GAC, it does not provide the same level of filtration efficiency as RGF or biological activity or filtration efficiency seen in SSFs (Hijnen et al., 2010). Consequently, while GAC is excellent for adsorbing organic compounds, its efficiency in virus removal is typically lower than the other filtration methods. Taken together, these insights highlight the importance of choosing the

appropriate filtration method in water treatment processes based on the specific requirement for virus removal.

In drinking water treatment, membrane processes like microfiltration, ultrafiltration, nanofiltration, and reverse osmosis are highly effective in removing viruses through mechanisms such as size exclusion and adsorption. Microfiltration and UF work predominantly on the principle of size exclusion, physically blocking viruses based on their size relative to the pore sizes of the membranes. The smaller the pore size, as seen in ultrafiltration, the more effective the removal of even smaller virus particles. Nanofiltration and reverse osmosis, with even finer membranes, provide a higher level of purification, effectively removing viruses through a combination of size exclusion and adsorption. In these processes, the tight membrane structure not only physically blocks viruses but also allows for the adsorption of viruses onto the membrane surface, further enhancing removal efficiency. These membrane technologies can ensure the production of safe, virus-free drinking water (Goswami and Pugazhenthir, 2020). As these technologies are not considered in subsequent chapters as they are unavailable to test in this study, the reader is referred to reviews in this topic (Chen et al., 2021a).

The most commonly utilised method to inactivate viruses in drinking water treatment is the application of oxidative treatments such as ozone, chlorine, and UV-based disinfection processes (Jalali Milani and Nabi Bidhendi, 2022). Less frequently applied treatment processes are AOPs commonly applied for reuse-based water treatment flowsheets. These methods are highly effective at inactivating and removing a broad spectrum of viruses, by disrupting the outer protein/lipid coat, capsid, or genetic material (DNA or RNA) of viruses. This damage prevents the virion from replicating or attaching to host cells, thus rendering them non-infectious or sufficiently inactivated to be non-detectable by standard methods (i.e., culture-based approaches) (Kong et al., 2021).

1.4.2 Impact of High Turbidity and NOM on Viral Disinfection

In oxidative drinking water treatments such as chlorination, turbidity presents a significant challenge to the disinfection of viruses (Heffron, Ryan and Mayer,

2019). The suspended particles inherent in turbid water can shield viruses from the disinfectant, thereby reducing its efficacy. This protective effect is more pronounced for particle-associated viruses due to their direct attachment to the particles (LeChevallier, Evans and Seidler, 1981). Even bacteria-associated viruses, which have an additional layer of protection from their bacterial hosts, can be further shielded by the physical barriers created by turbidity. Elevated levels of turbidity can necessitate adjustments in the chlorination process, specifically in terms of increasing the disinfectant concentration to ensure effective viral inactivation (Jalali Milani and Nabi Bidhendi, 2022).

The presence of organic matter in water undergoing chlorination has a two-fold impact on viral disinfection. Firstly, organic compounds can react with chlorine, leading to the formation of disinfection by-products (DBPs), which have potential health implications (Mazhar et al., 2020). Secondly, this reaction reduces the concentration of available free chlorine, diminishing its capacity to effectively inactivate viruses (Gray, 2014). Furthermore, organic matter in downstream drinking water distribution systems facilitates the formation of biofilms, which can harbour viruses and provide them with additional protection against chlorine (Ndiongue, Huck and Slawson, 2005).

To counteract the challenges posed by high turbidity and organic matter, pre-treatment processes such as coagulation, flocculation, and filtration are essential to condition water (Melo et al., 2019). This is achieved by reducing the levels of turbidity and organic matter before the application of chlorine or UV (Jarvis et al., 2019). The treatment process also requires continuous monitoring, including the assessment of turbidity, organic matter content, and chlorine residuals, to dynamically adjust the treatment strategy and maintain the efficacy of disinfection or effective viral inactivation (Rachmadi et al., 2020). A balanced approach is mandated, one that ensures effective viral disinfection while minimizing DBP formation and maintaining water quality (Mazhar et al., 2020). This necessitates a careful evaluation of the initial water quality and ongoing adjustments to the treatment parameters to ensure that the disinfection goals are attained. Overall, chemical disinfection following other oxidative processes is typically utilised as

the final barrier against viruses in drinking water treatment however, assessing physico-chemical methods such as coagulation for removing particulate matter and viral load may be equally, if not more important from the viewpoint of risk management (Asami et al., 2016). Asami (2016), suggested that true disinfection efficiency of a system is difficult to establish due to the low concentrations of indigenous viruses in treated water. Therefore, establishing the efficiency of upstream processes on the reduction of viral load is essential to optimise disinfection processes and increase viral risk management for water utilities and drinking water treatment operators.

Accordingly, this research aims to explore the effects of viral load under scenarios where physico-chemical processes like coagulation are operating 'optimally' versus conditions whereby process parameters such as turbidity are not being removed effectively i.e., 'suboptimal.' Additionally, it will assess how each stage of the water treatment process influences subsequent stages in the overall treatment of the flowsheet under different concentrations of virus challenge. This study will employ coliphages as a surrogate for pathogenic virus as explained in the subsequent section.

1.5 Enumerating somatic coliphages

The International Organization for Standardization (ISO) Standard ISO10705 2:2000 (British Standards Institution., 2000) details methodology for isolating, detecting, and quantifying the somatic coliphage Phi-X 174 (Φ -X 174). Φ -X 174 is part of the *Microviridae* family of bacteriophages and is detailed as a small, non-tailed, icosahedral virus with a circular single-stranded DNA which infects the host *E. coli*. In the ISO standard, standardised strains of *E. coli* (Migula) Castellani and Chalmers (ATCC 13706) and Φ -X 174 are applied used (Figure 1) (Michel et al., 2010).

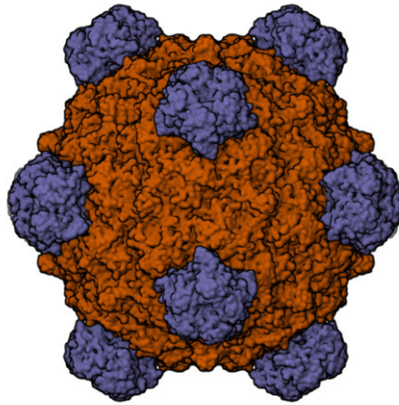


Figure 1: Φ -X 174 atomic X-ray structure. Φ -X 174 is a single-stranded bacteriophage which infects the host organism *E. coli* (ATCC 13706) (Michel et al., 2010).

This process involves introducing the water sample into a culture of the host bacteria, thereby creating an environment conducive to the replication of somatic coliphages. To quantify the coliphages present in the sample, enumeration techniques such as plaque assays or PCR methods are utilised. The ISO method relies on the ability of a single virus particle, or phage, to infect a bacterium, initiating a cycle of replication that culminates in the destruction of the cell (Abedon, Duffy and Turner, 2009; British Standards Institution., 2000). When this process manifests across a bacterial lawn on an agar plate, it results in the formation of clear zones, known as plaques. Each plaque is essentially the footprint of a single virus infectious unit and spread (British Standards Institution., 2000) which manifest as clear zones against the opaque background of the bacterial lawn. After a suitable period of incubation, these plaques are counted.

Each plaque observed in a plaque assay theoretically represents a colony of a single somatic coliphage from the original water sample. Counting these plaques, while considering dilution factors, allows for the determination of coliphage concentration in the sample. In summary, the plaque assay is a specific and sensitive method for detecting and quantifying somatic coliphages, playing a key

role in water quality assessment, especially for testing faecal contamination (British Standards Institution., 2000).

1.5.1 Concentrating drinking water viruses

Due to the dilute nature of the matrix, especially from water at inter-stage of the drinking water treatment plant and final water, the concentration of viruses is often necessary. Several methods are commonly applied, these include electronegative filtration, electropositive filtration, ultrafiltration, and techniques like polyethyleneglycol precipitation and centrifugation. The efficiency of these methods can be influenced by factors such as the type of virus, the water matrix, and the sample volume. The choice of concentration method is important as it affects the recovery of viruses and, consequently, the reliability of detection methods like the plaque assay. Different methods might be more suitable depending on the specific viral type targeted and the water source being tested. Each method's efficiency in concentrating viruses can directly influence the ability to detect phages in the water sample reliably (Cashdollar and Wymer, 2013). The volumes of water range from 100 ml to 10 L in raw waters to several thousand litres in final treated drinking water. Concentration of viruses whilst important was not the focus of this research as viruses were spiked into experiments at levels whereby, they could be reliably quantified without significant extra quantification.

1.5.2 Limitations

The somatic coliphage method has certain limitations that are important to consider, particularly in terms of viral aggregation and sub-lethal damage. One of the limitations arises from viral aggregation, a phenomenon where multiple virus particles clump together. This can be problematic in a plaque assay as these aggregated viruses may form a single plaque, leading to an underestimation of the actual virus count. This single plaque is often counted as originating from one virus particle, rather than the multiple ones which could be present. Factors such as the ionic strength of the water, the presence of organic matter, or the sample preparation procedures can influence this aggregation (Pradhan et al., 2022).

Another significant limitation is the occurrence of sub-lethal damage to the viruses. This refers to a state where the viruses are partially inactivated but still capable of initiating an infection (Sharan, Chhibber and Reed, 2011). Such viruses may not efficiently form clear plaques, which can lead to an underrepresentation of their presence in the water sample. Environmental stresses like UV radiation or chemical disinfectants can cause this sub-lethal damage, affecting the plaque assay's reliability in quantifying viable viruses. In contrast, light and dark repair mechanisms are fundamental cellular processes employed by viruses and other cells to rectify DNA damage. These mechanisms maintain the integrity and stability of genetic information. Light Repair, known as photoreactivation, is a mechanism designed specifically to address DNA damage caused by UV light, such as pyrimidine dimers, notably thymine dimers, within DNA (Weinbauer et al., 1997). This process relies on light exposure, particularly UV-A and visible light, to activate the enzyme photolyase. When photolyase binds to the damaged DNA, it breaks the dimer bonds and restores the DNA's original structure (Bowman et al., 2000). In contrast, dark repair mechanisms, notably Nucleotide Excision Repair (NER), operate independently of light conditions (Reardon and Sancar, 2005). NER is adept at recognizing various damages that distort the DNA helix, including UV-induced dimers. This repair process involves several stages: identifying the damage, excising a short segment of single-stranded DNA containing the damage, synthesizing a replacement DNA segment using the undamaged strand as a template, and finally, rejoining the DNA to ensure its continuous double-stranded structure (Reardon and Sancar, 2005). Viruses lack their own repair mechanisms, including light repair, as they depend on host cells' machinery for replication and repair. When a virus infects a host cell, it may indirectly benefit from the host's DNA repair mechanisms if its genetic material is damaged. For example, if a bacteriophage infects a bacterium that has light repair mechanisms, the bacteriophage's DNA might be repaired by the host bacterium's enzymes. Given these limitations, careful interpretation of the results from plaque assays is essential.

Recognizing the limitations of the plaque assay, including viral aggregation and sub-lethal damage, is key to accurately interpreting its results and ensuring

reliable water quality assessments. Factors like virus type and differing infection dynamics for host strain and assay conditions affect the assay's sensitivity and quantification limits. Reported limits of detection for somatic coliphage are not well established. However, data for Lymphocytic Choriomeningitis Virus (LCMV) suggest that the assay's sensitivity can be quite high, detecting as low as 10 PFU/mL of the virus under optimal conditions (Cordey et al., 2011; Korn Johnson and Homann, 2012). This level of sensitivity is achieved through careful optimization of the assay conditions, particularly when using techniques like reverse transcription RT-PCR for the detection of LCMV. This method is known for its high reproducibility and sensitivity, capable of detecting very low quantities of viral RNA, which corresponds to low levels of infectious virus. However, the number of viral RNA copies present cannot always be directly correlated with infectious virus. This is especially true in the case of LCMV, where the presence of defective interfering virions can be a factor. Despite these complexities, advancements in detection methods have significantly improved the sensitivity and efficiency of viral detection in various samples. However, these values can vary based on the method and duration of incubation used in the assay. Additionally, it's important to consider the susceptibility of the host to the phage and the possibility of phage replication in the environment, which can lead to false positives. These factors highlight the need for careful interpretation of the results and, in some cases, the use of supplementary methods to confirm findings.

1.6 Policy and Governance

Accurately assessing the risk of viral infection from tap water requires quantifying the concentration of viruses in the final water post-treatment. However, monitoring viruses through treatment is practically challenging due to their low concentrations (Kreißel et al., 2014). As such, the management of drinking water quality has shifted towards a risk-based approach, prompting water utilities to consider enhanced monitoring of viral indicators in response to proposed regulations at both European and UK levels. In 2022, the Public Water Supplies (Scotland) Regulations introduced a control threshold of 50 plaque-forming units (PFU) per 100 mL of somatic coliphage in raw water, instigating a risk-based

strategy to assess viral characterization in the source water. In essence, it separates pristine groundwater likely unaffected by faecal contamination with either surface water contaminated by agricultural run-off or wastewater inputs, or groundwater affected by surface water ingress. This approach triggers a comprehensive risk assessment of the extended treatment process upon surpassing the threshold, aiming to detect and manage potential pathogenic virus risks in line with the revised EU drinking water directive (European Union, 2022). Currently, there is no specific legislation covering somatic coliphages in England, Wales, or Northern Ireland.

The WHO guidance and EU legislation also emphasizes verification monitoring of final water using somatic coliphage, setting viral treatment targets from 1-2.5 log removal values (LRV) for media and membrane filtration processes in their Drinking Water Parameters guidelines (WHO, 2017). The risk-based approach from the WHO guidelines serves the purpose of identifying optimal management interventions and control measures, effectively mitigating hazards to prevent potential risks to consumers. This strategy focuses on the implementation of operational monitoring systems that continuously evaluate and address potential threats to water quality. This encompasses establishing treatment performance targets based on source water monitoring to ensure the required log removal of pathogens. Verification monitoring, particularly for somatic coliphage in finished drinking water, serves as a step to confirm the water supply system's efficacy in eliminating enteric viruses and maintaining water safety standards (WHO, 2017). The end product standard of 0 PFU per 100 ml is the final part of the EU legislation. Exceedance of this standard suggests non-compliance due to unacceptable risks of pathogenic viruses.

Determining the necessary treatment performance involves aligning with WHO's definition of safe drinking water, which equates to meeting a disease burden target of 10^{-6} disability-adjusted life years (DALY). This is translated to concentrations of reference pathogens in drinking water that correspond to implied safety levels of the water for operational source water monitoring. One approach entails determining the concentrations of reference pathogens in the

source water by undertaking site-specific assessments, which provides a good evidence-base for establishing performance targets in treatment (WHO, 2017). However, the WHO report acknowledges, that this is a high cost and skill requirement for this methodology. Thus, the source appraisal approach is mandated via establishment of faecal contamination levels based on catchment via with *E. coli* monitoring in the source water (WHO, 2017). This, 'risk-based' monitoring of *E. coli* to establish the relative levels of contamination and likely frequency of events which could compromise source waters quality or veracity of drinking water treatment processes such as heavy rainfall periods. Treatment performance targets are inferred from the established level of faecal contamination and frequency of monitoring for somatic coliphage through catchment appraisal (WHO, 2017) (Appendix A). Central to this information is the standard requirements for less than 50 PFU/ 100 mL in the source water and the subsequent requirement to demonstrate the veracity of the treatment works in regard to LRV targets alongside verification monitoring of somatic coliphage of the end product translates into a framework for frequency of testing for somatic coliphage in different water sources to minimise viral risk to acceptable levels.

