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ALYAA MOHAMMED ZYARA

REMOVAL OF VIRUSES FROM DRINKINGWATER BY CHLORINE AND UV DISINFECTIONS

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Alyaa Mohammed Zyara

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ABSTRACT

Chlorine and UV disinfections are common methods used to ensure the safety of drinking water. However, some viruses and other pathogenic microorganisms can be Cl- and/or UV-resistant. Therefore, it is important to find new methods to disinfect water. UV light emitting diodes (UV-LEDs) and combined treatment with chlorine and UV are newer methods that may be effective in the inactivation of Cl- and/or UV-resistant viruses. The aim of this thesis is to evaluate the use of traditional chlorine and UV methods, as well as the up-to-date applications of UV-LEDs and the combination of chlorine and UV against viruses in drinking water. This should yield scientific knowledge for the further development of drinking water disinfection.

Five methods were studied to inactivate coliphages that had been isolated from Kuopio municipal wastewater. In total, 18 different coliphages, which were either RNA or DNA coliphages, were isolated. Seventeen of them were used in chlorine disinfection experiments along with the F+ specific RNA virus MS2 as a surrogate virus. The coliphages were spiked into drinking water and treated with different dosages of chlorine during different contact times. The UV inactivation of the MS2 and 18 isolated coliphages was studied by using a mercury UV-lamp (Hg-UV) at 254 nm with different UV doses. In addition, inactivation efficiency of UV-LEDs at 270 nm wavelength was analyzed using five Cl- and/or UV-resistant coliphages which were examined by using transmission electron microscope. The inactivation of these coliphages was also analyzed with the combined chlorine and UV methods, by using first chlorine followed by UV (Cl/UV), or by using first UV and then chlorine (UV/Cl).

In chlorine disinfection, no reduction was achieved for the six most resistant coliphages in 10 min contact time at the chlorine dosage of 0.3 – 0.5 mg/L (free Cl-dosage of 0.12 - 0.21 mg/L), while the 11 sensitive coliphages achieved more than 99 % (2 Log_{10}) reductions.

With Hg-UV disinfection, 10 UV-resistant strains achieved less than 99 % (2 Log_{10}) reductions after exposure to a UV dose of 22 mWs/cm^2, while the nine UV-sensitive or intermediate strains achieved up to 99.99999 % (7 Log_{10}) reductions with the same doses. UV-LEDs reduced the numbers of four UV- and/or Cl-resistant coliphages by 99.99 % (4 Log_{10}) in 7 min contact time, which corresponded to the dose of 70 mWs/cm^2 in Hg-UV. MS2 was UV-resistant against both Hg-UV and UV-LEDs; thus, it is a good indicator for viruses in UV-disinfection experiments.

In the combined disinfection experiments, total chlorine of 0.05 - 0.25 mg/L (free Cl- dosage of 0.02 - 0.08 mg/L) followed by a UV dose of 14 - 22 mWs/cm^2 caused 99.9 – 99.999 % (3 - 5 Log_{10}) reductions for all UV- and/or Cl-resistant coliphages tested including MS2. The combined treatment was more effective than chlorine or UV alone, and also more effective than the sum of the individual chlorine and UV treatment showing high synergy effect. The synergy was absent or lower when UV was applied first and then followed by chlorine. Thus, in the combined treatment the order of disinfectants is important and should be taken into account in the future for developing drinking water disinfection methods.

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CAB Thesaurus: water treatment; drinking water; disinfection; viruses; bacteriophages; chlorine; chlorination; ultraviolet radiation; light emitting diodes; combination; synergism; transmission electron microscopy

Yleinen suomalainen asiasanasto: vedenpuhdistus; juomavesi; desinfiointi; virukset; bakteriofagit; kloori; klooraus; ultraviolettisäteily; ledit; yhteisvaikutukset; synergia; elektronimikroskopia

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Alyaa Mohammed Zyara

LIST OF ABBREVIATIONS

ADWG	Australian Drinking Water Guidelines
AGI	Acute gastroenteritis of unknown etiology
APHA	American Water Works Association
BOD	Biochemical oxygen demand
CDC	Centers for Disease Control and Prevention
Cl	Chlorine
COD	Chemical oxygen demand
CT	Free chlorine concentration multiplied by contact time
DBPs	Disinfection by-products
DNA	Deoxyribonucleic acid
ds	Double-stranded
E. coli	*Escherichia coli*
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
F-RNA	Male-specific or F+ coliphage ("F" refers to the genetic fertility factor that is required for bacteria to produce a sex pilus necessary for conjugation)
HAV	Hepatitis A virus
HEV	Hepatitis E virus
Hg-UV	Mercury UV lamp
ISO	International Organization for Standardization
LP	Low pressure UV lamp
MF	Microfiltration
MP	Medium pressure UV lamp
NSF/ANSI	National Academy of Science/American National Standards Institute
NWRI	National Water Research Institute
PCR	Polymerase chain reaction
PFUs	Plaque-forming units
RNA	Ribonucleic acid
RNase	Type of nuclease that catalyzes the degradation of RNA into smaller components
TEM	Transmission electron microscope
UF	Ultrafiltration
UNICEF	United Nations Children's Emergency Fund
US	United States
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UV-LEDs	UV light emitting diodes
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referrred to by the Roman numerals I-III.

I Zyara AM, Torvinen E, Veijalainen A-M, Heinonen-Tanski H. (2016). The effect of chlorine and combined chlorine/UV treatment on coliphages in drinking water disinfection. Journal of Water and Health, 4: 640-648, doi: 10.2166/wh.2016.144.

II Zyara AM, Torvinen E, Veijalainen A-M, Heinonen-Tanski H. (2016). The effect of UV and combined Chlorine/UV treatment on coliphages in drinking water disinfection. Water, 8: 130, doi:10.3390/w8040130, open access.

III Zyara AM, Torvinen E, Veijalainen A-M, Heinonen-Tanski H. (2017). UV-LEDs efficiently inactivate DNA and RNA coliphages. Water, 9: 46, doi:10.3390/w9010046, open access.

AUTHOR'S CONTRIBUTION

I Alyaa M Zyara performed the experiments and analyzed the data under the supervision of Torvinen, Veijalainen, and Heinonen-Tanski. She wrote the first draft of the paper. All authors contributed to the writing and approved the final manuscript.

II Alyaa M Zyara performed the experiments and analyzed the data under the supervision of Torvinen, Veijalainen, and Heinonen-Tanski. She wrote the first draft of the paper. All authors contributed to the writing and approved the final manuscript.

III Alyaa M Zyara performed the experiments and analyzed the data under the supervision of Torvinen, Veijalainen, and Heinonen-Tanski. She wrote the first draft of the paper. All authors contributed to the writing and approved the final manuscript.

CONTENTS

1 INTRODUCTION

Water is called the elixir of life. It covers about 75 % of the earth's surface. However, even though the total volume of water is high, only 2.5 to 3 % of it is fresh water. Moreover, only 1 % of fresh water can be used for human consumption without treatments (WHO, 2011). Agriculture is the largest user of fresh water, consuming 69 % it, while the industry consumes 19 %, and municipalities 12 % (FAO, 2016). It is estimated that the global population will reach 8 billion by 2030, which will increase the need for fresh water. In the same time, climate change will reduce surface water and ground water resources, especially in dry subtropical regions. Climate change will also decrease water quality at high latitudes due to increased rainfall, and cause risks to drinking water production (IPCC, 2014).

Improved water means drinking water, and it must meet several parameters when supplied from the source via treatments and disinfections to the consumer (Zuane, 1997; Pandit and Kumar, 2013). In 2015, more than 91 % of the human population had the possibility to use improved drinking water, but only 68 % had access to sanitation. Poor sanitation causes the contamination of water, which results in waterborne diseases. It has been estimated that contaminated water causes 1.7 billion diarrhea cases world widely and around 525,000 deaths of children under five each year (Ashbolt, 2004; Cairncross et al., 2010; WHO, 2017).

A major challenge in reducing waterborne disease is controlling pathogenic agents, such as viruses. The first human pathogenic viruses were discovered in water in the 19th century, when the poliovirus was detected in the East River flowing in the western side of New York City (Grabow, 2007), and the hepatitis E virus was detected in water leading to an outbreak in New Delhi in 1955 (Bosch, 1998). Many types of enteric viruses have contaminated water and caused waterborne epidemics since the 1950s (Sinclair et al., 2009). Besides gastroenteritis, which means inflammation in the stomach and intestines accompanied by vomiting and diarrhea, these viruses cause different diseases, e.g. liver disease and respiratory tract infections.

Removing pathogenic organisms from drinking water is thus essential for the protection of human health, and it can be done in several ways. Conventional drinking water treatment includes several processes, such as coagulation, clarification, filtration, and disinfection. Disinfection is the main and final key to the removal of pathogenic organisms from water. It can be done using chemicals, such as chlorine or ozone; physical treatments, such as UV; or combining chemical-chemical or chemical-physical treatments, where the treatment order can also vary.

Chlorination of the public water supply started in London in 1905 (Gerba and Pepper, 2015). Chlorine is the most common chemical disinfection method of drinking water (Zuane, 1997), since it has residual effect in water distribution systems (Lehtola et al., 2005; Pizzi, 2010). Chlorine compounds are cheap and easily adjustable oxidative chemicals which stay in water for a long time. However, chlorine is not effective in removing some resistant viruses, such as adenoviruses, and some protozoa, such as *Cryptosporidium* (Thurston-Enriquez et al., 2003a; EPA, 2010). Chlorine can also produce disinfection by-products (DBPs), which are harmful to health (WHO, 2011). For these reasons, physical disinfection with UV has become more common.

UV-disinfection was first used in Marseilles in 1910 (Solsona and Méndez, 2003). Nowadays, it is considered to be safer than chlorine, since it can efficiently control Cl-resistant pathogens without producing any DBPs. However, traditional Hg-UV lamps have a short lifetime, they produce toxic mercury waste, and they consume much energy (Sobotka, 1993; Bonzongo and Donkor, 2003). Recently introduced UV light emitting diodes (UV-LEDs) may be a good solution to these problems (Crawford et al., 2005; Vilhunen et al., 2009).

Combined treatments using chemical-physical or physical-chemical disinfection methods are also an interesting possibility to remove Cl- and/or UV-resistant viruses. The combined methods can be used so that different treatments are simultaneous, meaning that the chemical and physical treatment are used at the same time without quenching the chemical compound. The combined method can also be sequential, meaning that the first chemical treatment step is finished before the next step begins.

The aim of this thesis is to evaluate traditional and modern disinfection methods against viruses in drinking water and to contribute scientific knowledge for further development of drinking water disinfection. The work focuses on disinfection with chlorine, UV, their combinations, and UV-LEDs against coliphages isolated from municipal wastewater.

2 LITERATURE REVIEW

2.1 Drinking water

Drinking water, also known as potable, improved, or purified water, is water that is safe for drinking and food preparation. The amount of drinking water required by one person depends on physical activity, age, body size, health issues, and environmental conditions. It is estimated that an average human drinks about one to four liters per day, and those who work hard in a hot climate can consume up to 16 liters a day. Children considering their body size, consume more water than adults do (Zuane, 1997; Bitton, 2014; WHO, 2015). According to the World Health Organization (WHO, 2015), about 4.2 billion people obtain water through piped connections, which are not always safe. Approximately 2.4 billion people access water through other improved sources, such as protected wells and public taps. The rest, 663 million people, rely on unimproved sources, including the 159 million people who are dependent on untreated surface water (UNICEF, 2015; WHO, 2015).

Drinking water may be contaminated by microorganisms, such as pathogenic enteric bacteria, viruses, and intestinal parasites, if it is in contact with human or animal feces. Contaminated water causes approximately 1.7 billion diarrhea diseases in the world and leads to 525,000 deaths for children under five each year (Ashbolt, 2004; Cairncross et al., 2010; WHO, 2017). In low-income countries, e.g. 38 % of health care facilities lack any water source, 19 % do not have improved sanitation, and 35 % lack water and soap for hand washing (WHO, 2015). The health risks caused by waterborne pathogens may lead to the need for additional water treatment steps, such as the boiling of drinking water (WHO, 2011), which is not possible due to the high price of fuel and water. Many women in poor countries must still walk kilometers daily to fetch water and fuel.

2.2 Drinking water legislation

Legislation means setting standards, and this can be used to ensure that drinking water quality is acceptable for consumers. The first international guidelines to ensure safe drinking water were proposed by the WHO: in 1958, the organization set international standards for drinking water after sending questionnaires to its member states to evaluate their national water quality standards (WHO, 1958, 2011). Since then the WHO guidelines have been updated for many times to reach the current 4th edition with the first addendum (WHO, 2017). The guidelines serve as basis for setting specific regulations and standards for water quality and monitoring in each country or region taking into account the local circumstances. The aim of the guidelines is to minimize the risks affecting drinking water quality by providing a comprehensive preventive risk management framework for health protection, from catchment to consumer, that in addition to standard setting, covers policy formulation, risk-based management approaches and surveillance (WHO, 2017).

In Europe, the Council Directive 98/83/EC sets the minimum requirements for water quality that all EU countries must follow. Additional or stricter requirements may be given considering the local conditions (Council Directive 98/83/EC). In Finland, the Council Directive has been implemented as Decrees of the Ministry of Social Affairs and Health on the quality and monitoring of drinking water (STM 2001, 2015). In general, all countries as well as individual states have standard regulations that vary depending on the source of water, climate, geographical location, and economic, political, and cultural issues (Zuane, 1997; WHO, 2011).

The hygienic quality of drinking water is monitored with indicator organisms, the presence of which indicates fecal contamination. *Escherichia coli*, fecal enterococci, and total coliforms are the most common indicators used (Council Directive 98/83/ EC; EPA, 2014), and e.g. in the EU, *E. coli* and fecal enterococci must not be detectable in any 100 mL water sample (Council Directive 98/83/EC). Usually the presence of these bacteria is a good indication of contaminated water and possibility of causing disease (Zuane, 1997). International rules and guidelines are reviewed and updated from time to time considering new research results and the changing global environmental scenario, including the emergence of new pathogens and pollutants as well as the sources of water (Pandit and Kumar, 2013).

The chemical parameters must also meet the WHO guidelines and regional statutes (e.g. Council Directive 98/83/EC; EPA, 2017), since many chemical contaminants may threaten human health, especially if the exposure time is long. Some of these contaminants may enter water naturally from the ground. For example, arsenic is harmful to humans even at low concentrations; it can already cause dermal lesions such as hyperpigmentation, peripheral

neuropathy, skin cancer, bladder and lung cancer, and peripheral vascular disease at concentrations below 50 μg/L (WHO, 2011). On the other hand, some natural compounds, e.g. iron and sodium, or manganese and humus compounds, may affect the acceptability of water due to changes to e.g. its taste and odor (WHO, 2011). Moreover, other parameters, such as pH or alkalinity, are important to control because values that are too low may cause corrosion in the pipes (Tam and Elefsiniotis, 2009).

2.3 Waterborne pathogens

2.3.1 General

Water may contain many different enteric pathogens, which are pathogens originating from feces and causing mainly gastrointestinal diseases (Kolling et al., 2012; Pandit and Kumar, 2013; EPA, 2015a). The main sources of enteric pathogens in drinking water are feces due to lack of sanitation, municipal wastewater plant effluents, inadequate treatment of livestock waste, and on-site wastewater treatment systems (Gerba and Smith, 2005; Burkholder et al., 2007). Storm water runoff from surface water carrying animal waste or percolated as ground water are also important ways of contamination (Cole et al., 1999).

Enteric pathogens can be transmitted to humans by a fecal-oral route, which means that the microorganisms enter the human body via mouth by water or food contaminated with feces from infected persons or animals. Pathogens transmitted in this way from water sources are called waterborne pathogens. Some pathogens can survive in water distribution systems and some can multiply in favorable conditions, such as in warm water rich in nutrients. More than 1,000 species of pathogens have been seen to be transmitted via water and infect humans (Bitton, 2014). For this reason, the WHO has paid attention to microbiological water quality.

Waterborne pathogens can cause many types of diseases, most of which are diarrheal (EPA, 2015a; WHO, 2015). For example, cholera is an epidemic disease caused by *Vibrio cholera* bacteria transmitted via unsafe drinking water mainly in South-East Asia, Africa, and Latin America (WHO, 2000; Lee, 2001). Typhoid and paratyphoid fevers are common diseases caused by bacteria *Salmonella typhi* and *Salmonella paratyphi,* respectively (Levantesi et al., 2012). Dysentery (bloody diarrhea) can be caused by bacteria *Shigella* or some *Escherichia coli* strains, or protozoa *Entamobea histolytica*. Furthermore, many parasites such as *Giardia lamblia* or *Cryptosporidium parvum* may cause long-lasting gastroenteritis (Gerba and Pepper, 2015). According to Gerba (1996), the etiology of agents causing waterborne disease is often unknown (Figure 1).