1.7 Optimal and sub-optimal treatment for virus removal

While bench-scale investigations offer controlled settings to manipulate virus concentrations and system variables, their limitations lie in replicating real plant operational conditions accurately. For instance, during batch bench-scale coagulation/sedimentation trials, minor disturbances within the confined space of small containers can significantly impact virus removal, potentially leading to variable results – detected from full scale systems, which tend to be larger and have continuous plug flow. Conversely, larger-scale processes are less susceptible to these errors, providing a more accurate reflection of removal efficiencies (Asami et al., 2016).

Furthermore, operational dynamics within full-scale water treatment plants, such as variations in raw water quality and the consequential adjustments in plant operational parameters, exert considerable influence on virus removal (Havelaar, van Olphen and Schijven, 1995). Therefore, comprehensive assessments of

virus removal efficacy conducted at pilot-scale drinking water pilot rigs operating under near-real-world conditions become imperative to accurately gauge the microbial risk. Currently, studies undertaking these controlled simulations appear limited in the literature. Evaluating virus removal performance in these operational environments would ensure a more realistic assessment of the efficiency and reliability of treatment processes in mitigating potential microbial and specifically viral threats. While viral removal through treatment processes has been reported previously (Albinana-Gimenez et al., 2009; Ali, Al-Herrawy and El-Hawaary, 2004; Chen et al., 2021b), there is a gap in the literature regarding the fate of viruses through conventional treatment both under optimised operation, and the instance where a process upstream is operating under sub-optimal conditions.

1.8 Rationale

This work aims to evaluate the effectiveness of the drinking water treatment train as a means to govern operational guidelines for water utilities to optimise their viral removal via conventional water treatment. 'Challenge' trials using somatic coliphage were conducted under both normal and sub-optimal operating conditions, where conditions could be conducive for viral breakthrough to occur. Treatment challenges are determined by bench-scale testing including pH and conventional jar testing for coagulation processes which govern removal capability of individual treatment processes when tested at near-real scale. Lastly, the study aims to determine the correlation, if any, between flow cytometric analysis, water chemistry data, such as turbidity or particle counts and the passage of viruses through the treatment processes. These parameters were selected as key operational parameters which are routinely assessed by water utilities in the UK. In instances where pathogenic microorganisms are detected in water or granular media, the results of this study will help establish effective treatment strategies for their removal. The experimental approach taken in this study offers a novel contribution to the field, further advancing the practical aspects of virus removal in water treatment processes and identify strategies for

water utilities to control pathogenic microorganisms in drinking water. Finally, future research is suggested to advance this nascent field of research.

1.9 Aim

The primary objective of this project is to assess the effectiveness of current drinking water treatment processes in mitigating the risks associated with viral contaminants through quantifying the removal in individual treatment processes and an integrated flowsheet. The anticipated outcome is to provide water utilities with data-driven insights and recommendations for optimizing their treatment strategies for virus risk management. Ultimately, contributing to the development of more robust and efficient protocols for ensuring the safety and reliability of drinking water from a virological perspective. A series of objectives and their corresponding hypothesis is presented (Table 1).

1.10 Objectives

Table 1: Brief objectives and their related hypotheses

Objective	Hypothesis
Undertake a series of challenge trials using suitable virus(es) and somatic coliphage under treatment challenges and normal operating conditions.	The concentration of somatic coliphage will increase under sub-optimal coagulant dose. Optimal coagulant dose will be effective in removing large quantities of somatic coliphage by ensuring better particle removal efficiency.
Identify the removal capability of individual treatment processes considering standard approaches for risk reduction.	At pilot-scale, most somatic coliphages will be removed by coagulation and clarification and corresponding particle aggregation and removal mechanism, disinfection will produce 0 PFU/100 mL in the final water through action of chlorine reducing coliphage (viral)viability.
Identify the correlation, or lack of, between water chemistry data such as	Log removal of somatic coliphage will be positively correlated to removal of (a)

turbidity, UV₂₅₄ or particle counts and the passage of coliphage and viruses through treatment processes.

turbidity, and (b) total cell count (TCC) and intact cell counts (ICC) as most of the virus will be particle-associated therefore correlated with particle removal mechanisms and measurements but not directly with UV₂₅₄ as a surrogate for organic material.

To achieve these objectives, bench-scale and pilot-scale testing was achieved to determine the effect of coagulation under optimal and sub-optimal conditions and the impact this has on treatment processes downstream. Coagulation was optimised for bench-scale and pilot-scale experiments for coagulant dose, pH, and removal of somatic coliphage using water derived from Chicheley Brook, England (52.079049, -0.627952). This water is not typical, being partly derived from runoff of nearby Cranfield campus and airport and surrounding reach. Thus, the water from this source has relatively low alkalinity, turbidity, and NOM. The NOM is challenging to treat on account of it being low molecular weight and relatively hydrophilic. However, with several wastewater and agricultural inputs upstream of this intake, there is the potential for significant microbial contamination – reflecting most lowland water sources in England.

2 Addressing Viral Risk in Drinking Water: Evaluating the Impact of Treatment Processes in Drinking Water

2.1 Introduction

The provision of safe, wholesome drinking water encounters significant challenges in the presence of pathogenic viruses, which challenge water utilities' efforts to ensure compliance of safety standards (Favere et al., 2020). The presence of these pathogens primarily arises from faecal contamination or inadequacies in drinking water treatment processes (Gibson, 2014). Despite the effectiveness of conventional treatment methods for virus removal, inefficiencies or failures in these processes poses a potential and poorly quantified public health risk (Asami et al., 2016).

Conventional water treatment encompasses a series of steps, including coagulation, filtration, and disinfection, largely aimed at removing NOM and pathogens (Garfí et al., 2016; Hurst, 1991; Piri, Homayoonnezhad and Amirian, 2010). The removal of viruses primarily occurs through mechanisms such particle adhesion and size exclusion during these treatment phases, reinforced by subsequent oxidative disinfection such as ozone and chlorine (Kong et al., 2021) and physical methods such as UV disinfection. Notably, the assessment of viral log reduction via physico-chemical methods, especially coagulation, remains critical for comprehensive viral load reduction and effective risk management strategies due to its vital role in condition the water for filtration and disinfection (Asami et al., 2016).

Monitoring viral indicators, notably somatic coliphages plays a key role in identifying viral contamination, aiding in rapid faecal pollution detection within water sources. Φ -X 174 is a bacteriophage commonly used as a surrogate for quantifying viral pollution in drinking water treatment, enumerated by the methodology ISO-10705 2:2000 (British Standards Institution., 2000). Regulatory bodies such as the WHO advocate for enhanced monitoring of viral indicators and have set stringent treatment log removal targets of pathogenic viruses to ensure sufficient removal of viruses and water safety (WHO, 2017). Emphasizing

a risk-based approach, regulatory standards such as the Public Water Supplies (Scotland) Regulations suggest continuous assessment and a limit of 50 PFU / 100 mL in the source water an approach adapted from the recast drinking water directive of the EU (European Union, 2022).

To investigate viral removal, bench-scale studies offer controlled evaluations but are often limited in replicating full-scale conditions. A crucial knowledge gap exists in comprehensively assessing treatment efficacy at near-real-scale and its correlation with broader water quality indicators within the whole treatment train. As such, evaluating viral loads at pilot-scale, within representative operational environments offers a distinct understanding of treatment process removal efficiency (Asami et al., 2016). To further supplement this, the research establishes correlations between somatic coliphage removal and key water quality indicators such as turbidity, UV₂₅₄, TCC, and ICC. This analytical approach identifies potential predictive markers for viral passage through treatment processes, aiding in the early detection of operational inefficiencies or viral breakthroughs.

This study aimed to systematically evaluate the efficacy of drinking water treatment processes at pilot-scale in mitigating viral risks, focusing on Φ -X 174 as a representative indicator – also used within the ISO standard for enumeration. The primary objectives involved controlled challenge trials simulating optimal and sub-optimal coagulation treatment conditions. These trials clarify how varying operational scenarios affect viral concentration, providing insights into the treatment process' robustness. The investigation includes bench-scale and pilot-scale experiments to optimise coagulation parameters, focusing on coagulant dose and pH. This optimisation seeks to determine the most effective operational conditions that maximise the removal of Φ -X 174, contributing to enhanced viral risk management strategies.

In addition, emphasis was placed to evaluate practical application of WHO directives in real-scale treatment scenarios, specifically assessing the suitability of somatic coliphages for verification monitoring. Instead of solely pursuing specific LRV targets, the focus lies in comprehensively understanding treatment

efficacy and correlations between key water quality indicators and viral removal. By exploring these aspects, this project seeks to contribute evidence for refining and implementing effective regulatory standards in line with the risk-based approach.

2.2 Materials and Methods

2.2.1 Virus

The viral indicator organism used throughout this study was the somatic coliphage ϕ -X 174 a bacteriophage host-specific to a susceptible *E. coli* strain ATCC 13706 (American Type Culture Collection, 2023). Preparation and enumeration of the somatic coliphage was conducted as per the British Standards ISO 10705-2:2000 (British Standards Institution., 2000) where coliphages were enumerated using the double agar overlay plaque assay method. In short, 2.5 mL of semi-solid Modified Scholtens' Agar spiked with calcium chloride (1mol/L) and 1 mL of the sample containing the somatic coliphage was diluted in series and added to 1 mL of the *E. coli* previously cultured in Modified Scholten's Broth and poured on a petri dish containing 10 mL of pre-prepared complete Modified Scholten's Agar. The plates were incubated at (36 ± 2) °C for 18 hours and the plaques counted, and converted to a volume tested in PFU/mL using Equation 1.

Equation 1: Plaque count procedure

$$X = \frac{N}{F \times (100 \text{ mL})}$$

Where:

X is the number of plaque-forming units of somatic coliphage per 100 mL (PFU/ 100 mL)

F is the dilution factor

N is the total number of plaques counted on the plate

2.2.2 Flow cytometry assessment for bacteria

Flow cytometry provides an indirect assessment of the total bacterial population in a sample. With dyes and gating used to distinguish live and total cells from background (Cheswick et al., 2020). TCC and ICC was measured via flow cytometry for each sample. Cell concentration was determined via flow cytometry alongside viability assessment. If a sample displayed a TCC or an ICC concentration surpassing 1,000,000 and 800,000 cells/mL respectively, it was diluted using sterilised Evian water filtered through a 0.2 μm polyethersulfone filter. Following dilution, samples were prepared for TCC and ICC detection via fluorescence staining. For TCC detection, a stain was produced by diluting SYBR Green I (10,000 $\times$ stock. S-7567; Thermo Fisher Scientific, UK) with Dimethyl sulfoxide (DMSO) filtered to 0.22 μm (Z290807, Sigma-Aldrich, UK), yielding a working concentration of 100 $\times$ SG. ICC detection stain was created by combining five parts of 100 $\times$ SYBR Green I and one part of Propidium Iodide (1mg.mL⁻¹, corresponding to 1.5 mM; Thermo Fisher Scientific, UK). To attain this measurement, water samples were pipetted into a 96-well plate and stained with the fluorescent stain SYBR Green I for total cells and SYBR Green I and Propidium Iodide for intact cells according to (Claveau et al., 2024). The samples were incubated for 15 minutes at 37°C in the dark. The plate is transferred to the flow cytometer (BD Accuri C6) and each sample run at fast flow rate (66 $\mu\text{L}/\text{min}$) with 50 μL sample size. Industry standard fixed gates (Gatza, Hammes and Prest, 2013) were applied to determine the TCC and ICC separated from low nucleic-acid background noise (Hassard et al., 2019).

2.2.3 Background *E. coli* levels in the water

To assess which source water category the water used for the experiments are, the background *E. coli* was measured by taking a sample aseptically from the abstraction point of Chicheley Brook. The standard method for enumeration of *E. coli* by the American Public Health Association was undertaken (Rice, Baird and Eaton, 2017) In short, 100 mL of the sample is filtered through a sterile, 0.45 μm membrane filter under a partial vacuum. The filter paper is aseptically transferred to a petri dish of McConkey agar and incubated at 35°C $\pm$ 0.2°C for 18-24 hours.

The colonies were counted, and the colony-forming units (CFU)/100 mL calculated. For quality control, triplicate petri dishes were inoculated alongside a negative control and two sampling events were undertaken. It is acknowledged that this is unlikely to reflect the *E. coli* concentration throughout the trial, rather provides a snapshot of the faecal loading for this catchment. For comparison purposes it is recommended to have extensive longitudinal data on faecal pollution within drinking water sources. However, this was not available for the NRF-WWT.

2.2.4 Background *E. coli*

In order to establish the classification of the source water according to the WHO guidelines (WHO, 2017), the background *E. coli* was quantified using the river water from the abstraction point. Two sampling events were undertaken, one following a period of rainfall and another following dry weather (Table 2**Error! Reference source not found.**).

Table 2: Background *E. coli* levels from Chicheley Brook after two sampling events

Sampling event	<i>E. coli</i> results (CFU/ 100 mL)
Post-rainfall	1.6-2.0x10 ³
Post-dry weather	1.7-2.0x10 ³

Based on the assessment within Appendix A, the upper bounds of detected *E. coli* i.e., 2000 align with a contaminated which was on the threshold for a heavily contaminated watershed. Appendix A suggests that at this contamination level, we would expect it to be located in a region with villages and intensive agriculture using manure or wastewater sludge, processes human waste before releasing it into the water however the area sampled is not directly affected by wastewater discharges.

2.2.5 Bench-scale coagulation testing

Bench-scale coagulation trials were conducted using jar tests (Figure 2) (programmable jar tester PB-900, Phipps and Bird, United States). Water was obtained from the National Research Facility in Water and Wastewater Treatment (NRF-WWT) storage tank. Water was abstracted ($\sim 20 \text{ m}^3$) from Chicheley Brook (52.079049, -0.627952) circa monthly with 5 m^3 abstracted daily until tank reached capacity. 5 m^3 was abstracted to comply with abstraction licences. This storage reservoir was at ambient temperature, which was $4\text{-}14^\circ\text{C}$, the raw water was stored for up to 4 weeks and the tank was briefly drained and then cleaned via pressure wash between subsequent abstractions. Real raw water was used to provide the pilot rig with a feed representative to full scale to ensure that the results obtained have bearing on the complexity and dynamic situation experienced at full scale drinking water treatment plants. This is compared many other studies which are primarily undertaken at either bench or pilot-scale using artificial drinking water matrices. Prior to pilot scale testing, jar tests were undertaken to determine the optimum coagulant dose and pH for the real water and for the removal of spiked somatic coliphage. Iron (III) Chloride (45% purity) (Sigma Aldrich, 2023) was selected as the coagulant for all experiments.