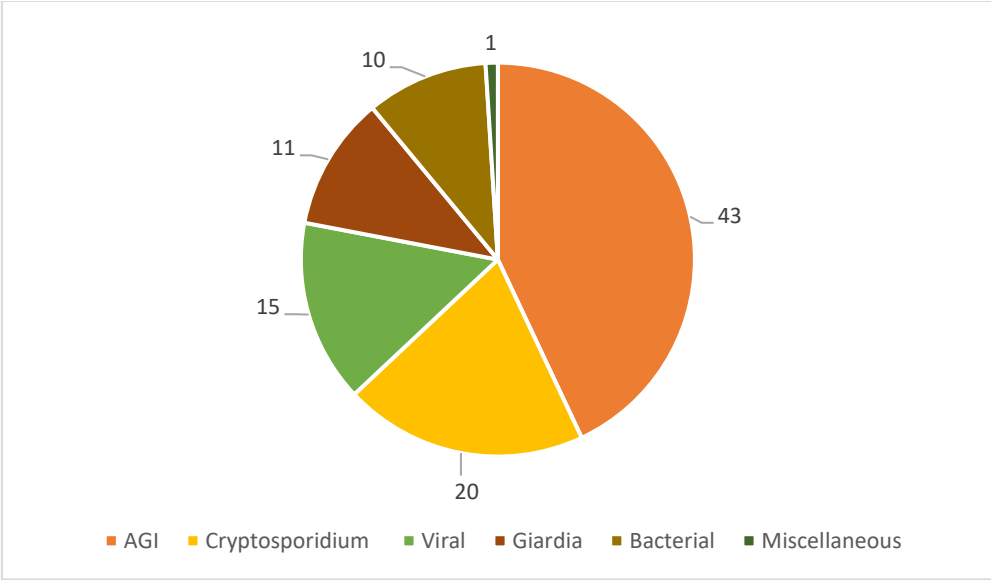


Figure 1. The percentage of etiological agents associated with cases of waterborne disease. AGI = acute gastroenteritis of unknown etiology (Gerba, 1996).

Enteric viruses cause the greatest concern among waterborne pathogens due to their ease to transfer, low infectious dose, and long survival time in the environment. Enteric viruses represent a wide range of taxonomic groups which are characterized by their small size and can include both RNA and DNA viruses (Table 1) (Yezli and Otter, 2011).

Approximately 140 of more than 200 human enteric viruses cause gastroenteritis disease and diarrhea (Melnick, 1984; Bitton, 2014). The most important among these viruses are adenoviruses, rotaviruses, astroviruses, and human noroviruses (Glass et al., 2001, Lopman et al., 2003). In addition, some enteric viruses can infect the human body without causing diarrheal diseases. These include e.g. the hepatitis A (HAV) and E viruses (HEV), poliovirus, and coxsackie virus (Ashbolt, 2004).

Vaccination can be used to control some waterborne viral diseases. Vaccination experimentation against polio started in Finland in 1954. Later, many other countries adopted polio vaccinations and the number of polio cases was greatly reduced globally. In 1988, the WHO launched a global program to eradicate polio, and today the number of cases is very low (Monto, 1999; Baicus, 2012). Later (1996), e.g. in the US, vaccination was recommended for HAV (CDC, 2016). On the other hand, there is still no vaccine available against the coxsackie B3 virus, and there is no drug that specifically kills this virus (Henke et al., 2003).

Table 1. Most common human enteric viruses in drinking water according to Grabow (2007); Bitton (2014); WHO (2011); and Miller (2016) (ds = double stranded; ss = single stranded)

Viruses	Family	Genetic material	Diameter (nm) of virus
Adenoviruses	Adenoviridae	dsDNA	70 – 120
Astroviruses	Astroviridae	ssRNA	27 – 43
Enteroviruses (polio, echo, coxsackie)	Picornaviruses	ssRNA	28 – 30
Hepatitis A and E viruses	Picornaviruses	ssRNA	27- 32
Norwalk agent (calicivirus or norovirus)	Caliciviridae	ssRNA	27 – 40
Rotaviruses	Reoviridae	dsRNA	60 – 80

2.3.2 Human enteric viruses

The largest group of enteric viruses are **enteroviruses**, which are picornaviruses. Enteroviruses are currently divided into seven major groups of human pathogens, including the poliovirus, coxsackieviruses, echovirus, and rhinoviruses. They can cause many human diseases that are not gastrointestinal diseases, such as severe paralysis and aseptic meningitis, myocarditis, respiratory illnesses, conjunctivitis, and severe generalized disease of infants (Miller, 2016). Infected persons excrete high numbers of enteric viruses in their stools, typically between 10^5 and 10^{11} virus particles/gram of feces (Fog and Lipp, 2005).

Adenoviruses were discovered by Wallace Rowe and his colleagues in 1953. These viruses are associated with animals, including mammals (Grabow, 2007). Human adenoviruses have been classified into six groups (A-F) and 51 antigenic types (Pond, 2005). These viruses are transmitted to humans via wastewater, surface water, swimming pool water, and drinking water (Percival et al., 2004; Albinana-Gimenez et al., 2006; Jiang et al., 2007; WHO, 2011; Bitton, 2014). An infected person can excrete as much as 10^{11} adenovirus particles/gram of feces, so that one infected person can transmit the disease to many other individuals. The incubation time is generally less than 10 days but may be up to 24 days (Pond, 2005). A wide range of human diseases can be caused by adenovirus, such as gastroenteritis, respiratory diseases, urethritis, and conjunctivitis (Albert, 1986; Grabow, 2007; WHO, 2017). The symptoms of these diseases differ, but generally include fever, vomiting, and diarrhea. The

estimated prevalence of acute adenovirus gastroenteritis in infants and children in developing countries is 5 - 20 % (Albert, 1986).

Astroviruses were first observed in 1975 using an electron microscope to examine stool specimens from infants with gastroenteritis. Globally, there are eight human astrovirus serotypes, and some of these cause gastroenteritis (Jeong et al., 2012, Bosch et al., 2014). After a one- to four-day incubation period, the symptoms of astrovirus appear as fever, headache, abdominal pain and watery diarrhea for two to three days, and vomiting leading to weight loss (Dennehy et al., 2001; Jeong et al., 2012). These viruses especially infect children in their first two years (Herrmann et al., 1991; Glass et al., 1996; Jeong et al., 2012), and adults can also become infected after being exposed to high doses of the virus (Guix et al., 2005). In one study, astroviruses were detected e.g. in eight of 68 French drinking water systems, and it has been found that the presence of this virus means an increased risk of an endemic level of gastroenteritis (Schwab, 2007).

Hepatitis viruses are a virus group that infects the liver, causing a disease called jaundice. The hepatitis viruses B, C, and D are transmitted via blood, while HAV and HEV are transmitted via the fecal-oral route directly through person-to-person contact or contaminated water. The incubation time of HAV and HEV is usually two to six weeks (Cuthbert, 2001; Ashbolt, 2004; Martin and Lemon, 2006; Yong and Son, 2009; Jacobsen and Wiersma, 2010; Bitton, 2014; CDC, 2015; Miller, 2016). Hepatitis viruses cause high risks because up to 90 % of infected persons, particularly children, show no clinical symptoms but do excrete the virus (Grabow, 2007). Jaundice is more common in children so that the ratio of disease between adults and children is usually 1 to 3 (Miller, 2016). Globally, at least 1.4 million cases of HAV appear each year, which means that this virus is at least 100 times more common than typhoid fever or cholera (WHO, 2017). Polluted water in Shanghai caused more than 300,000 cases of HAV in 1988 (Miller, 2016). In the US, the number of acute hepatitis cases was estimated to be 3,473 in the year 2013 (CDC, 2015).

Noroviruses belong to the family of caliciviridae, which causes the majority of cases of gastroenteritis in the world. Gastroenteritis of "unknown etiology" is often considered to have been caused by noroviruses. Nowadays, human noroviruses are divided into at least six genogroups and over 40 genotypes (Donaldson et al., 2010; Robilotti et al., 2015). Norovirus can infect humans through contaminated wells, small and community water systems, and groundwater (Taylor et al., 1981; Beller et al., 1997; Maunula et al., 2005). An infected person can excrete 10^9 norovirus particles/gram of feces (Atmar et al., 2008), so that one infected person can transmit the disease to many other individuals. The virus has a very low infective dose of 1-100 particles (Yezli and Otter, 2011). The incubation time of norovirus is short, from one to two days (Lee et al., 2013), and the symptoms start suddenly. In the US, 23 million cases of norovirus appear each year (Mead et al., 1999).

Rotaviruses consist of seven groups, of which A, B, and C have been reported to be human pathogens (Estes, 2001). The infective dose of rotaviruses is approximately 1 – 100 virus particles (Gerba et al., 1996; CDC, 2014b) and an infected person can excrete more than 10^{12} rotaviruses particles/gram of feces (Grabow, 2007; Miller, 2016). The incubation time is approximately 48 hours (Lee et al., 2013). Rotaviruses cause viral gastroenteritis in infants, children, and adults (Anderson and Weber, 2004). These viruses cause approximately 111 million cases of gastroenteritis and over 60,000 deaths in children under 5 years old annually (Parashar et al., 2003, 2006).

2.3.3 Factors controlling the survival of human viruses in water

The survival of viruses in the environment including water is affected by several factors, the interactions of which can be highly complicated and not yet fully understood. Temperature is probably the most important factor that affects the survival of enteric pathogens. Their survival time is usually longer at low temperatures (Hurst et al. 1980; John and Rose, 2005; Gerba, 2007; Rodríguez-Lázaro et al., 2012; Gerba and Pepper, 2015). E.g. in one study, the decay rates (Log_{10}/day) of coliphage MS2 in groundwater were approximately 10 times higher at 23 °C than at 4 °C (Yates et al., 1985). Longer survival in different types of water at 4 °C than at 15 °C or 22-25 °C has also been detected for many other viruses, such as human adenoviruses (Enriquez et al., 1995; Moresco et al., 2016), the murine norovirus (Moresco et al., 2016), human norovirus (Ngazoa et al., 2007), poliovirus (Enriquez et al., 1995), HAV (Enriquez et al., 1995), and coliphage PRD-1 (Yahya et al. 1993). Temperature affects the protein and nucleic acids by denaturization, which can be the reason for shorter survival in higher temperature (Gerba, 2007).

Most enteric pathogens are stable, with a pH range between 6 and 9 (Gerba and Pepper, 2015). In a study by Feng et al. (2003), the inactivation of MS2 and Qβ coliphages increased when the pH decreased to below 6 or increased to above 8. Extreme pH values can affect the virus surface by direct oxidation of capsid proteins and affect its nucleic acids by hydrolyses (Feng et al., 2003). pH may also impact the survival of viruses by affecting the adsorption of viruses to particles (Hurst et al., 1980; Yates, 2003; Gerba, 2007). The adsorption usually increases at an acidic pH, since the surface charges of the virus and the solid particle by acidic pH lead to electrostatic attraction between them (Yates, 2003).

The adsorption to suspended solids, such as clays, sand, particulate organic matter, or sediment, may prolong the survival of viruses (Smith et al., 1978; Hurst et al., 1980; Gerba, 2007), by e.g. increasing the stability of the viral

capsid, preventing aggregate formation, or offering protection from enzymes, other degrading factors, and UV inactivation (Fong and Lipp, 2005; Gerba, 2007). E.g. the coxsackievirus B3, adenovirus 1, echovirus 7, and HAV have survived for longer periods of time in soil-groundwater mixtures than in groundwater alone (Yates, 2003). Moreover, organic material could affect the survival of viruses through adsorption (Moore et al., 1981; Powelson et al. 1991; Yates et al., 2003). However, the effect of organic matter on survival of viruses is unclear: according to some sources (Gerba, 2007), survival is enhanced by the presence of organic material, while according to others it is not (Hurst et al. 1980).

The results regarding the influence of other water microbes on the survival of viruses are also variable. Filtering or other sterilization of water has prolonged the survival of viruses in many studies (Wetz et al., 2004; John and Rose, 2005; Gerba, 2007), but not in all (Hurst et al., 1980; Yates et al., 2003; John and Rose, 2005). The negative effect of other microbes could be due to e.g. the antiviral substances that they excrete (Yates et al., 2003).

Survival of enteric viruses has usually been longer in fresh than seawater (Enriquez et al., 1995), and longer in ground than surface water (Gerba, 2007). E.g. norovirus can be detected in groundwater for more than three years even if stored at 25 °C, and it can remain infective for as long as 61 days (Seitz et al., 2011). Higher salinity or antagonistic microbial flora in seawater and a lack of antagonistic microbial flora in ground water have been suggested as reasons for these differences (Enriquez et al., 1995; Gerba, 2007). UV in sunlight can inactivate viruses in environmental waters by damaging these viruses' nucleic acid (Fong and Lipp, 2005) or by photooxidation of the viral genome via photosensitizing substances in water (Gerba, 2007; Kohn and Nelson, 2007).

Biofilm is a layer of mucilage adhering to a solid surface in which microorganisms in water attach and develop (Gupta et al., 2016). It usually protects bacteria against unfavorable environmental conditions and may play a role in survival of viruses in water environment. Viruses are known to accumulate to biofilms of drinking water distribution systems (Lehtola et al., 2004; Långmark et al., 2005; Skraber et al. 2005; Lehtola et al., 2007; Helmi et al., 2008) and stay there for a long time (Skraber et al. 2005; Helmi et al., 2008), according to some studies (Lehtola et al. 2007) even for a longer time than in the water phase.

2.3.4 Coliphages

Coliphages are non-human pathogenic viruses that infect coliforms and related bacteria. They are found in the intestine and feces of humans and warm-blooded animals (Sobsey et al., 1995; Grabow, 2001; Jofre, 2007; EPA, 2015b). Coliphages are divided into several morphological groups, consisting of F-specific coliphages and somatic coliphages (Figure 2, Table 2). F-specific coliphages infect *E. coli* via sex pilus on the host, and they are known as F-RNA or F+ phages or male-specific coliphages. The somatic coliphages infect *E. coli* through receptors on the host cell wall (Sobsey et al., 1995; Cole et al., 2003; Vinjé et al., 2004; Jofre, 2007; Mesquita et al., 2010; EPA, 2015b). The viral genome of coliphages is either RNA or DNA, and it can be recognized by their response to RNase, an enzyme that degrades RNA and can be found in specific cultivation tests (Hsu et al., 1995).

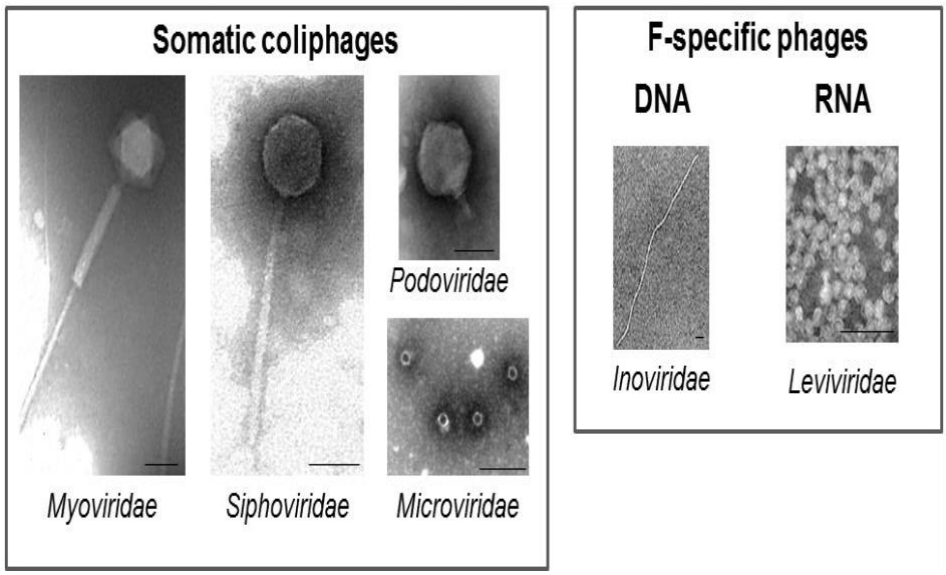


Figure 2. The most common morphological types in somatic coliphages and F-specific coliphages. Bar 50 nm (Jofre et al., 2016).

Table 2. Major groups of indicator coliphages, adapted from Leclerc et al. (2000), Jofre (2007), Mesquita et al. (2010), Jończyk et al. (2011), and EPA (2015b) (ds = double stranded; ss = single stranded).

Family	Nucleic acid	Type	Structure	Phage examples
Inoviridae	Circular ssDNA	F-specific	Nonenveloped, filamentous	SJ2, fd, AF-2, M13
Leviviridae	Linear ssRNA	F-specific	Nonenveloped, isometric	Group 1: MS-2, f2, R-17, JP501 Group 2: GA, DS, TH1, BZ13, KU1, JP34 Group 3: Qβ, VK, ST, TW18 Group 4: SP, F1, TW19, TW28, MX1, ID2
Microviridae	Circular ssDNA	Somatic	Nonenveloped, isometric	φX174, S13
Myoviridae	Linear dsDNA	Somatic	Nonenveloped, contractile tail	T2, T4, T6
Podoviridae	Linear dsDNA	Somatic	Nonenveloped, short noncontractile tail	T3, T7, P22
Siphoviridae	Linear dsDNA	Somatic	Nonenveloped, long noncontractile tail	λ, T1, T5
Tectiviridae	Linear dsDNA	F-specific	Nonenveloped, cubic capsid, no tail	PRD1, PR722

The life cycle of coliphages can be divided into lytic and lysogenic cycles. In the lytic cycle, coliphages infect their host and reprogram the host cell to produce high amounts of new phage particles before the lysis of the host cell, which leads to the latter's death. During the lysogenic cycle, the phage is combined with the host genome, or it may exist as plasmid in the host cell and may alter the phenotype by expressing new genes (Grabow, 2001; Clokie et al., 2011).