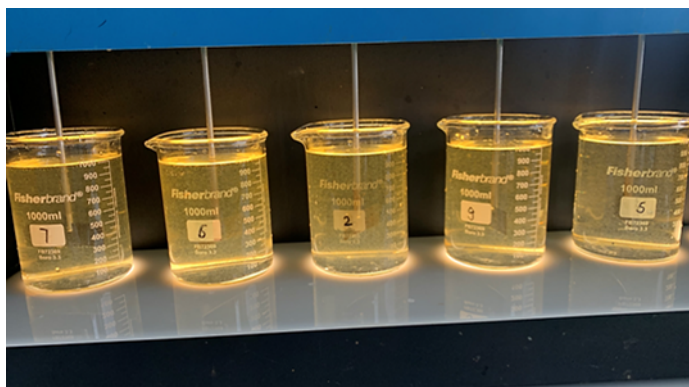


Figure 2: Jar tester setup using the Phipps & Bird PB-900 jar tester.

A standardised jar testing protocol was followed for all tests (Figure 2). In short, 1 L Pyrex® glass beakers were placed under the bench-scale jar tester (Phipps and Bird PB-900) and pre-programmed as followed: a 1.5-minute rapid mix at 200 rpm where the coagulant was added, and the pH was adjusted after

coagulant addition, using small drops of 1M sodium hydroxide or 1M hydrochloric acid (Sigma Aldrich, 2023), and validated using ion selective pH probe. Then a 15-minute mix at 30 rpm and a 10-minute floc settling time at 0 rpm was undertaken (Fisher Scientific, 2023). Samples were then taken at half-depth and settled turbidity and UV₂₅₄ were measured based on Standard Methods 2130 B and 5910 (Standard Methods Committee of the American Public Health Association, 1995). The range of coagulant doses tested included 0, 5, 10, 12, 15, 17 and 20 mg-Fe³⁺/ L at pH 5, 6, 7 and 8. The dosage of the coagulant was determined based on its mass, considering the molar mass of Iron (III) chloride. After establishing the optimal coagulant dose and pH levels, the experiment was repeated to evaluate their effectiveness in the removal of the somatic coliphage Φ-X 174 at each coagulant dose and pH. Enumeration of the coliphage was conducted as per section 2.2.1. Coagulant doses comprised of 0, 2, 5, 10, 15, 20 and 30 mg-Fe³⁺/ L at pH 5, 6, 7 and 8, with pH 5-7 being most reflective of coagulation conditions using FeCl₃ in lowland surface waters from the East of England Region.

2.2.6 Pilot Testing

2.2.6.1 Pilot rig set-up

To assess the scalability of bench-scale jar tests to a larger, continuous drinking water treatment system, comprehensive experiments were conducted using a near-real scale, continuous flow drinking water pilot rig. The raw water for these experiments was sourced from Chicheley Brook. It was stored in advance to ensure an adequate supply throughout the experimental phase of the project. For each experimental run, a specific portion of this water was channelled through a main line into the drinking water treatment pilot plant (Figure 3A). This drinking water rig served as an experimental model, closely simulating the treatment processes typical of a UK-based surface water-derived drinking water treatment plant. The treatment steps replicated in this pilot setup included coagulation and flocculation, followed by clarification via dissolved air flotation (DAF). Subsequently, the process involved rapid gravity filtration (RGF) using sand media over a gravel bed, ozone treatment, granular activated carbon (GAC)

filtration, and finally, chlorination. These steps and their arrangement within the treatment system are detailed in Figure 3B and 3C.



Figure 3: A. The location of the National Research Facility for Water and Wastewater Treatment (NRF-WWT) at Cranfield University, coupled with the designated abstraction point along Chicheley Brook. This specific point was utilized for sourcing water in the pilot-scale experiments. B. A detailed depiction of the near-real scale drinking water treatment rig. This photo showcases the rig's design and layout to mimic real-world water treatment processes. C. Underside of the drinking water treatment rig's structural and functional components.

In an effort to closely replicate full-scale water treatment processes, the pilot plant at our facility was designed to emulate key parameters of a typical UK surface

water treatment plant. These parameters include hydraulic residence time (HRT), chemical dosing, mixing, and surface loading rates for filters. The scale of our pilot plant falls between 1:30th and 1:100th of the size of full-scale systems, providing a representative model for experimentation. As depicted in Figure 4, the pilot plant operates with a raw water flow rate of 200 L/h, having a total volume of 510.5 litre. During these trials, the nominal HRT was approximately 153 minutes. However, the effective HRT varied based on dynamic factors such as the DAF recycle ratio, pH control, and somatic coliphage dosing. To ensure steady-state conditions, the plant was operated for approximately two HRTs prior to sampling.

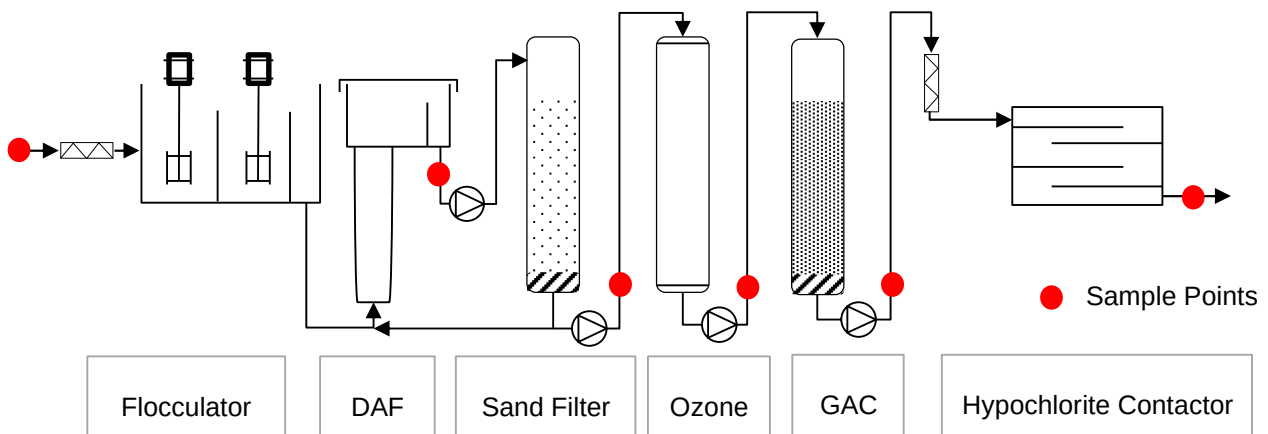


Figure 4: Schematic of the pilot rig flowsheet

Raw water was drawn from the holding tank and fed into the pilot rig at a rate of 3333 mL/min (Figure 4). Adjustable peristaltic pumps (600 series closed-couple pump, Watson-Marlow Ltd., UK) from 25 L holding tanks controlled the addition of coagulant, pH adjustment chemicals, and somatic coliphage doses. These components were introduced into the raw water using an inline static mixer, followed by a divided wall flocculator tank. This tank, consisting of two coagulation/flocculation chambers totalling 124.8 litres, was equipped with variable-speed picket-fence type paddle mixers set to a mixing intensity of 15 rpm. The proportional integral derivative controller (Strata, UK), which received input from an online pH sensor and UV₂₅₄ (Endress and Hauser, UK) located directly after the static mixer, regulated the dosing of pH and coagulant. Post-coagulation, the water was gravity-fed into a DAF column with a volume of 15.7

litres. Here, small air bubbles attached to the flocs, and desludging was achieved hydraulically by overflowing a weir in the 36-liter DAF header tank. A saturated air-water mixture was fed into the base of the DAF column at a rate of 400 mL/min with a 2-bar air flow, enhancing the separation process.

The water is subsequently pumped from the outlet of the DAF clarifier to the top of a sand filter comprising of a support structure of 15 cm coarse gravel, 15 cm fine gravel and 1 m of sand held by a wire mesh holding grate. The chamber is gravity-fed by the DAF. The RGF consisted of an acrylic column (78 L) containing 20 cm of gravel support structure above a wire mesh holding grate, topped with 100 cm of sand. The sand filter was fed by gravity from the outlet of the DAF clarifier. The pilot plant has two filter cleaning operations may be triggered by level in the filter, outlet turbidity reading, time, or manually. In these experiments, the filter cleaning was not undertaken during the trial. Instead, at the end of the experiment, one of two solenoid valves opens and transfers fresh mains water into the bottom of the sand filter alongside compressed air to perform a combined air/water backwash for 5 min to clean the RGF media and restore filter performance. Dirty wash water overflows via a top outlet on the filter which is discarded to wastewater treatment. Environmental risk managers were engaged and consulted to ensure oversight.

The water was pumped to the top of the ozone chamber (0.2 m diameter by 3 m height) with an ozone generator set to 8 normal air litre/min oxygen flow rate. This is followed by the water pumped to the inlet at the top of the GAC column, with the same dimensions and bed height as the sand filter but with Filtrasorb 400 agglomerated coal-based GAC as the absorbent (Chemviron, UK). The ozone dose was validated via Indigo Method: The indigo method is a colorimetric test that uses the blue dye indigo carmine, which decolorizes in the presence of ozone. The change in colour intensity is measured spectrophotometrically and is inversely proportional to the ozone concentration. The ozone residual was set at 8 normal air litre/min. In the post ozone samples, the ozone was quenched via addition of molar excess of sodium sulphite (Na_2SO_3).

The last stage in the treatment process is the hypochlorite contactor following an inline static mixer fed with pH adjustment and sodium hypochlorite pumped from 25 L peristaltic pumps. The contactor is formed of a 100 L baffled tank with 4 off-centre internal baffles arranged in serpentine flow arrangement. Prior to the water being sent to drain (Figure 4). Chlorine concentration was optimised through breakthrough trials and measured via DPD test for free chlorine (Hach, Germany) based on standard methods 4500-Cl (G) (Standard Methods Committee of the American Public Health Association, 2023). The chlorine breakthrough point for water treatment was determined by collecting water samples before and after chlorine addition and administering a calculated chlorine dose. Free chlorine concentration was measured immediately and after 1 hour in these samples using colorimetric method as above. The breakthrough point, marked by a steady rise in free chlorine levels, indicated minimal consumption by oxidizable organics. This data helped to adjust the chlorine dosing to achieve the desired residual concentration. The experiment was repeated to confirm the effectiveness of the new dosing, with all steps and results thoroughly documented for future analysis. The breakthrough was tested after 1 hour which reflected the contact time ranges tested (0.5-1 hour). The pH was adjusted using dedicated chlorine pH adjustment as for the coagulant pH control system.

In each test, the plant was brought to a steady state before introducing additional somatic coliphages (Φ -X 174). At this stage, 1-litre control samples were taken to establish a baseline of the natural somatic coliphages in the source water. These controls and experimental samples were obtained via grab samples from flushed sample taps at six locations: at the inlet (post somatic coliphage addition), after DAF, post RGF, following ozonation, post GAC, and after the chlorine contact tank (Figure 4). Subsequently, coliphages were added to the raw water and, after two HRTs, sampling was resumed on an hourly basis for 6 hours. Sample lines were flushed so that at least 3-fold the volume of the sample line was wasted prior to taking a sample. The samples were transported to the laboratory under darkened conditions at room temperature and were assayed for somatic coliphages (same day) as previously described. Physicochemical parameters were monitored by online sensors (turbidity, pH, UV₂₅₄ nm). Between

tests, the pilot plant was cleaned via pressure wash with tap water. Then, approximately >1 HRT of tap water was permitted through the plant prior to the next experiment starting. For practical reasons, the pilot plant was not disinfected (i.e., to inactivate residual coliphages), between experiments as the water being treated is inherently non-axenic but samples were regularly taken as 'negative' controls to understand the baseline levels of somatic coliphages coming into the pilot plant from the raw water.

2.2.6.2 Coagulation optimisation at pilot-scale

Once the optimum coagulation dose was determined at bench-scale, a pilot-scale experiment was undertaken. Coagulant doses included 0, 5, 10, 12, 17, 20 and 29.6 mg-Fe³⁺/L at the pre-determined optimum pH based on the coagulation jar tests. The inline turbidity sensors take readings every 10 seconds with the coagulant dose being adjusted every 60 minutes. The change in turbidity data was extracted and plotted over time accounting where possible for variations in HRT.

2.2.6.3 Coagulation optimisation at pilot scale with somatic coliphage

To investigate the removal of coliphage at near-real scale, the pilot rig was run under the same conditions as section 2.2.6.2 but with the additional dosing of Φ-X 174 into the raw water feed. Somatic coliphage dosing was optimised by adding concentrated Φ-X 174 in phosphate buffer in the 20L dosing chamber. Regular inlet samples were collected to understand where the inlet concentration of somatic coliphage changed through each ~8-hour experiment. 1 L Samples are taken (from each sample point) in sterile 1L borosilicate glass beakers every 15 minutes, with samples for coliphage analysis aliquoted into 1 mL sterile centrifuge tubes. Then 50 mL of sample was aliquoted into sterile polypropylene centrifuge tubes for additional analysis. Turbidity data was extracted and plotted over time accounting for HRT of the system and plug flow nature of the continuous flow pilot plant.

2.2.6.4 Interstage LRV assessment

To assess the removal of somatic coliphage at each stage of water treatment, the coliphage was spiked into the water before coagulation. The reduction of coliphage was measured at each treatment step by calculating the LRV from the PFU/100 mL of the preceding stage. This approach assumed that the concentration of coliphages remained constant between stages, apart from changes due to the treatment itself. Additionally, turbidity and UV₂₅₄ data were processed for each sample, necessary due to some damage to the rig's online sensors and for calibration.

2.2.7 Statistics

Statistical analyses were conducted using SPSS software (Version 29, IBM, 2023). Initially, the Shapiro-Wilk test was applied to assess the normality of the data distributions, with a significance threshold set at $p > 0.05$ to denote normal distribution. For datasets conforming to normal distribution, inferential statistical techniques including one-way ANOVA, two-way ANOVA, and multiple regression analysis were employed. The level of statistical significance for all tests was established at $p < 0.05$. Multiple linear regression analysis was undertaken to examine which variables influenced the dependant variable. In this case the dependent variable was either somatic coliphage concentration or the bacterial cell concentration determined via flow cytometry. The analysis ensured that the dependent variable was measured on a continuous scale and that the model included both continuous and categorical independent variables. Independence of observations was confirmed using the Durbin-Watson statistic. A thorough examination of linearity between the dependent and independent variables was conducted, alongside checks for homoscedasticity to ensure similar variances along the best fit line. The issue of multicollinearity was addressed by confirming that no independent variables were excessively correlated. The presence of outliers, leverage points, or influential points was meticulously investigated and managed. Lastly, the residuals' normal distribution was verified using appropriate statistical methods, completing the essential steps for a valid multiple regression analysis. Modified or standardized regression coefficients, commonly known as

Beta coefficients, are crucial in interpreting regression models. These coefficients are scaled to have a uniform unit of measurement, enabling direct comparison of the influence of each independent variable on the dependent variable. When comparing the impact of different variables, Beta coefficients provide a clear understanding of which variables have a more significant effect. However, caution is required when dealing with highly correlated independent variables. In cases where variables show a high degree of correlation (multicollinearity), it's advisable to retain only one variable from the correlated group in the multiple regression analysis. Including multiple highly correlated variables can distort the model's accuracy and the interpretation of the coefficients. Additionally, the correlation coefficient, denoted as 'r', is an essential concept in understanding the relationship between variables. It ranges from -1 to +1. A correlation coefficient of +1 indicates a perfect positive correlation, meaning that as one variable increases, the other variable also increases at a consistent rate. Conversely, a coefficient of -1 signifies a perfect negative correlation, where an increase in one variable corresponds to a proportional decrease in the other. Lastly, a correlation coefficient of 0 indicates no correlation, meaning there is no linear relationship between the two variables.