F-specific RNA coliphages can be used as indicators for human enteric viruses such as enteroviruses, caliciviruses, astroviruses, and HAV and HEV (Grabow, 1986, 2001; Chung et al., 1998). The adenovirus shares similarities with some somatic coliphages (King et al., 2011). The indicator value of coliphages is based on the fact that their composition, structure, size, morphology, and resistance to environmental conditions and/or disinfection treatments are similar to those of the enteric pathogenic viruses (Grabow, 1986; 2001; Leclerc et al., 2000; Cole et al., 2003; Nappier et al., 2006; EPA, 2015b). In addition, the detection and quantification of coliphages is cheaper, easier, more accurate, and faster than the detection of enteric viruses (Havelaar, 1986, 1987; Bosch 1998; Lin and Ganesh, 2013).

MS2 (ATCC 15597-B1) is a bacteriophage often used as an indicator for human enteric viruses in water (Grabow, 1986, 2001; Mamane et al., 2007; Shin and Sobsey, 2008; Rattanakul et al., 2014). MS2 is a small F-specific coliphage with a diameter ranging between 22 and 28 nm, linear single-stranded RNA, and icosahedral symmetry; it belongs to the genus Levivirus related to the *Leviviridae* family (NWRI, 2012).

The concentrations of coliphages can be in the range of 10^3 to 10^7 plaque-forming units per liter (PFU/L) in domestic raw and treated wastewater; thus, coliphages indicate fecal contamination (Leclerc et al., 2000; EPA, 2001a, b; Cole et al., 2003; EPA, 2015b). Somatic coliphages are more persistent in sewage and polluted waters, and they are found in higher numbers than F-specific RNA coliphages (Grabow, 2001; Jofre, 2007). Even though coliphages are commonly found from fecally contaminated waters, correlation between concentration of coliphages and detection of human enteric viruses has not always been detected (Leclerc et al., 2000; Jiang and Chu, 2004; EPA, 2015b).

Coliphages can be analyzed using different methods. In a double-layer agar method, approximately 1 mL of sample and a *Salmonella typhimurium* or *E. coli* host are typically added in temperated soft agar and then poured over the surface of solid agar (Adams, 1959; ISO 1995, 2001; EPA 2001a, b). Rajala-Mustonen and Heinonen-Tanski (1994) modified the method by adding 0.1 mL of 2,3,5-triphenyltetrazolium chloride (TTC) solution to increase the contrast between background and plaques. In a single agar layer method, the volume of water can be increased up to 100 mL. The agar, sample, and *E. coli* host are mixed together and poured on the plate (Grabow and Coubrough, 1986; EPA, 2001a, b). In a spotting most probable number technique, a small amount of enriched sample, typically 10 µL, is spotted onto the surface of solid agar containing the *E. coli* host (EPA, 2001a, b).

Nowadays, molecular methods have been developed for the detection and quantification of coliphages. So far, however, there are no applicable polymerase chain reaction (PCR) methods for the detection of all somatic coliphage groups in water (Jofre et al., 2016). Quantitative or qualitative (reverse transcription) PCR methods (RT-PCR) suitable for different kinds of water samples are available for groups 1 to 4 of F-specific RNA coliphages (Ogorzaly and Gantzer, 2006; Kirs and Smith, 2007; Friedman et al., 2009; Wolf et al., 2010) and for F-specific DNA coliphages (Long et al., 2005). Fast methods have also been developed based on different molecules, such as ß-galactosidase and adenylate kinase, released by coliphages from the infected cell after lysis (Ijzerman et al., 1993, Guzmán et al., 2009). If the number of coliphages in a sample is low, the sample can be concentrated by different methods, such as traditional membrane filtration (Sobsey et al., 1990; Méndez et al., 2004) and flocculation with chemicals (Chang et al., 1958; John et al., 2011) or, most recently, ultrafiltration enabling concentration of water up to 100 L (Hill et al., 2007; Ikner et al., 2011).

2.4 Drinking water treatment

Surface water, such as rivers, lakes, and reservoirs, and ground water can serve as drinking water sources (USEPA, 2015). A conventional drinking water

process can efficiently purify raw water of enteric viruses and other microorganisms that cause waterborne diseases (WHO, 2011). These process steps together can remove up to 99.9 % (3.4 Log_{10}) of enteric viruses in raw water (Hurst, 1991; Bell et al., 1998; Le Chevallier and Au, 2004; WHO, 2017). This removal efficiency can further increase by 99 % (2 Log_{10}) if chlorine disinfection is added (Hurst, 1991; Bell et al., 1998; Le Chevallier and Au, 2004; WHO, 2017).

The surface water treatment process often starts with coagulation using iron or aluminum salts with a positive charge to neutralize the negative charge of colloidal particles in water. Due to the action of coagulation salts, the neutralized particles aggregate and form large floc particles (flocculation), which are heavy enough to be separated from water (Zuane, 1997; Gao et al., 2002; Le Chevallier and Au, 2004; Pandit and Kumar, 2013). Flocculation is the slow mixing of the water particles with chemicals to build up floc particles. It can be affected by mixing rate and time (Le Chevallier and Au, 2004; Pizzi, 2010). The flocs can be removed in the clarification step, which is usually sedimentation (Edzwald and Kelley, 1998; Pizzi, 2010) but which can also be flotation, where the particles are carried to the surface of the water with air bubbles (Le Chevallier and Au, 2004).

Flocculation and clarification are often followed by filtration, which can remove the rest of the suspended solids and microflocs that cause turbidity. Rapid sand filtration is a common physical process in which water is filtered through one or several layers. Filtration material is often anthracite, sand, or active carbon (Cornwell et al., 2003). Slow sand filtration is a biological process in which biofilm is formed on the surface of the material. For instance, New York adopted this method for use of Hudson River water in 1870 (Zuane, 1997).

Membrane filtration is a newer method; it provides a direct physical barrier to remove microorganisms larger than 0.2 μm, including *Giardia* and *Cryptosporidium* (John et al., 2012). The membrane processes used in drinking water treatment for microbe removal are microfiltration (MF), ultrafiltration (UF), and nanofiltration (LeChevallier and Au 2004; John et al., 2012). Different filtrations can be combined with disinfection.

2.5 Drinking water disinfection

Disinfection is used to inactivate or reduce pathogenic microorganisms during drinking water treatment (John et al., 2012; Pandit and Kumar, 2013; ADWG, 2015). Chemical methods are most common, and they include e.g. chlorination (see 2.5.1.), ozonation, and iodination (Engelbrecht et al., 1980; Li et al., 2002; Ballester and Malley, 2004; Fang et al., 2014; Shin and Sobsey, 2008; Cromeans et al., 2010). Chemical disinfectants can also control color, taste, and odor, and

sometimes oxidize iron and manganese (WHO, 2011). Physical methods include e.g. boiling water (on a household scale) and UV irradiation (on all scales) (Meng and Gerba, 1996; Thurston-Enriquez et al., 2003b; Hijnen et al.; 2006; WHO, 2011). UV-LEDs are a method under development and, similarly to traditional Hg-UV, they can effectively reduce the densities of microbes including bacteria, phages, and human viruses (Chevremont et al., 2012a, b; Nelson et al., 2013).

Combined treatment of UV and chlorination is a common practice at waterworks but other combinations using chemical and physical methods are only under development, but they have shown to be promising (Cho et al., 2011; Fang et al., 2014; Lee and Shin, 2011; Rand et al., 2008; Rattanakul et al., 2014, 2015). The aim in all disinfection treatment is to maintain pipe safety, and therefore the disinfectant compounds must be added to distribution systems and they should stay on the pipe walls for an extended period (CDC, 2013).

2.5.1 Chlorination

Chlorination of public water supply started in London in 1905 (Gerba and Pepper, 2015). Later, chlorine was adopted for global use, and it is nowadays the most common disinfection method used in waterworks (Gerba and Pepper, 2015; CDC, 2014a). Different chlorine forms, such as chlorine gas, chlorine dioxide, chloramine, sodium hypochlorite solution (bleach), and solid calcium hypochlorite, are used in disinfection (Solsona and Mèndez, 2003; Pandit and Kumar, 2013). The typical chlorine concentrations in drinking water are between 0.2 and 0.5 mg/L to obtain around 0.2 mg/L of residual-free chlorine in the distribution system (ADWG, 2015; WHO, 2011).