3 Results and Discussion

3.1 Coagulation Optimisation

3.1.1 Coagulation optimisation jar tests

To optimise the coagulant dose and pH for the source water, bench-scale jar tests were undertaken. Coagulant dose ranged from 5 mg-Fe³⁺/L to 20 mg-Fe³⁺/L and pH ranges from pH 5 to pH 8. Tests were undertaken in duplicate with triplicate measurements taken for each parameter to ensure robustness and reliability of the data. The relationship between UV₂₅₄ (Figure 5A) and turbidity (Figure 5B) with variable coagulant doses and pH was established. The results show an optimum coagulant dose of 10 mg-Fe³⁺/L for both the turbidity and UV₂₅₄ data evidenced by consistently lower residual turbidity and organics. The turbidity did not fall below WHO thresholds recommended for disinfection of 1 NTU. However, as this was only the first stage of the treatment process, it is anticipated that other treatment operations would reduce turbidity accordingly. The data from these trials had practical implications for optimizing the pilot scale water treatment processes for efficiency. The optimum pH for removal of turbidity and UV₂₅₄ was pH 6. There was minor variation in coagulant doses 10, 12 and 17 mg-Fe³⁺/L for UV₂₅₄ analysis, similarly with 5 and 15 mg-Fe³⁺/L for both turbidity and UV₂₅₄ indicating a degree of tolerance in the coagulant dosing. This could imply that slight deviations from the optimal dose may not significantly impact the treatment efficacy within this range, providing some flexibility in the coagulation process. The smallest reduction in UV₂₅₄ was shown at the highest coagulant dose of 20 mg-Fe³⁺/L but the lowest coagulant dose of 5 mg-Fe³⁺/L for turbidity (Figure 5).

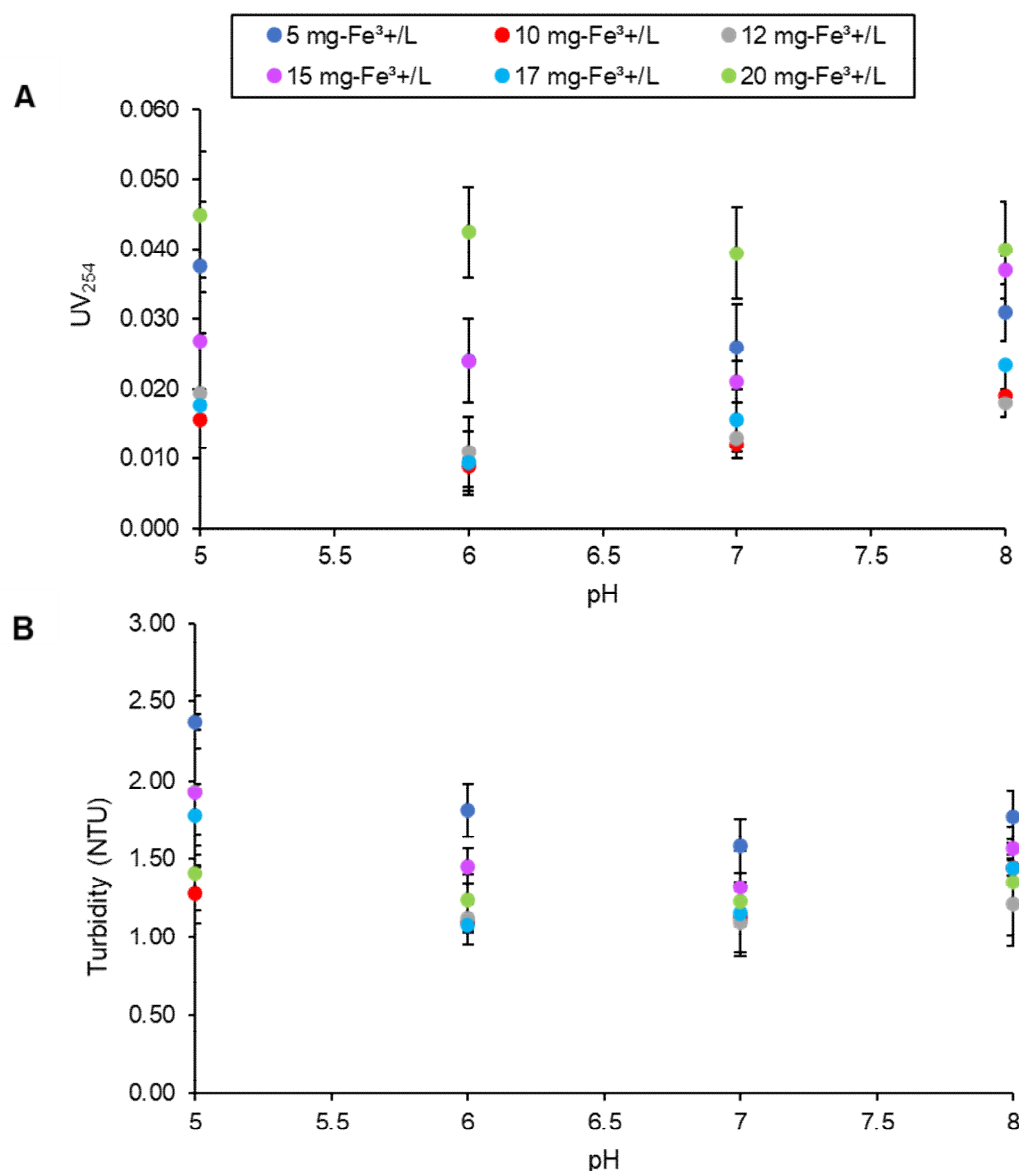


Figure 5: Comparative Analysis of (A) UV₂₅₄ Absorbance and (B) Turbidity across varied pH Levels and Coagulant Dosages depicting an optimum coagulant dose of 10 mg-Fe³⁺/L at a pH of 6. The values presented are averages from two independent experiments (n=2), and the error bars represent one standard error of the mean. Each sample was measured in triplicate to ensure the reliability of the results.

Statistical analysis confirmed a normal distribution for both datasets using the Shapiro-Wilk test for normality (p-value>0.05). Utilizing a two-way ANOVA analysis for significance between coagulant dose and both turbidity and UV₂₅₄, statistical significance emerged in response to variations in coagulant doses.

Turbidity and UV₂₅₄ reductions both had a statistically significant response to the coagulant dose (p-value=0.007, p-value<0.001 respectively) and pH (p-value=0.003, p-value=0.022 respectively). The strong statistical relationship between coagulant dose and pH to turbidity and UV₂₅₄ removal (p-values ranging from 0.003 to <0.001) emphasizes their well-established importance controlling coagulation efficiency. Overall, the water tested was extremely recalcitrant to coagulation probably attributed to its hydrophilic nature of the NOM and low molecular weight nature of the organics (Finkbeiner et al., 2020; Özdemir, 2022). In an ideal experiment, an easier source of water would have been tested for coagulation experiments, however, due to the large volumes required for the pilot scale experiments, the closest available water was taken, despite its challenging nature and relatively unrepresentative NOM (compared to standard UK lowland surface waters) which tend to be more hydrophobic with a high molecular weight distribution. This is due to the greater presence of humic substances, which are large, complex molecules formed through the decomposition of organic matter. After validating the coagulant dose at bench scale, the next experiment was to validate this using the continuous flow pilot plant in the NRF-WWT.

3.1.2 Coagulation optimisation pilot trial

The objective of the coagulation optimization pilot trial was to ascertain the optimal coagulant dosage for efficient drinking water treatment, closely mirroring full-scale operational conditions. Initial jar test experiments aimed at coagulation optimization revealed that the most conducive pH level for coagulation is around pH 6. This discovery informed the pH adjustment strategy employed in the pilot trial to achieve the best coagulation results. Nevertheless, given the variations in coagulant dosage and the shift from a batch process in jar tests to a continuous flow system in the pilot plant, it was deemed necessary to conduct a trial exploring various coagulant dosages in the pilot setup.

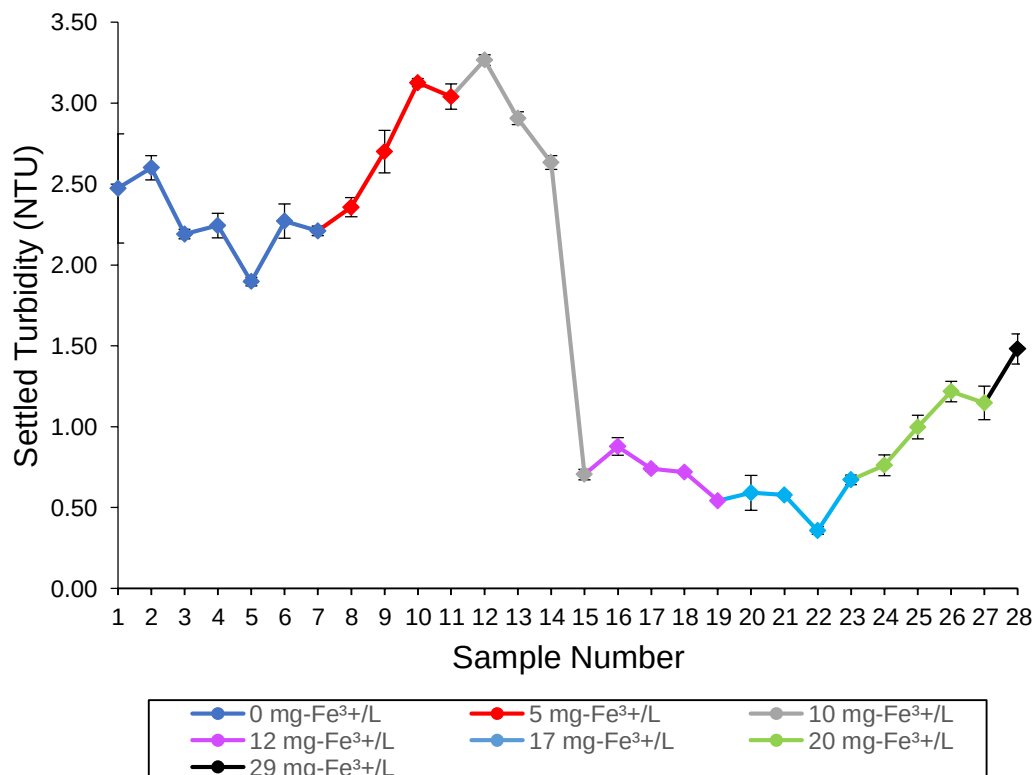


Figure 6: Evaluation of Coagulant Dosing in a Simulated Drinking Water Treatment Process. This figure depicts data from a near-real scale drinking water treatment rig, focusing on optimizing coagulant dosing. Samples were collected at 15-minute intervals. The coagulant dosage was adjusted hourly, with a 30-minute period at 0 mg-Fe³⁺/L coagulant dose preceding each change to establish a stable baseline. The graph shows the average settled turbidity (n=3) at various coagulant doses, with error bars representing one standard error of mean. It was found that the most effective coagulant dose for minimizing settled turbidity is 17 mg-Fe³⁺/L, achieved at a pH of 6.

Despite initial challenges with online turbidity sensors post-DAF and post-sand filter operation, alternate bench-top turbidimeter measurements were used to provide reliable data. It was determined that the absence of a coagulant dose resulted in very low settled turbidity possibly due to pre-sedimentation within the holding reservoir. However, upon introducing a 5 mg-Fe³⁺/L coagulant dose, there was an observed increase in turbidity from 2.25 NTU to ~3 NTU, which

suggests that this dosage was insufficient for effective flocculation (Figure 6). This increase could be part of the natural variability in the feedwater expected with full scale pilot plant operations as the inlet settled turbidity for this experiment is only approximately 2 NTU.

Importantly, at a 10 mg-Fe³⁺/L dose, there was a notable reduction in turbidity, indicating effective removal of particulates via flocculation. The reduction trend continued for doses of 12 mg-Fe³⁺/L and 17 mg-Fe³⁺/L, with the latter dose yielding the lowest turbidity at 0.36 ± 0.02 NTU, suggesting an optimal flocculation efficiency at elevated dose delivery compared to jar test. Conversely, higher doses of 20 mg-Fe³⁺/L and 29 mg-Fe³⁺/L corresponded with increased turbidity 0.75-1.50 NTU. This could be due to re-stabilization of colloids, where these excessive coagulant causes the particles to repel each other again, thus hindering floc formation and possibly resuspending settled particles (Matilainen, Vepsäläinen and Sillanpää, 2010). Statistical analysis supports these observations, with the Shapiro-Wilk test confirming normal distribution of data, and one-way ANOVA revealing a significant relationship between coagulant dose and settled turbidity (p-value < 0.001). This statistical significance underscores the importance of coagulant dose optimization for achieving clarity in water treatment processes and provided a framework for testing virus removal.

3.2 Somatic coliphage coagulation test

3.2.1 Coagulation optimisation jar test with somatic coliphage

The dose of coliphage was determined by an experiment using varying concentrations of Φ -X 174 and determining the removal using both 0 mg-Fe³⁺/L and the 'optimum' coagulant dose of 10 mg-Fe³⁺/L (based on residual turbidity from both jar tests and pilot scale validations). The final concentration of Φ -X 174 which was dosed was approximately 3×10^7 PFU/100 mL following a preliminary test dosing varying concentrations of the somatic coliphage until the concentration measured post-flocculation was detectable. Similar test procedures were undertaken as per section 3.1.1. However, to ensure that data reflected changes between coagulant doses, pH ranged from pH 5 to pH 8 and

coagulant dose range from 0 mg-Fe³⁺/L to 29 mg-Fe³⁺/L. Log reduction of Φ-X 174 for each coagulant dose was calculated from 0 mg-Fe³⁺/L.