When chlorine is added to water, two main chemical species are formed: hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) (Solsona and Mèndez, 2003; APHA et al., 2005; WHO, 2011; Pandit and Kumar, 2013; Bitton, 2014; EPA, 2016).

Chlorine first dissociates into HOCl, which in turn dissociates into a hypochlorite ion (OCl^-) and hydrogen ion (H^+), depending on the pH of the water (equations 1 and 2).

$$Cl_2 + H_2O \Leftrightarrow HOCl + HCl \qquad (1)$$

$$HOCl \Leftrightarrow H^+ + OCl^- \qquad (2)$$

HOCl dominates at acidic pH from 2 to 7.5, while the hypochlorite ion (OCl^-) dominates at alkaline pH above 7.5 (Solsona and Mèndez, 2003; WHO, 2011; Pandit and Kumar, 2013; Bitton, 2014; EPA, 2016). These two forms of

chlorine, HOCl and OCl^-, are called free chlorine. They are extremely reactive, degrading organic matter (Galal-Gorchev, 1996; WHO, 2011; Pandit and Kumar 2013; EPA, 2016). HOCl is more efficient than OCl^- in inactivating microbes because it is a stronger oxidant and more stable. It destroys metabolic enzymes and damages protein synthesis pathways (Pereira et al., 1973; McKenna and Davies, 1988; Solsona and Mèndez, 2003). Chlorine can modify purine and pyrimidine bases, leading to genetic defects in microbes (Patton et al., 1972; Hoyano et al., 1973) and damages in DNA (Pandit and Kumar, 2013).

Chlorine is applied at one or many points to maintain an efficient chlorine concentration in the water distribution system. Temperature has an important effect on disinfection, and increasing it enhances the disinfection efficiency (EPA, 1999). Chlorine at temperatures of 25 to 28 °C has been found to inactivate polio virus types MK500, 2, 3, and coxsackievirus B5 within 6, 2, 2, and 1 min, respectively, while chlorine at the temperatures of 1 to 5 °C inactivated the same viruses within 30, 60, 30, and 16 min, respectively (Kelly and Sanderson, 1958).

"Ct value" refers to disinfectant effectiveness, which is explained by the Chick-Watson model based on chemical reaction kinetics of linear log-survivor time curves (Chick 1908; Watson 1908). To obtain Ct (free Cl × min/L), C, meaning the disinfectant concentration, is multiplied by t, the time required to achieve a certain dose (Chick 1908; Watson 1908). This value is the most important parameter in disinfection. When the Ct value increases, the ability of chlorine to oxidize and disinfect increases accordingly (Thurston-Enriquez et al., 2003a; EPA, 2016).

The effect of chlorine inactivation varies depending on the virus type and the concentration of free residual chlorine, as presented in Table 3. WHO, (2011) concluded that generally, Ct values of 2 mg free Cl × min/L to more than 30 mg free Cl × min/L are needed to achieve 99 % inactivation of enteric viruses. Some viruses are not inactivated with chlorine (Engelbrecht et al., 1980, Thurston-Enriquez et al., 2003a), and at least strains of polioviruses, coxsackie viruses, and echoviruses have been reported to be resistant to chlorination (Engelbrecht et al., 1980; Cromeans et al., 2010).

Table 3. The efficiency of chlorine on enteric viruses transmitted via drinking water

Virus	Ct (free Cl × min/L)	Log_{10}-reduction	References
Adenovirus types 2, 5, 40, 41	0.01-1.4	3-4	Thurston-Enriquez et al., 2003a; Ballester and Malley, 2004; Cromeans et al., 2010; Page et al., 2010
Coxsackie B3, B5	2.2-7.4	2-4	Engelbrecht et al., 1980; Cromeans et al., 2010
Echoviruses 1, 5, 11	0.6-1.5	2-4	Engelbrecht et al., 1980; Cromeans et al., 2010;
Hepatitis A	300-600	complete	Li et al., 2002
MS2	0.3-0.8	2-5	Shin and Sobsey, 2008; Rattanakul et al., 2014
Murine norovirus, human norovirus, and feline calicivirus	<0.07-0.3	2-4	Thurston-Enriquez et al., 2003a; Shin and Sobsey, 2008; Cromeans et al., 2010
Polio types I, 2	0.3- 0.6	2- 3	Engelbrecht et al., 1980; Thurston-Enriquez et al. 2003 a

The main disadvantage of chlorination is its potential to form carcinogenic DBPs when reacting with organic material (Ates et al., 2007; Yang et al., 2013). Therefore, alternative disinfection methods are needed.

2.5.2 Ultraviolet irradiation

UV irradiation was discovered by Downes and Blunt in 1877 after they noticed the germicidal effect of sunlight. Mercury lamps were then developed in 1901 (Solsona and Mèndez, 2003; Schmelling, 2006), and UV-LEDs in 2000s. UV is that portion of the electromagnetic spectrum that lies between X rays and visible light. It is divided into four regions according to wavelength: vacuum UV between 100 and 200 nm; UVC between 200 and 280 nm; UVB between 280 and 315 nm; and UVA between 315 and 400 nm (Wright and Cairns, 1998; Masschelein and Rice 2002; Schmelling, 2006; Malato et al., 2009; Hunter and Townsend, 2010; Choi and Choi, 2010; WHO, 2011; Bitton, 2014). UVC has a germicidal effect on microorganisms and it is applied to disinfect water in different doses, which are calculated from the UV intensity multiplied by the exposure time. Usually, the unit used for the UV dose is milliwatt seconds per centimeter squared (mWs/cm^2), which is the same as millijoule per centimeter squared (mJ/cm^2).

The efficiency of UV disinfection is affected by water quality, including increases in turbidity; organic matter, which absorbs UV; and hardness, which may affect the lamp function by forming precipitates on the lamp surface. Some chemicals, such as iron, nitrites, and phenols, can absorb UV so that in the presence of these compounds, there will be a need for higher UV intensity (EPA, 1999).

The efficiency of UV disinfection is also affected by the length of irradiation time, adsorption, lamp intensity, reflection in the interface of air and water, and beam divergence (Bolton and Linden, 2003; EPA, 2010; Hijnen, 2010). Other factors are related to the microorganisms and strain variation, repair mechanisms, and physiological state (pre-culturing, growth phase) (Hijnen, 2010).

UV inactivates viruses by damaging the nucleic acids (DNA/RNA) with irradiation of near 260 nm (Schmelling, 2006; EPA, 2010; Hijnen, 2010; Bitton, 2014), causing thymine dimerization (von Sonntag et al., 2004). UV inhibits both replication and transcription, and prevents multiplication of the viruses in host cells causing their death (Schmelling, 2006; EPA, 2010; Hunter and Townsend, 2010; WHO, 2011). Studies of viruses have demonstrated that the initial site of UV damage is the viral genome, followed by structural damage to the virus coat (Nuanualsuwan and Cliver, 2003; Simonet and Gantzer, 2006). The repair of thymine dimers in DNA viruses could occur through a dark-repair or photo-reactivation of host cells, the latter requiring exposure to visible light for some time (Hunter and Townsend, 2010; EPA, 2010; Hijnen, 2010). RNA viruses, are not capable for the repair of thymine dimers (von Sonntag et al. 2004; Schmelling, 2006 Hijnen et al., 2006; Hijnen et al, 2010).

2.5.2.1 Mercury-UV (Hg- UV)

Mercury lamps (Hg-UV) operate by transforming electrical energy into UV radiation. The electric current ionizes mercury vapor and produces either monochromatic or polychromatic radiation (EPA, 1999; Pizzi, 2010). Monochromatic radiation at wavelength of 253.7 nm is emitted by low-pressure lamps, while polychromatic radiation at wavelength of 180 to 370 nm is emitted by medium-pressure lamps. The intensity of low-pressure (LP) lamps is lower than that of medium-pressure (MP) lamps (EPA, 1999; Schmelling, 2006).

Pulsed UV is a new type of UV which uses a flashlamp filled with inert gases such as xenon or krypton. Electrical current is discharged into the lamp in a series of very short pulses of nanoseconds (1 – 20 pulses/second). The electric current ionizes the gas which produces polychromatic radiation with wavelength of 100 - 1100 nm (Pizzi, 2010; Zhang et al., 2011). Total energy of this type of UV is much higher than in Hg-UV. Pulsed UV has been used in water and wastewater to inactivate resistant parasites and *Bacillus* endospores (Garvey et al., 2014; Garvey and Rowan, 2015).