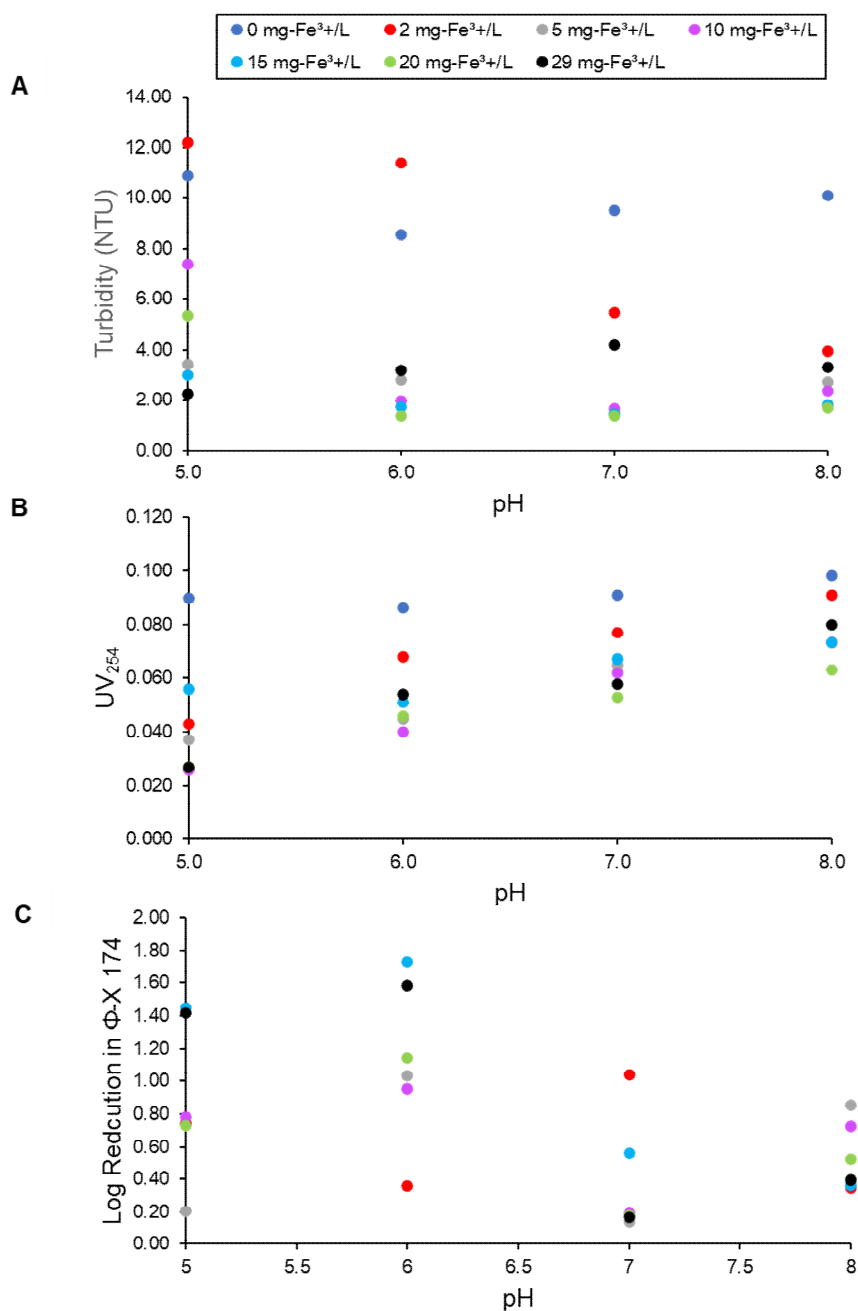


Figure 7: Coagulation dose and pH on the LRV of Φ-X 174. Measured (A) turbidity, (B) UV₂₅₄ and (C) log reduction of Φ-X 174. Optimum coagulant dose for Φ-X 174 was 15 mg-Fe³⁺/L at pH 6. Due to technical restraints, duplicates of only failed runs were performed.

The log reduction of Φ -X 174 is calculated from the 0 mg-Fe³⁺/L dataset at each pH. The removal efficiency of turbidity and organics from water was influenced by both the pH and the coagulant dose delivered (Figure 7). The highest LRV of Φ -X 174, which indicates the most effective viral removal, occurs at a coagulant dose of 15 mg-Fe³⁺/L and a pH of 6 with 1.73 LRV. This suggested that the treatment process was most efficient at these conditions for somatic coliphage removal. This was similar for the maximum turbidity removal but not the UV₂₅₄ where the peak removal was at pH 5. The peak organic removal at pH 5 in the presence of iron coagulants can be attributed to the chemistry of iron in aqueous solutions. At lower pH values, iron coagulants are more likely to be in their soluble Fe (III) form, which is more effective for coagulation because it readily hydrolyses and subsequently forms flocs via charge neutralisation, therefore, as the organic compounds are sometimes more stable than particles, this could be why an optimum pH for Fe is needed. As these flocs can effectively adsorb and precipitate organic molecules, leading to enhanced removal from water. As the pH increases, the solubility of iron decreases, reducing the formation of these active coagulation species, thus diminishing the removal efficiency. For turbidity and UV₂₅₄, the trends are less consistent across different pH levels, but pH significantly affects their reduction, indicating the importance of pH control in water treatment processes (Naceradska, Pivokonska and Pivokonsky, 2019). The formation of flocs is more efficient at lower pH levels due to the charge neutralization mechanism, which facilitates the aggregation of organic matter and its subsequent removal from the water with optimum for iron-based coagulants normally around pH 5.

The pilot residual turbidity and UV₂₅₄ values were higher than the Coagulation optimisation jar tests. For all coagulant doses above 2 mg-Fe³⁺/L, pH 5 and 6 show similar removal, whilst this decreases for pH 7 and 8. 2 mg-Fe³⁺/L did not follow this trend, with the highest LRV of the somatic coliphage at pH 7, whilst pH 5,6 and 8 maintain a stable log reduction. The statistical analysis shows statistical significance between turbidity and pH (p-value<0.05) whilst there is no statistical significance between the LRV of Φ -X 174 or UV₂₅₄ and the dose of coagulant (p-value=0.579 and 0.759 respectively). On the other hand, pH significantly

influenced both the UV₂₅₄ reduction and Φ-X 174 LRV (p-value<0.05) but not the turbidity (p-value=0.165). The lack of statistical significance between the coagulant dose and the LRV for Φ-X 174 and UV₂₅₄ suggests that factors other than the coagulant dose may influence the efficiency of contaminant removal. However, the pH level significantly influenced the reduction of UV₂₅₄ and Φ-X 174, reaffirming the role of pH in optimizing water treatment. Next the somatic coliphage measurements were assessed in the pilot scale treatment train.

3.2.2 Pilot-scale coagulation optimisation to determine somatic coliphage removal.

The pilot rig was operated with samples taken every 20 minutes and the coagulant dose changed every hour with an additional sample of 0 mg-Fe³⁺/L taken to determine a baseline. The settled turbidity was measured and the enumeration of somatic coliphage undertaken as per section 2.2.1. the LRV was calculated based on the reduction of the spiked concentration of Φ-X 174 of 6.54×10^7 PFU/ 100 mL – a value far in excess of most water sources but needed to adequately determine the LRV by sufficiently challenging each unit process – in this case coagulation. The coagulant doses range from 0 mg-Fe³⁺/L to 29 mg-Fe³⁺/L. There appeared to be a general decrease from 1-1.40 NTU to 0.4-0.6 NTU as the coagulant dose increased from 0 to 10 mg-Fe³⁺/L, after which the trend fluctuates from 0.6-0.8 NTU (Figure 8A). The Φ-X 174 was lowest in sample 3 (0 mg mg-Fe³⁺/L) of 1.73 (Figure 8B). This suggests that the removal with clarification was reasonable even without substantial coagulant added. This can be attributed to flocculation due to pH adjustment and subsequent density separation of turbidity / virus in the clarification system (DAF). The turbidity removal trend was similar to the jar test data but less apparent possibly due to the plug flow nature of the pilot rig and time delay between dosing and observed changes to turbidity values. The lowest turbidity was observed at a dose of 10 mg-Fe³⁺/L, suggesting optimal coagulation efficiency at this concentration, compared to 15 mg-Fe³⁺/L at bench scale. The LRV of Φ-X 174 peaked at 10 mg-Fe³⁺/L before plateau of between 2.3-2.8 at all subsequent sample points with coagulant doses ranging from 15-29 mg-Fe³⁺/L suggesting further virus removal

was not possible within the constraints of the pilot rig dosing experiment when using coagulation/clarification alone.

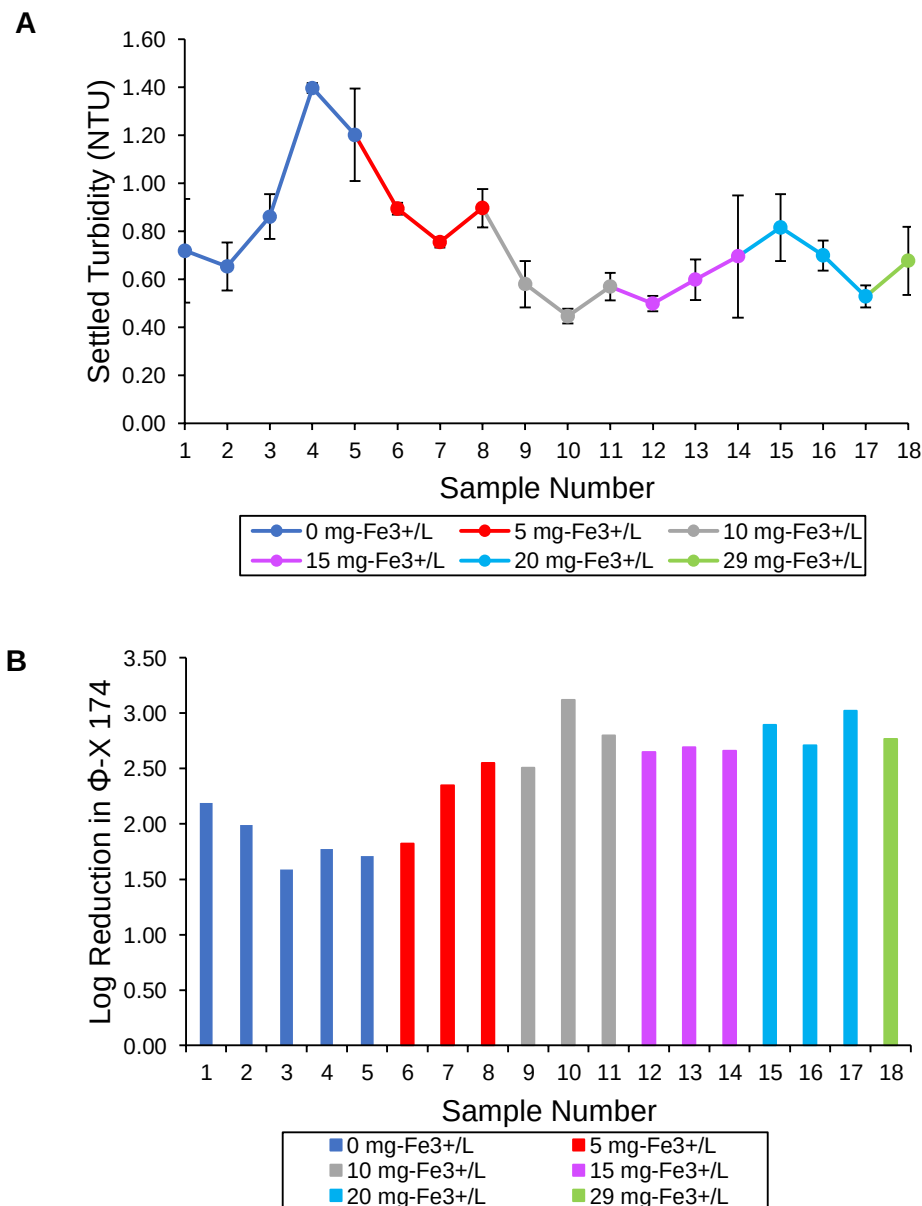


Figure 8: The (A) settled turbidity and (B) LRV of Φ -X 174 for different coagulant doses. Sample number denotes a sample taken every 20 minutes with the coagulant dose changed every hour. Settled turbidity measurements are averaged ($n=3 \pm$ one standard error). The LRV represents the average of triplicated samples. The optimum coagulant dose is 10 mg-Fe³⁺/L for both log reduction and settled turbidity.

3.3 LRV pilot-scale trials

The log reduction of Φ -X 174 was calculated based on the concentration in the outlet of the prior treatment process and turbidity, UV_{254} , TCC and ICC reported for all samples. The test conditions for coagulant dose include 0 mg- Fe^{3+} /L to represent a baseline condition, the optimum dose determined in section 3.2.2 of 10 mg- Fe^{3+} /L and a dose of 2 mg- Fe^{3+} /L to represent the case where in full-scale conditions, the coagulation process is running under sub-optimal conditions. Two pilot runs were conducted, the first with a lower final concentration of Φ -X 174 of an average of 5.33×10^{10} PFU/100 mL and the second with an average of 1×10^{14} PFU/100 mL. The pilot-scale trials for the interstage LRV occurred over 7 hours for each test. Samples were taken at 6 sample points throughout the treatment process including: post-mix (post- inline mixer prior to the flocculation tank), post DAF, post-sand, post-ozone, post-GAC, and the post chlorine contact tank (final water) (Table 3).

Figure 9 delineates the efficacy of various drinking water treatment processes in achieving LRV for somatic coliphage. Beginning with the effect of coagulation/clarification, (post-DAF), there is a clear variability in effectiveness of removal, with LRV ranging from about 0.23 to 2.25, the highest reduction being observed in the third sample but the ranges of removal being similar to earlier trials involving only this treatment process (see 3.1.2) during the period of 10 mg- Fe^{3+} /L coagulant dose. During minimal coagulant dose, the reduction due to this process was around 0.503. Moving on to the sand filtration stage, the LRV reductions appear generally lower than for the coagulation/clarification, mostly falling into negative log reduction (-0.088 to -1.3) possibly due to ineffective filter ripening (due to short ripening period). This implies that the sand filtration's, impact in this trial is comparatively moderate which is not what was anticipated. Further work would be needed to optimise the filter operation to assess the peak removal conditions. The influence of ozonation, marked by pink bars, also shows variability in the reduction of somatic coliphage with LRVs extending from 0.288 at suboptimal coagulant doses (i.e., 0 mg Fe^{3+} /L) to roughly 2.5 at 10 mg Fe^{3+} /L (accounting for time delay between upstream changes to coagulant dose and its impact on ozone). The samples 5-7 in post ozone process being equivalent to

samples 2-4 in the post-coagulant water. This indicates a moderate to good efficacy in decreasing the concentration of the virus. The subsequent stage of GAC treatment, shown in blue bars, demonstrated a more consistent and notable reduction with LRVs clustering between 0.476 towards the start of the run and peaking at 4.749 at the end of the experiment. This consistency suggests that GAC is quite effective in curtailing levels of somatic coliphage. This is probably a facet on the use of virgin GAC, likely to have amply active sites for adsorption of virus. In practise, the effect of ozone / GAC is unlikely to be decoupled. The flexibility of the pilot plant permitted this comparison.