Nowadays, all these UV lamp types are used in drinking water disinfection without forming the disinfection by-products associated with chlorination (Wright and Cairns, 1998). In addition, UV treatment needs only a short

contact time, leading to minimal space requirement, and it does not cause corrosion in the water distribution system.

Hg-UV has been noticed to control many waterborne bacteria, viruses, and protozoa which can be resistant to chlorine (Cotton et al., 2001; Masschelein and Rice 2002; Schmelling, 2006; Hijnen et al., 2010). It has been applied in the Netherlands since 1980 (Kruithof et al., 1992) due to its significant efficacy against the Cl-resistant protozoa *Cryptosporidium* spp. (Clancy et al., 1998; Mofidi et al., 2001; Rochelle et al., 2004; Dotson et al., 2010; Pandit and Kumar, 2013) and *Giardia* (WHO, 2011; Pandit and Kumar, 2013).

However, many viruses are resistant to UV, and adenoviruses are among the most resistant microbes against UV (Meng and Gerba, 1996; Thurston-Enriquez et al., 2003b; Nwachuku et al., 2005; Baxter et al., 2007; EPA, 2010; Rattanakul et al., 2014). In addition, the non-pathogenic bacteriophage MS2 and *Bacillus subtilis* spores have been classified as standard challenge organisms due to their high UV resistance (EPA, 2010).

The typical UV dose in water disinfection recommended by the National Academy of Science/American National Standards Institute (NSF/ANSI) is 40 mWs/cm^2 (Choi and Choi, 2010; NSF/ANSI, 2012; Bitton, 2014). This dose provides 3 – 4 Log_{10}-inactivation of most waterborne pathogens (Yates et al., 2006). However, many countries recommend a UV dose between 16 and 40 mWs/cm^2 (Masschelein and Rice, 2002), but in the case of UV-resistant viruses even the dose of 40 mWs/cm^2 is not adequate. In fact, enteric viruses such as adenoviruses 40 and 41 may need UV doses of up to 222 mWs/cm^2 (Gerba et al., 2002; Thurston-Enriquez et al., 2003b; Ko et al., 2005; Baxter et al., 2007; EPA, 2010) to achieve 2-4 Log_{10}-inactivation (Table 4). Other enteric viruses are much more sensitive to UV (Table 4).

Table 4. The reduction of viruses at different Hg-UV doses in drinking water

Viruses	UV dose (mWs/ cm^2)	Log_{10}-reduction	References
Adenovirus types 1, 2, 5, 6, 15, 40, 41	40 - 222	1-4	Meng and Gerba, 1996; Gerba et al., 2002; Thurston-Enriquez et al., 2003b; Ballester and Malley, 2004; Ko et al., 2005; Nwachuku et al., 2005; Hijnen et al., 2006; Baxter et al., 2007; EPA, 2010; Hunter and Townsend, 2010; Bitton, 2014; Rattanakul et al., 2014, 2015
Coxsackie virus B3, B5	20 - 36	3- 4	Battigelli et al., 1993; Gerba et al., 2002; Hijnen et al., 2006; Bitton, 2014
Echovirus 1, 2	16 - 33	3- 4	Gerba et al., 2002; Bitton, 2014
Hepatitis A	12 - 30	3- 4	Battigelli et al., 1993; Hijnen et al., 2006; Hunter and Townsend, 2010; Bitton, 2014
MS2	5- 116	1- 4	Battigelli et al., 1993; Meng and Gerba, 1996; Thurston-Enriquez et al., 2003b; Nwachuku et al., 2004; Hijnen et al., 2006; Schmelling, 2006; EPA, 2010; Cho et al., 2011; Fang et al., 2014; Rattanakul et al., 2014
Poliovirus 1	13-31	3- 4	Meng and Gerba, 1996; Gerba et al., 2002; Nwachuku et al., 2004; Hijnen et al., 2006; Hunter and Townsend, 2010; Bitton, 2014
PRD-1	31.6	4	Meng and Gerba, 1996
Rotavirus SA11	23-44	3- 4	Battigelli et al., 1993; Hijnen et al., 2006; Hunter and Townsend, 2010; Bitton, 2014
φX174	2- 9	4	Battigelli et al., 1993; Schmelling, 2006

2.5.2.2 UV-LEDs

Recently, UV-LEDs have garnered more attention in water disinfection; they may provide a solution to UV mercury lamps. The benefits of UV-LEDs are that they consume less energy, they do not produce toxic mercury waste, and the materials can be recycled. UV-LEDs can be repeatedly turned on and off without waiting times, and have a potential lifetime of approximately 100,000 h, i.e. 10 times longer than that of Hg-UV lamps. In addition, they are small and fit in many places (Crawford et al., 2005; Vilhunen et al., 2009; Chatterley and Linden, 2010; Aoyagi et al., 2011; Bowker et al., 2011).

LEDs consist of two semiconductor types, p silicon and n silicon, connected to move the electrons from the n-type into the holes of the p-type material to emit light at the p-n junction (Crawford et al., 2005; Khan, 2006; Hu et al., 2006). The semiconductors vary in the material types and wavelengths. The basic structures of UV-LEDs consist of aluminum nitride or/and aluminum gallium nitride (Tamulaitis, 2011).

UV-LED irradiation with a wavelength ranging from 265 to 405 nm and low power outputs have demonstrated efficient inactivation in *E. coli* (Crawford et al., 2005; Hamamoto et al.,2007; Mori et al., 2007; Vilhunen et al., 2009; Bowker et al., 2011; Chatterley and Linden, 2011; Nelson, 2013; Oguma et al., 2013; Gross et al., 2015). On the other hand, generally higher UV doses were needed for virus disinfection compared to that of bacteria (Tamulaitis, 2011).

UV-LEDs have been studied with viruses, such as different type of coliphages or adenovirus, at wavelengths between 255 and 285 nm (excluding 270 nm) (Aoyagi et al., 2011; Bowker et al., 2011; Jenny et al., 2014, 2015; Oguma et al., 2016a, b; Sholtes et al., 2016; Song et al., 2016; Beck et al., 2017). The Log_{10}-reductions have been between 1 and 5 Log_{10} depending on the coliphage type and UV dose and exposure time, test design, and virus type (Table 5).

Table 5. Reduction of viruses at different doses of UV-LEDs operated between 255 and 285 nm wavelengths

Virus	Wavelength of UV-LED (nm)	UV-LED doses (mWs/cm^2)	Log_{10}-reduction	References
Adenovirus 2	260 280	91 91	2.5 3	Beck et al., 2017
Adenovirus 5	280	43.5	1	Oguma et al., 2016b
MS2	255	40	3	Aoyagi et al., 2011
	255	45	1.7	Bowker et al., 2011
	260	43	3	Beck et al., 2017
	260	60	2.5	Jenny et al., 2014
	260	58	4	Sholtes et al., 2016
	275	45	2	Bowker et al., 2011
	280	43	2.5	Beck et al., 2017
	280	60	1.9	Aoyagi et al., 2011
	285	34.5	1	Oguma et al., 2016a
Qβ	255	30	2.5	Aoyagi et al., 2011
	260	45	4	Jenny et al., 2014
	280	30	1.5	Aoyagi et al., 2011
	285	27	1	Oguma et al., 2016a, b
T7	255 275	20 20	3.5 4.7	Bowker et al., 2011
φX174	255 280	6.4 8.9	3.7 3.2	Aoyagi et al., 2011

2.5.3 Combined disinfection treatment

When two different disinfection processes are used together, the reduction of microorganisms can be higher than the sum of the two methods used separately, i.e. synergy may be obtained. Chemical/chemical and chemical/physical methods are examples of combined disinfection treatments. For instance, chlorine can be as used as a primary disinfectant and ozone (O_3), UV irradiation, or chlorine dioxide as a secondary disinfectant (Jung et al., 2008; Lee and Shin, 2011; EPA, 2016).

The simultaneous process means that both disinfection methods are started at the same time, or that the secondary treatment is started directly after the primary treatment time without stopping the reaction of the first treatment, so that both disinfections are done at least partly at the same time (Koivunen and Heinonen-Tanski, 2005; Shang et al., 2007; Vankerckhoven et al., 2011; Rattanakul et al., 2014). The sequential process involves stopping the reaction of the primary disinfection step (e.g. chlorine or UV) before using the secondary disinfectant (e.g. UV or chlorine) (Shang et al., 2007; Rand et al., 2008; Cho et al., 2011; Lee and Shin, 2011; Rattanakul 2014, 2015).

Previous studies have shown synergistic disinfection effects on microorganisms in the combined treatment of drinking water or wastewater using two treatments, such as Cl/UV (Shang et al., 2007; Rand et al., 2008; Wang et al., 2011; Rattanakul et al., 2014, 2015), peracetic acid (PAA)/UV (Rajala-Mustonen et al., 1997; Koivunen and Heinonen-Tanski, 2005), and ozone (O_3)/UV (Jung et al., 2008).