The highest log reduction of Φ -X 174 in the post-ozone samples occur during the optimal coagulant dosing window, although there is a decline in the log reduction in samples 5,6 and 7 where the dose of coagulant is 10 mg-Fe³⁺/L. in contrast, the post-GAC samples still had good removal suggesting log removal of Φ -X 174 from sample 3 onward where the effective optimal coagulant dose of 10 mg-Fe³⁺/L does not come into effect until sample 7, due to delay in water flowing through the pilot plant (Table 3). The final water samples suggested limited removal of Φ -X 174 for samples 1 and 2 but no further log reduction for the subsequent samples. As the log reduction is calculated as per the PFU/ 100 mL of the treatment process prior, the post-GAC samples presented with 0 PFU/ 100 mL for these samples in addition to the final water samples showing no somatic coliphages above the limit of detection. As such, the log reduction of Φ -X 174 has no integer value.

The effect of these treatments is most evident in the final water quality, with the green bars representing an LRV 4.1-4.2 of Φ -X 174, suggesting a substantial overall reduction of somatic coliphage in the chlorination. The sum of the LRV of Φ -X 174 provided ranges from a minimum of 5.58 to a maximum of 6.63. On average, the LRV sum is approximately 5.96, indicating a relatively narrow spread in the effectiveness across the different samples or treatment processes represented by these numbers (Table 3). This consistency reflects a reliable performance in the system or method being assessed for reducing the concentration of Φ -X 174 and probably limits to the overall removals which can

be achieved. Principally, this is a facet of the treatment processes being investigated and the final concentration of phages which were dosed and thus which could be removed. A point of interest was the similar overall LRV of the treatment works during periods of optimal versus suboptimal coagulation dose (Table 3). This implied that the multi-step treatment process, when taken as a whole, is highly efficient at reducing viral contamination, ensuring the safety of the drinking water. The graph underscores the importance of a multi-barrier approach to water treatment, with each stage incrementally contributing to the overall reduction of contaminants to deliver water that is safe for consumption. The presence of a concentration of 10^{14} of the virus suggests that there was amply virus which could be removed. Further discussion about the Σ -LRV of Φ -X 174 linked to the EU standards for LRV is considered in the overall thesis discussion.

A two-way ANOVA analysis shows a statistically significant relationship between the coagulant dose for both the log reduction of Φ -X 174 and the settled turbidity (p -value<0.05) but not UV_{254} – aligning with observations from bench scale (Figure 10A, B). The effective coagulant dose at the outlet of each treatment process as a product of the HRT (Table 3). The time taken to reach each coagulant dose increases along the treatment train. The post-GAC and final water samples are representative of coagulant doses 0 mg-Fe³⁺/L and 10 mg-Fe³⁺/L but were not run for a period of time that would represent 2 mg-Fe³⁺/L.

Table 3: Effective coagulant dose for each treatment process based on the HRT.

EFFECTIVE COAGULANT DOSE BASED ON HRT (MG-FE³⁺/L)							
Timepoint (Hour)	Post DAF	Post Sand	Post Ozone	Post GAC	Final Water	Σ -LRV of Φ -X 174 – low dose	Σ -LRV of Φ -X 174 – high dose
1	0	0	0	0	0	5.8	4.8
2	10	10	0	0	0	6.63	4.49
3	10	10	0	0	0	5.66	3.78
4	10	10	10	0	0	5.71	5.49
5	2	2	10	10	0	6.13	9.51
6	2	2	10	10	10	5.58	9.71
7	2	2	2	10	10	6.18	0.68

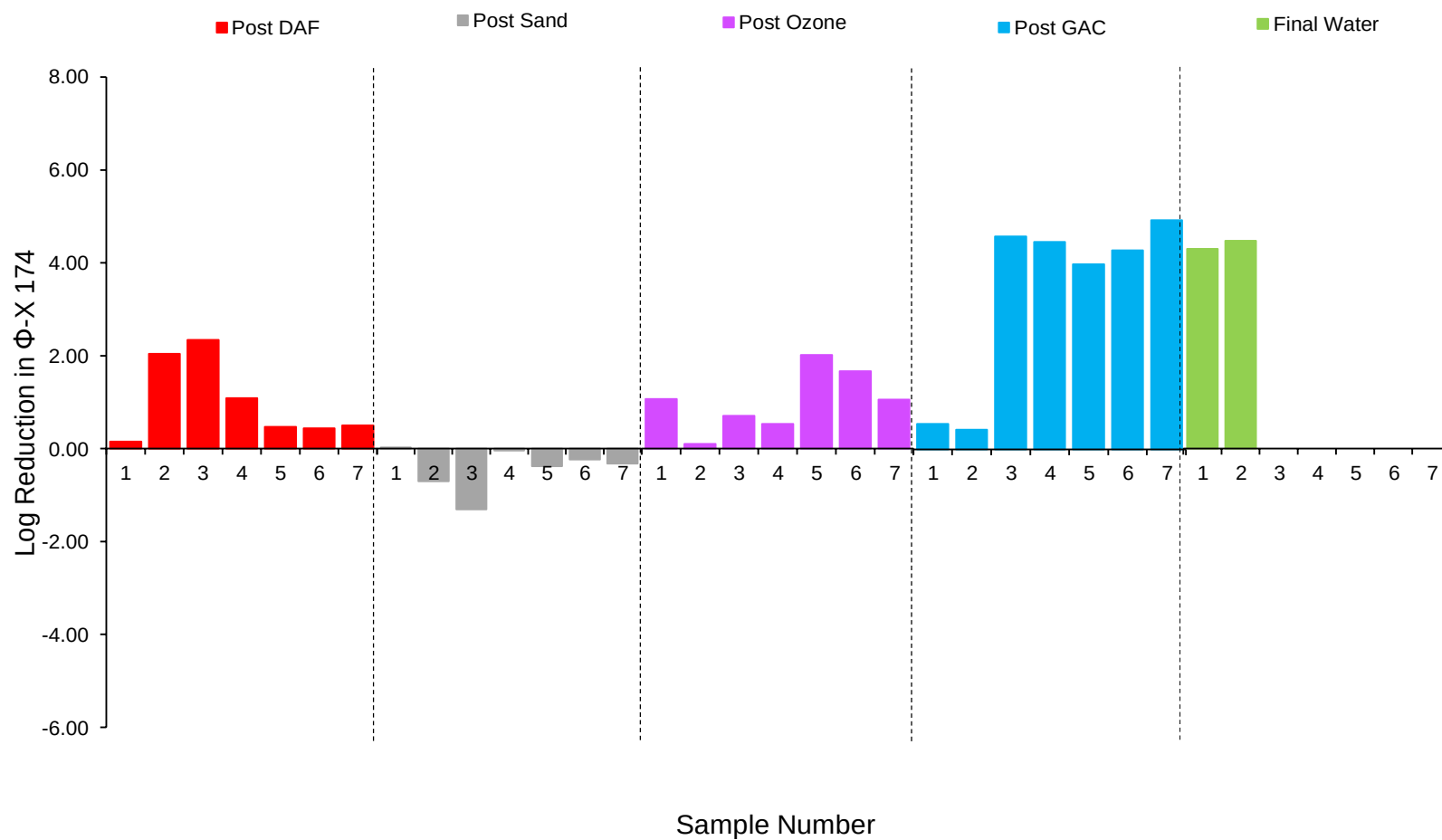


Figure 9: Log reduction value (LRV) of Φ -X 174 at each treatment stage under low coliphage dose conditions of 5.33×10^{10} PFU/100 mL. Positive values indicate a decrease in somatic coliphage and negative values an increase. Non detects are represented by nil LRV.

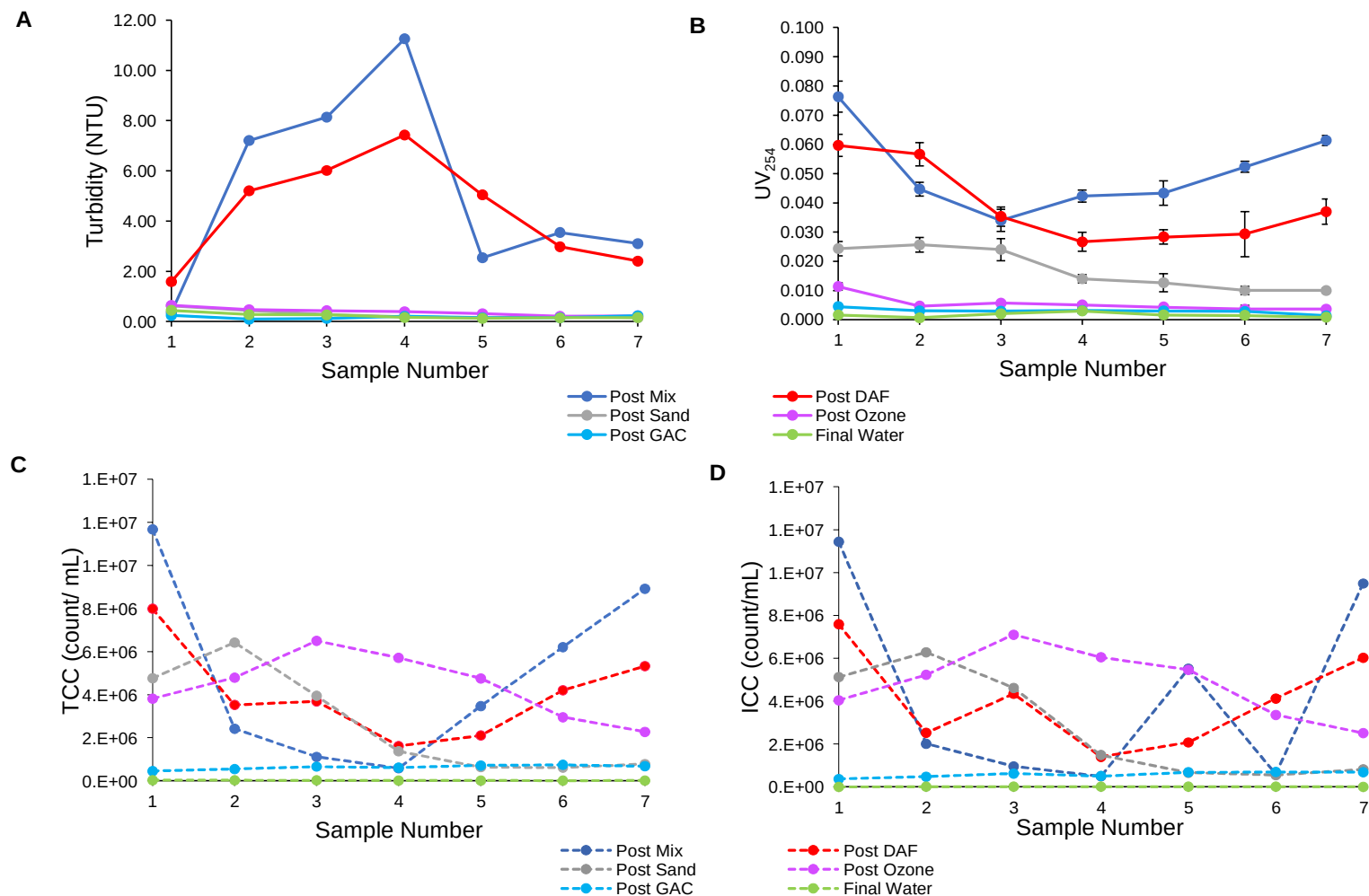


Figure 10: LRV trial with low dose of Φ -X 174 at 5.33×10^{10} PFU/100 mL. (A) turbidity, (B) UV_{254} , (C) TCC and (D) ICC for each sample. Averages for turbidity and UV_{254} (n=3) are plotted ($\pm$ one standard error).

The TCC and ICC was extremely variable throughout the trial as expected. The post GAC and post chlorine (final water) cell concentrations being substantially lower accounting for oxidative damage to the cell membrane by ozone and chlorine respectfully (Figure 10 (C) and (D) respectively). The post-mix and post-DAF samples have similar trends to the turbidity and UV₂₅₄ and the post-sand filter sample follows the same trend as the UV₂₅₄ data only. The post-ozone samples have an unexpectedly high count/mL across all samples suggesting inefficient ozone exposure. The post-GAC and final water samples have an average of 5.5×10^5 count/mL and 2.27×10^4 counts/ mL for TCC respectively and 4.96×10^5 and 6.13×10^3 counts/mL respectively for ICC.

To explore the relationships between factors within the drinking water treatment works a multiple linear regression was undertaken (Table 4). The following independent variables were selected: Turbidity, UV₂₅₄, ICC and Process stage (ordinal variable). TCC was not selected as it presented strong auto-collinearity to ICC. The overall model R^2 was 0.783, ANOVA, $F=11.38_{(5,36)}$, $p<0.001$ suggesting the predictor variables had a strong positive relationship with the Φ -X 174 concentration.

Table 4 Multiple Linear Regression output – Beta coefficients and statistical significance parameters obtained by regression analysis for Φ -X 174 concentration in the drinking water stages.

Dependent Variable: Log PFU Φ -X 174	Coefficients	Standardized Coefficients (β)	t	p-value
(Constant)	8.35		2.883	0.007
Turbidity	-0.21	-0.174	-1.016	0.316
UV ₂₅₄	72.64	0.631	2.637	0.012
ICC	-7.72	-0.017	-.024	0.981
Process	-0.89	-0.453	-1.561	0.013

According to the Beta coefficients, their degrees of influence were, from the greatest to the lowest: UV₂₅₄>Process treatment stage>Turbidity>ICC, however, only UV and the process type are statistically significant.. The UV₂₅₄ (p=0.012) and process (p=0.013) had a statistically positive influence on log PFU an increase this variable led to an increase in somatic coliphage concentration. The turbidity, ICC were found to have no impact on Φ-X 174 concentration within the range of parameters studied (Figure 12; Table 4). The link between concentrations of PFU and UV₂₅₄ are unsurprising, as elevated levels of organic compounds are indicative of poorer coagulation / treatment efficiency. This also corresponds to sample points where elevated virus levels were observed.

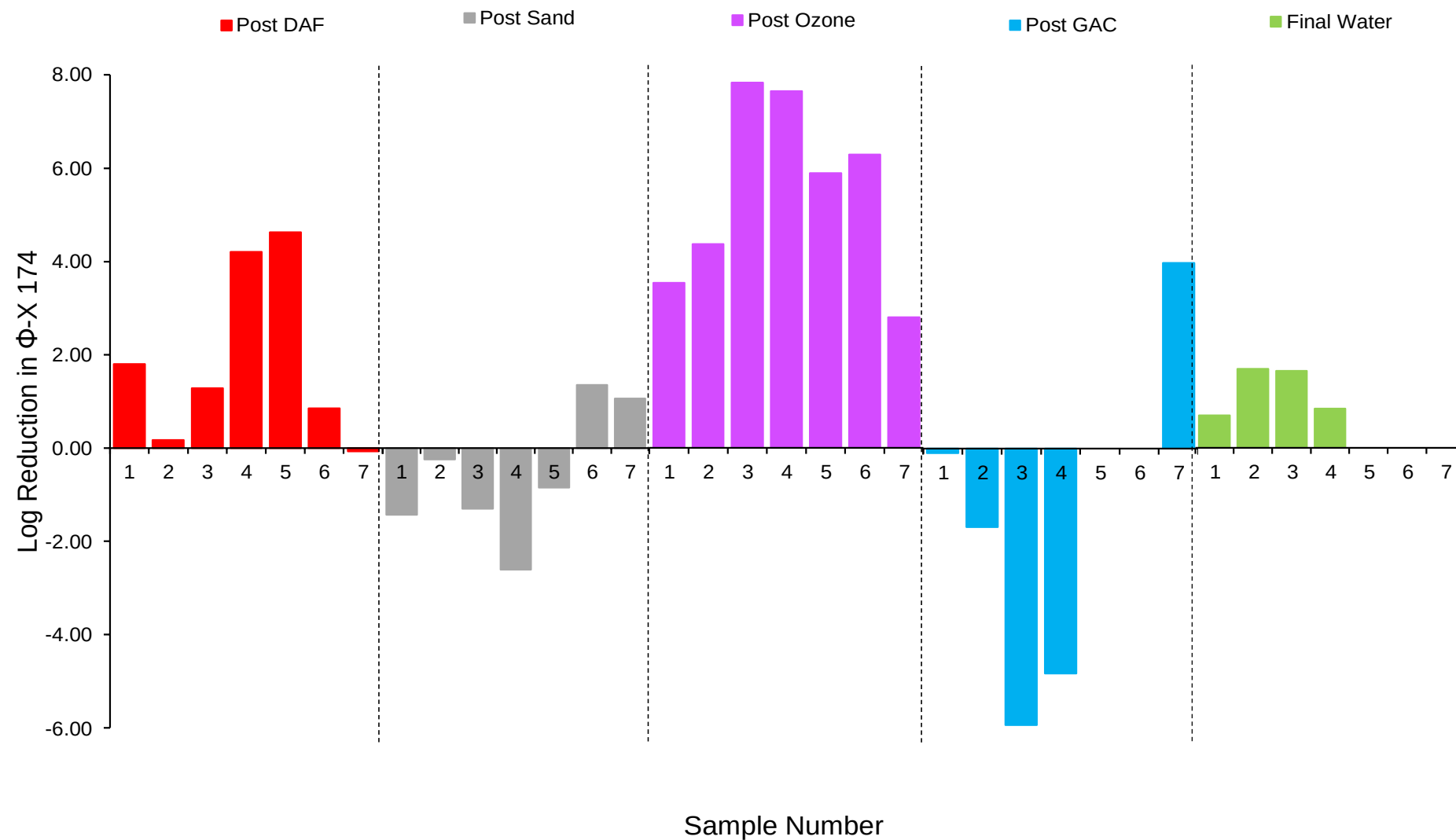


Figure 11: Log reduction of Φ -X 174 at each treatment stage under high coliphage dose conditions. Positive values indicate a decrease in somatic coliphage and negative values an increase.

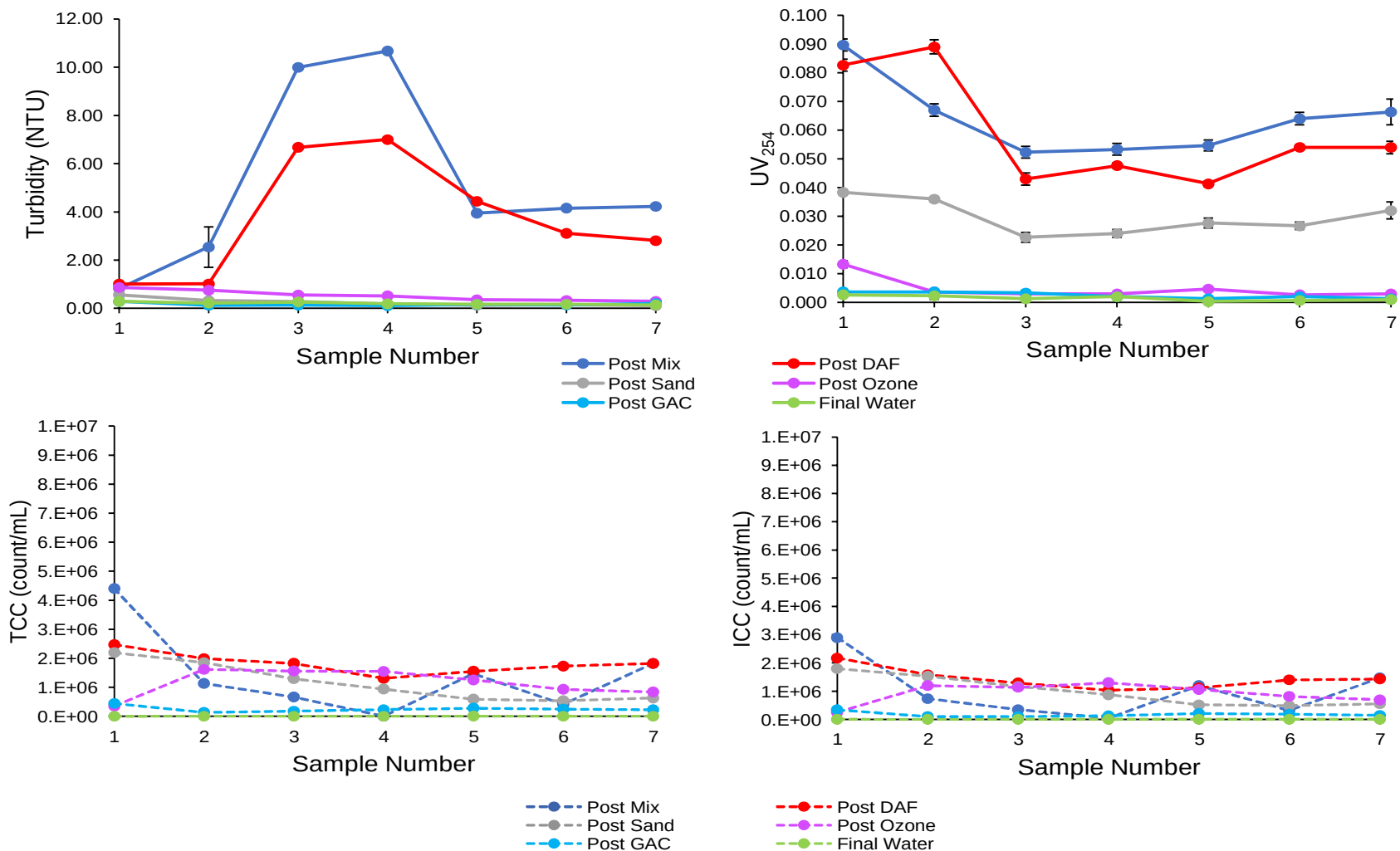


Figure 12: LRV trial with high dose of Φ -X 174 at 1×10^{14} PFU/100 mL. (A) turbidity, (B) UV₂₅₄, (C) TCC and (D) ICC for each sample. Averages for turbidity and UV₂₅₄ (n=3) are plotted ($\pm$ one standard error).

4 Overall Thesis Discussion

The study's exploration of coagulation optimisation echoes previous research emphasizing its crucial role in contaminant removal and highlights the relationship between coagulant types, dose of coagulant, and pH levels, reinforcing the importance of tailored approaches in optimising treatment efficacy (Alsaeed, Alaji and Ibrahim, 2022; Chiavola et al., 2023; Lee et al., 2017; Shivraj, Quraishi and Basu, 2023; Sillanpää et al., 2018).

The best coagulant doses for effectively removing the bulk turbidity from the water varies between each test. The optimum coagulant dose for the bench-scale jar tests and the pilot-scale test for coagulation optimisation in the absence of Φ -X 174 is between 10 mg-Fe³⁺/L for jar testing and 17 mg-Fe³⁺/L for the pilot-scale test, both at pH 6. The mechanisms for NOM removal have indicated the trends in the coagulation efficiency. Charge neutralisation occurs when cations from Iron (III) Chloride adsorb to the surface of particulates in the water and a precipitate of the cation alongside the constituent ions of the particulate surface form a solid solution (Duan and Gregory, 2003). This enables the process of sorption to take place continuously, involving both the creation of surface complexes and the precipitation of the sorbing ion in the bulk solution. As sorbate concentration increases, the surface precipitate increases until all surface sites are saturated. Moreover, as the concentration of the sorbing ion in the bulk solution increases, there is a notable increase in the quantity of precipitates forming on the surface. Consequently, when a metal adheres to a surface, a fresh hydroxide layer develops, facilitating the continued transfer of metal to the solid phase (Duan and Gregory, 2003). The outcome is small flocs forming in the water which can readily settle and be removed. This can be seen at coagulant doses of 10 mg-Fe³⁺ to 17 mg-Fe³⁺ where optimal turbidity and UV₂₅₄ removal occur for the coagulation optimisation tests.

Following this, there is a proportion of the coagulant doses which show similar turbidity and UV₂₅₄ removal as the minimum dose of coagulant applied. This is indicative of excessive coagulant dosing in water treatment which can prompt restabilisation, hindering particle aggregation. When coagulants are added

beyond the charge neutralisation range, it generates unstable larger flocs prone to disintegration. Additionally, excessive doses may reverse particle surface charges, compromising floc stability and contributing to restabilisation (Wei et al., 2015). The higher coagulant dose of 17 mg-Fe³⁺/l has similar removal capabilities to the optimum coagulant dose of 10 mg-Fe³⁺/L for the bench-scale coagulation optimisation experiment (section 3.1.1) due to sweep flocculation mechanisms when coagulant is over-dosed and ferric hydroxide precipitates, the effect is sweep floc with large floc size and more easily settleable flocs formed (Gao et al., 2011). In contrast, the changing coagulant dose post-optimum shows an increasing trend of settled turbidity, suggesting that a higher dose could be necessary than 29 mg-Fe³⁺/L for sweep flocculation for turbidity removal, although then presenting much higher than that reported in literature (Oriekhova and Stoll, 2014). Throughout all coagulation optimisation experiments, low doses of Iron (III) Chloride (5 mg-Fe³⁺/L) indicate an increase in both turbidity (settled turbidity presented for the coagulation optimisation pilot trials) and UV₂₅₄, much due to the poor formation of flocs and reduced contaminant removal (Shivraj, Quraishi and Basu, 2023). Overall, the water tested poses difficult to coagulate, much due to the overall low starting turbidity of approximately 4 NTU. This is due to the possibility of settling of suspended NOM in the holding tank which supplies both the pilot rig and the water used for bench-scale experiments. A series of pumps were installed into the tank but due to the design of the tank (short with a large surface area) the turbidity did not vary dramatically.

As shown in the results, the relationship between removal capabilities of Iron (III) Chloride and Φ-X 174 is positively correlated during coagulation. At pH levels around pH 5 and pH 6, the viruses are negatively charged and therefore adhere to the positively charged coagulant more readily. The isoelectric point of Φ-X 174 is 6.0 to 7.0 (Michen and Graule, 2010) and in the pH range studied (5.0-8.0), the Φ-X 174 virus should have a positively charged surface around pH 5 and pH 6, and a negative surface charge around pH 7 and pH 8. This literature coincides with the results of this study, suggesting that the optimum pH for maximum Φ-X 174 coagulation efficiency was acidic. The need for acidic pH to neutralise the zeta potential for coliphages has also been reported by (Aronino et al., 2009).

This is seen in the removal of Φ -X 174 in the bench-scale coagulation optimisation with somatic coliphage (section 3.2.1) where the log reduction of Φ -X 174 drastically decreases for all coagulant doses at pH 7 with the exception of 2 mg-Fe³⁺/L, much due to the coagulant dose being too small so hindering the removal capabilities. A reduction in the pH controlled by both the addition of Iron (III) Chloride and hydrochloric acid aids the removal efficiency by flocculation (Shamsollahi et al., 2019). As mentioned in section **Error! Reference source not found.**, coagulation removal is governed by three main mechanisms. In reference to removal of Φ -X 174 complementing the removal of settled turbidity, the LRV of Φ -X 174 at higher than optimum coagulant doses shows the same trend as the settled turbidity (3.2.2): slightly sub-optimal removal at higher coagulant doses but with no indication of sweep flocculation with secondary high levels of removal of Φ -X 174 and settled turbidity.

One objective of this study was to undertake challenge trials under optimal and sub-optimal treatment operation of coagulation and was hypothesised that the concentration of Φ -X 174 would increase under these sub-optimal conditions. The coagulant dose was successfully optimised at both bench-scale and pilot-scale, with 10 mg-Fe³⁺/L measured as the optimum dose for removing Φ -X 174 in near-real scale operating conditions. The Pilot-scale coagulation optimisation to determine somatic coliphage confirms that under sub-optimal conditions (namely under-dosing of coagulant) there is a reduction in the log removal of Φ -X 174 with the optimal, 10 mg-Fe³⁺/L achieving the highest log reduction of log 3.12 whilst 5 mg-Fe³⁺/L only shows the highest log removal of log-2.55.

The assessment of Φ -X 174 removal under varying concentrations reveals insights into its removal capabilities in treatment amidst fluctuating viral loading. In comparing low-dose and high-dose conditions (section 3.3), it becomes evident that despite variations in viral loading, the overall LRV of Φ -X 174 remains markedly consistent (Table 3). This discovery suggests a consistent removal efficiency, independent of changes in viral concentrations and or specific influence or a single unit process. This highlights the value of multiple barriers in reducing virus concentrations. The ~4 log reduction linked to chlorination is in line

with expectations from other studies at this Ct value. The unexpected results of elevated log reduction of Φ -X 174 in the post-GAC versus directly in the post-ozone sample point requires further investigation. As mentioned earlier, rarely would these unit process be distinct in terms of measuring microbial removals at full scale.

Examining this further, in the context of high-dose conditions, the post-ozone samples exhibited the highest log reduction, higher than the observed log removal of Φ -X 174 in other treatment processes (1). This could be due to an influx of Φ -X 174 from the sand filtration treatment process where viral loading commonly occurred, especially for sample numbers 3 and 4. In contrast, under low-dose conditions, the average log reduction for somatic coliphage in the post-ozone stage appears low in comparison to the post-GAC samples. Another observation was in the post-GAC stage (Figure 11), where higher virus levels were seen in the initial sample numbers in high-dose conditions of Φ -X 174 compared to consistent lower levels in the low-dose scenario. The high-dose run followed the initial low-dose run, implying that the media used in the high-dose of Φ -X 174 scenario might have already been primed or contaminated by the earlier passage of the virus through the system. The media-based treatment processes might have provided an environment for virus retention over the interceding days particularly in the GAC, subsequently influencing the removal dynamics during the subsequent high-dose run due to desorption of virus during either the tap water or acclimatisation phase of the experiment prior to virus dosing. In GAC systems, desorption under low pollutant concentrations is often driven by the competitive adsorption of NOM, which typically has a greater thermodynamic efficiency. This competition can lead to the displacement of previously adsorbed pollutants, causing their release back into the water.

In evaluating indicators for viral removal in water treatment processes, TCC and ICC exhibit a parallel trend with the log removal of Φ -X 174, particularly in post-DAF samples and especially in the high dose scenario. This alignment suggests a correlation, indicating their potential as reliable markers for assessing viral elimination during treatment processes. The influence of indigenous microbial

populations on virus survival within these systems is notable and comparable to literature (Rzeżutka and Cook, 2004). Fujioka, Loh and Lau, (1980) suggested that viral inactivation exhibits a consistent acceleration in raw water environments with higher background microbial community as opposed to sterilized water. When contrasting LRV pilot runs under high and low-dose conditions, a discernible difference in TCC and ICC between each run emerges. Specifically, in the high-dose scenario, TCC and ICC register a notable one-log decrease compared to the low-dose scenario. This could be attributed to the background presence of *E. coli*, potentially utilised by Φ-X 174 for replication, therefore impacting the native microbial population between each experiment. The temporal sequence of conducting the high-dose trial subsequent to the low-dose trial may have created conditions favourable for the multiplication of Φ-X 174, potentially at the expense of the indigenous microbial community. Further investigations are necessary to support this conclusion, needing additional tests to ascertain the precise dynamics governing the interplay between Φ-X 174 loading and the indigenous microbial population.

The novelty of this project included the use of near-real scale drinking water treatment processes in comparison to bench-scale testing. There is notable difference in the bench-scale coagulation optimisation with and without the addition of Φ-X 174 compared to the pilot-scale experiments. The optimum coagulant dose for the bench-scale coagulation optimisation experiments in the absence and presence of Φ-X 174 was 10 mg-Fe³⁺/L and 15 mg-Fe³⁺/L, respectively. In comparison, the pilot scale experiments under the same conditions saw optimal coagulant doses as 17 mg-Fe³⁺/L and 10 mg-Fe³⁺/L, respectively. This shows that, to scale to full-size treatment scenarios, optimisation is required at near-real scale to attain the most comparable results. Whilst not the first study to benchmark Φ-X 174 removal in WTW, this study has undertaken these tests via sequential scale up of complexity of process and number of stages, something which has to the author's knowledge not been readily reported before. As mentioned in the introduction, additional data would be required on the persistence (decay) of the virus within the treatment works and the role of pH adjustment and coagulant itself on the viability of the virus. An

additional line of enquiry would be to understanding if pre-conditioning virus, rather than dosing in relatively clean matrices would have an impact on the partitioning, transport and ultimately fate of viruses in such systems. One omission from this work due to time constraints, was understanding pathogenic virus removal within the pilot plant. This element is mentioned below as an area requiring future work.

While human pathogenic virus was not explicitly measured in this work, it is important to consider that the somatic coliphage is designed as a pathway indicator for the risks of these pathogens. In a scientific examination of virus removal during water treatment processes, this study documented variable log reduction ratios for different viruses. The observed general virus removal ratios ranged from 0.2 to 2.0-log₁₀ under specific treatment conditions. More specifically, the removal ratios for distinct viruses were as follows: Adenovirus exhibited a log reduction between 0.9 and 2.0, Coxsackievirus between 0.5 and 1.6, Hepatitis A Virus from 0.8 to 1.1, and Murine Norovirus ranged from 0.6 to 1.3-log₁₀. Importantly, these removal ratios were notably influenced by the characteristics of the source water. For instance, the effectiveness of coagulation in virus removal varied significantly across different source waters, with some demonstrating relatively high virus removals and others showing considerably lower ratios. This variation underscores the impact of specific water qualities and constituents, such as turbidity, dissolved organic carbon, and UV-absorbing NOM, on the efficiency of virus removal in water treatment processes (Shirasaki et al., 2018).

In alignment of the aim of this project, source water characterisation as undertaken in accordance with the WHO guidance of the Drinking Water Parameter Cooperation Project (WHO, 2017). The background *E. coli* was measured and enumerated at 1.6-2.0x10³ CFU/ 100 mL. This classifies the source water as 'contaminated' (Appendix A). In addition, with the source water characterised at 'contaminated', the treatment performance target shows a reduction of log-5 for enteric viruses (Appendix A). The trial to establish the log removal of Φ-X 174 when dosed with the lower final concentration of 5.3x10¹⁰

PFU/ 100 mL (Figure 10) shows an overall treatment log reduction of Φ -X 174 of 7-log for both optimal and sub-optimal conditions. However, for the high-dose LRV trial with a final concentration of 1.0×10^{14} PFU/ 100 ml had a log removal at optimal treatment of 14-log and log-10 for sub-optimal coagulation conditions. For this scenario, Φ -X 174 presented in the final water samples for coagulant dose 0 mg-Fe³⁺/L.

Due to the log-removal targets being exceeded, it can be concluded that at doses greater than background native enteric virus concentrations, the 10^{-6} DALY target has also been met, therefore suggesting that the conventional treatment applied at near-real scale, produced safe drinking water (WHO, 2017).

Although all scenarios reached the target of 5-log removal of somatic coliphage through the treatment process, the WHO (2017) details viral log removal credits for conventional filtration including coagulation, flocculation, DAF, and filtration as 2-log removal for each treatment step. This has not been upheld for the low viral dosing scenario, with the filtrate from the sand filter presenting positive for Φ -X 174 in both optimal and sub-optimal coagulant dosing conditions. In contrast, the higher dose of Φ -X 174 saw no log reduction for 0 mg-Fe³⁺/L but removal at both 10 mg-Fe³⁺/L and 2 mg-Fe³⁺/L. This suggests that at sub-optimal coagulation, in the case where there is an issue with the coagulation treatment process, but the system is still under-dosing coagulant in place of no coagulant, the log-removal target can still be met. Overall, the loading of somatic coliphage at high doses under sub-optimal coagulant doses will still meet the log removal target of log-5 for enteric viruses for the entirety of a conventional drinking water treatment flowsheet when using somatic coliphage as a representative.

The Public Water Supplies (Scotland) amendment (European Union, 2022) denotes a limit of 50 PFU/ 100 mL in the source water. If this limit is exceeded, the viral concentration must be analysed after each treatment process to assess whether the risk of breakthrough of pathogenic viruses is sufficiently under control. The study denotes that even at high doses far exceeding 50 PFU/ 100 mL, conventional drinking water treatment at pilot-scale can provide 0 PFU/ 100 mL in the final water when coagulation is optimised suggesting the reference

value of 50 PFU/ 100 mL is arbitrary when compared to full-scale works. In addition, only at doses of 10^{14} PFU/ 100 mL did Φ -X 174 present under conditions of 0 mg-Fe³⁺/L of coagulant where no Φ -X 174 was detected at lower doses of the virus. This implies that the viral loading needs to be more than 10^{10} PFU/ 100 mL to be an operational risk which varies drastically from the set limit of 50 PFU/ 100 mL. Clearly, further work is needed to validate this finding on varied water matrices which are evident in the UK. In addition, greater diagnostic appreciation of sub-optimal versus optimal treatment is needed going beyond FeCl₃ into other coagulants and also other treatment technologies. For example, understanding the role of filter backwashing would be especially valuable in challenge tests using pilot or full-scale filters.

Table 5: Summary of the objectives and related hypotheses and the outcome of each

Objective	Hypothesis	Results
Undertake challenge trials under optimal and sub-optimal conditions using somatic coliphage	Removal of somatic coliphage will decrease under sub-optimal treatment conditions	Somatic coliphage is greatly influenced by the coagulant dose. Sub-optimal coagulation showed an increase in the measured somatic coliphage
Identify the removal capability of treatment processes at pilot-scale	Most somatic coliphage will be removed in upstream NOM/particle removal stages, with disinfection providing 0 PFU/ 100 mL in the final water	0 PFU/ 100 mL was measured at lower dose of Φ -X 174 but exceeded when 10^{14} PFU/ 100 mL was dosed. Post-GAC and post-ozone had the highest removal with sand filters adding to viral load in no or sub-optimal coagulation conditions
Identify if there is a correlation between log removal of somatic	Log removal of somatic coliphage can be predicted by changes in turbidity, UV254	TCC and ICC provide an indication of log removal of somatic coliphage but measuring directly provides a more robust analysis

coliphage and other water quality indicators.	and bacterial cell counts measured via flow cytometry.
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5 Conclusion

The study presents significant findings in the field of water treatment, focusing on the removal of viruses from drinking water through various treatment processes. The effectiveness of these processes is closely examined, and their capabilities in reducing viral contamination in water are highlighted.

The thesis demonstrates that the optimal conditions for coagulation are crucial for effective virus removal. This includes identifying the right coagulant dose and understanding the correlation between different water quality indicators and the efficiency of virus removal. The research extends to assess the impact of treatment processes on the log reduction of Φ -X 174, a somatic coliphage, under different coagulant doses. The findings exceed the WHO's log-removal targets for enteric viruses, suggesting that the risks to consumers from viruses in treated drinking water are manageable with conventional water treatment methods.

The current guidance for the targeted viral removal of enteric or pathogenic viruses from drinking water treatment offers a risk-based approach using source water limits and log-removal targets. In this study, the somatic coliphage presence is significantly impacted by the coagulant dose, showing distinct variations. When employing sub-optimal coagulation, an escalation in measured somatic coliphage is observed. Specifically, the measurement registered 0 PFU/100 mL at lower Φ -X 174 doses but surpassed this value when dosed at 10^{14} PFU/100 mL. Notably, the post-GAC and post-ozone treatment processes demonstrated the highest removal efficiency. Furthermore, the sand filters contributed to an increase in viral load, particularly evident in conditions with no or sub-optimal coagulation. Further work is needed to elucidate reasons for this finding. Whilst TCC and ICC offer indications regarding the log removal of somatic coliphage, direct measurement yields a more robust analysis of their presence and removal trends. With regard to the regulations discussed in this project, far higher concentrations of Φ -X 174 is required to surpass the limit of risk to

consumers, with all log-reduction targets exceeded with higher doses of somatic coliphage than the operational limit in the source water.

Overall, the research highlights the need for policy and governance in water treatment, advocating for a risk-based approach to managing viral risks in drinking water. This includes continuous monitoring and operational adjustments in water treatment processes based on water quality assessments. The study's findings contribute valuable insights into the field of water treatment, offering practical strategies for water utilities to effectively manage pathogenic microorganisms in drinking water and enhance public health safety.

6 Future Research

This thesis focuses on the effectiveness of water treatment processes in removing viruses, future research could delve into several areas. An exploration of advanced filtration technologies such as nanofiltration or ultrafiltration could reveal their potential in offering better protection against viruses compared to conventional methods. The study of the impact of emerging viruses on current water treatment processes is crucial, especially in light of new viral threats. Additionally, understanding how environmental changes, including climate change or pollution, affect virus presence and removal in water sources is vital for adapting water treatment methods.

A significant area of exploration would be the incorporation of sensitivity analysis linked to different definitions of sub-optimal treatment for each unit process tested in the thesis. This analysis would investigate how varying degrees of treatment efficiency, influenced by factors like coagulant dose, filtration rates, backwash frequency or disinfectant levels, impact the overall effectiveness of virus removal. By examining the thresholds at which treatment processes become less effective, researchers can better understand the resilience and robustness of these processes under diverse operational conditions. This research is essential for water utilities to develop more adaptive and responsive treatment strategies, ensuring public health safety in a variety of scenarios. Future work could also

incorporate analysis aimed at comparing the removal of somatic coliphages with human pathogenic viruses or/alongside other surrogates to determine the risk to human populations.

By systematically evaluating these factors, the research could provide valuable insights into the resilience and adaptability of water treatment processes. For instance, understanding at what point a decrease in coagulant dosage significantly reduces virus removal efficiency can guide water treatment facilities in maintaining effective virus control even in resource-limited scenarios. Similarly, examining how changes in filtration rates influence the removal of different types of viruses can inform the design of more robust filtration systems that can maintain efficacy under varying operational conditions.

Lastly, conducting a comprehensive cost-benefit analysis of implementing advanced water treatment technologies, as well as assessing the long-term public health outcomes of improved water treatment processes, would provide a holistic view of the economic and health impacts. This approach is crucial for effective decision-making in public health policies and the sustainable management of water resources.

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Appendix A

Classification of source water of surface water supplies. Information obtained from Table 10 from the Drinking Water Parameter Cooperation Project (WHO, 2017)

Characterisation (sanitary survey)	E. coli level (90%) (CFU/100 mL)	Source water category
The watershed lies in an area without human settlements and activities like agriculture. Wildlife may be present but not in high densities	<2	Very Pristine
The watershed lies in an area with little or dispersed human settlement and small-scale agricultural activities. No direct input of human or livestock wastes is present.	2-20	Pristine
The watershed lies in an area with villages and extensive agricultural activities (animal-based or food crops using manure or wastewater sludge for fertilization) are undertaken. Human faecal wastes are collected and treated before discharged into the watershed. The intake of water for production of drinking water is not under the direct influence of wastewater discharges	20-200	Moderately Contaminated
Small cities, villages and agricultural (animal-based or food crops using manure for fertilization, feedlots) areas are present in the watershed. Wastewater is collected and treated before discharge in the watershed. Sewer overflows and agricultural run-off enter the watershed, but the water intake is not directly under the influence of these discharges.	200-2000	Contaminated
Many and large urbanized areas and intensive agriculture (feedlots, large manure-storage facilities, intensive fertilization with manure) are present in the watershed. Wastewater is generally collected and treated before discharge into the watershed, but significant untreated discharges may occur frequently.	2000-20000	Heavily Contaminated

Many and large urbanized areas and intensive agriculture (feedlots, large manure-storage facilities, intensive fertilization with manure) are present in the watershed. Wastewater is generally collected but not or marginally treated before discharge into the watershed, heavily impacted by sewer overflows and manure discharges	>20000	Grossly Contaminated
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Suggested treatment performance targets for the different source water categories and enteric pathogens. Information taken from Table 11 from the Drinking Water Parameter Cooperation Project (WHO, 2017)

Source water category	Treatment performance target (in log ₁₀)		
	Enteric protozoa	Enteric bacteria	Enteric viruses
Very pristine	3	5	2
Pristine	4	6	3
Moderately contaminated	5	7	4
Contaminated	6	8	5
Heavily contaminated	7	9	6
Grossly contaminated	Not suitable as source for drinking-water supply; if no alternative sources are available, very extensive treatment barriers are needed.		

Appendix B Ethical Approval Letter



8 March 2023

Dear Miss Barry ,

Reference: CURES/17917/2023

Project ID: 20735

Title: Real-time virus detection using drinking pilot plant and online flow cytometry

Thank you for your application to the Cranfield University Research Ethics System (CURES).

We are pleased to inform you your CURES application, reference CURES/17917/2023 has been reviewed. You may now proceed with the research activities you have sought approval for.

If you have any queries, please contact CURES Support.

We wish you every success with your project.

Regards,

CURES Team