Only a few studies have examined the combined treatment of chlorine and UV against viruses in drinking water (Table 6). Shang et al. (2007) and Rattanakul et al. (2014, 2015) got very high synergy using simultaneous treatment to inactivate MS2 and adenoviruses even if the chlorine dose (0.15 - 1 mg/L for approximately 1 min) and UV doses (10 and 23 mWs/cm^2) were low. In contrast, the sequential treatment using first UV followed by chlorine (UV/Cl) showed less or no synergy effect against MS2 and adenovirus 5 (Shang et al., 2007; Lee and Shin, 2011; Rattanakul et al., 2014, 2015). Therefore, Rattanakul et al. (2015) suggest that if chlorine and UV are combined in real scale drinking water disinfection, it is better to start with chlorine and then follow by UV to enhance the inactivation of adenovirus.

Table 6. Reduction of viruses in different doses of the combined Cl/UV or UV/Cl treatment

Viruses	Combined type	UV-dose (mWs/cm^2)	Cl (mg/L)	Time of Cl (min)	Log_{10}-reduction	Synergy
Adenovirus 5[(a)]	UV/Cl sequential	40	3.3	5	6	not analyzed
Adenovirus 5[(b)]	UV/Cl sequential	20	0.17	0.4- 1.5	2.4- 4	No
Adenovirus 5[(c)]	UV/Cl sequential	50	0.15	0.13- 0.37	3- 4	No
Adenovirus 5[(c)]	UV/Cl simultaneous	10	0.15	0.17- 0.67	2- 3.5	No
Adenovirus 5[(c)]	Cl/UV simultaneous	10	0.15	0.17- 0.5	3.1- 4.5	very high synergy
MS2[(d)]	UV/Cl sequential	23 46 69	1 1 1	0.4- 1.1 0.4- 0.95 0.25- 0.5	2- 4.5 3.2- 5 4.2- 5	yes yes yes
MS2[(d)]	UV/Cl simultaneous	23	1	0.1- 0.7	1.5- 4	high synergy
MS2[(d)]	Cl/UV simultaneous	23	1	0.1- 0.7	2.5- 6.5	very high synergy
MS2[(e)]	UV/Cl sequential	17 51	1 1	0.4- 2.2 0.4- 1.1	2.2- 4 4.5-6.5	no yes
MS2[(e)]	Cl/UV simultaneous	17	1	0.25- 0.9	2.5- 3.2	Yes

[(a)] Ballester and Malley, 2004
[(b)] Lee and Shin, 2011
[(c)] Rattanakul et al., 2015
[(d)] Rattanakul et al., 2014
[(e)] Shang et al., 2007

In the simultaneous inactivation process, UV and Cl damage microorganisms at the same time. The UV process damages the nucleic acids (Nuanualsuwan and Cliver, 2003) at wavelength of 260 nm (EPA, 2010; Hijnen, 2010; Bitton, 2014) and causes thymine dimerization (von Sonntag et al., 2004). Thereby, it inhibits nucleic acid replication and transcription and leads to cell death (Hunter and Townsend, 2010; EPA, 2010; WHO, 2011). Chlorine has also other targets, damaging and destroying the structure of viral protein capsid by oxidizition, and disrupting the nucleic acids (Hunter and Townsend, 2010; Wigginton et al., 2012). This destruction successfully occurs in these processes, enhancing the inactivation. These results support the assumption that viral capsid may be damaged by chlorine in the first step and the virus subsequently be more sensitive to UV, as discussed earlier by Rattanakul et al. (2014).

2.6 Transmission electron microscopy

The transmission electron microscope (TEM) uses an electron beam instead of visible light. The electron beam can be transmitted through an ultra-thin sample, interacting with that sample and producing highly magnified images called micrographs.

The term TEM was first used in 1931 by Kroll and Ruska, who presented the idea of electron lenses in their paper and soon built them in practice (Williams and Carter, 1996). Just four years later, TEM was developed by commercial companies (Ayache et al., 2010). Currently, TEM is one of the most efficient tools for several purposes, and especially in microbiology to detect details at the cell size level (Ayache et al., 2010). Its disadvantages are its high price and need for a large, specially designed room with temperature adjustment, expensive pieces of equipment, and trained personnel to operate them. In contrast, the major advantage is its ability to magnify more than 1.5 million times, which is needed in biology, medicine, and for many other purposes. The greatest resolution can be 0.2 nm, which allows the user to see tiny detail. Thus, TEM enables the study of virus morphology, including the shape, size, and different parts of organisms (Vale et al., 2010).

3 THE AIMS OF STUDY

The aims of this study were to evaluate different disinfection methods for the inactivation of 18 coliphages isolated from municipal wastewater, and to find a method that can efficiently inactivate viruses by using a low concentration of chlorine or a low dose of UV. To achieve these aims, three independent studies were conducted, and their specific aims were listed below.

Study I: (i) To study the effect of chlorination on 17 coliphages isolated from wastewater and compare the Cl-resistance to that of the MS2 virus; and (ii) to evaluate the efficiency of the simultaneous chlorine and UV treatment with low concentration and dosages on the most Cl-resistant coliphages isolated from drinking water.

Study II: (i) To determine the effects of different UV doses on inactivation of 18 coliphages isolated from municipal wastewater and known to have different resistances to chlorine; and (ii) to evaluate whether there is some synergism in the inactivation of these coliphage strains when the chlorine is combined with a following UV treatment (Cl/UV), or when the UV is combined with the following chlorine treatment (UV/Cl).

Study III: (i) To compare the efficiency of UV-LEDs at 270 nm with that of a traditional Hg-UV lamp at 254 nm to inactivate UV- and/or Cl-resistant coliphages.

In addition, the aim of this study was to characterize the isolated coliphages according to their nucleic acids, and morphology of Cl- and/or UV-resistant coliphages by using transmission electron microscopy.

4 MATERIALS AND METHODS

4.1 Isolation and purification of coliphages

Eighteen coliphages were isolated from the effluent of a wastewater plant in Kuopio, Finland using a double-layer technique (Adams, 1959). The coliphage MS2 (*Escherichia coli* ATCC 15597-B1) and these isolated coliphages were used in the studies described in Papers I, II, and III and in unpublished studies. The host bacteria used in cultivation were *E. coli* ATCC 13706 and *E. coli* ATCC 15597. Different plaques were picked up from the plates, depending on their size and on the morphology of lysogenic and lytic zones (Tan et al. 2008; Paper I, Table 1); inoculated into fresh host solutions in tryptose yeast extract glucose (TYG) broth; and incubated in a shaker at 37°C for 24±3 h. This overnight suspension was rejuvenated for 2 h and then centrifuged at 3250 x g for 15 min to separate coliphages and cell debris (Rajala-Mustonen and Heinonen-Tanski 1994). The purity of the coliphage suspension was verified by re-cultivating the solution 2 – 3 times with the same double layer technique as used in isolation.

4.2 RNase spot test

RNase enzyme (Ribonuclease Type I-A, ≥ 50 Kunitz units/mg protein, Sigma-Aldrich, St. Louis, MO, USA) was used to distinguish the nucleic acids of the isolated coliphages (Paper III Table 1). A fresh host bacterium and RNase or water were added to melted TYG agars, and then 3 µl of a pure coliphage solution was spotted onto the solid agar and incubated at 37°C for 24 ± 3 h. The presence of plaques on plates both with and without the RNase enzyme indicated a DNA coliphage, while the presence of plaques only on the plates without RNase indicated an RNA coliphage (Hsu et al., 1995).

4.3 TEM

Six coliphages proven to be resistant against Cl and/or UV, and MS2 were selected for analyses with TEM. The negative staining technique was used to increase the contrast between the sample and background. The grids with a diameter of 3.05 mm (Agar scientific) were coated with a Formvar film and reinforced by a thick carbon layer for 30 min (Ayache et al., 2010). Formvar- and carbon-coated grids were floated with 3 µl of the coliphage suspension

with an approximate density of 10^{12} PFU/mL for 2 min. The excess suspension was blotted with an edge of filter paper and left the grid surface lightly moist. A drop of the negative stain phosphor tungstic acid (5 %) was immediately added onto the grid, which was incubated for 1 min, and then the excess stain was removed with a filter paper. The grid was air dried for about 1 - 2 min before it was examined using TEM (JEM-2100F, JEOL, Tokyo, Japan) with magnifications up to 1.5 million (Figure 3). Three grids were prepared for each coliphage isolate. The length and width of the coliphage head and the length of the tail were measured using the Image Transmission Electron Microscope (iTEM, USA) program.

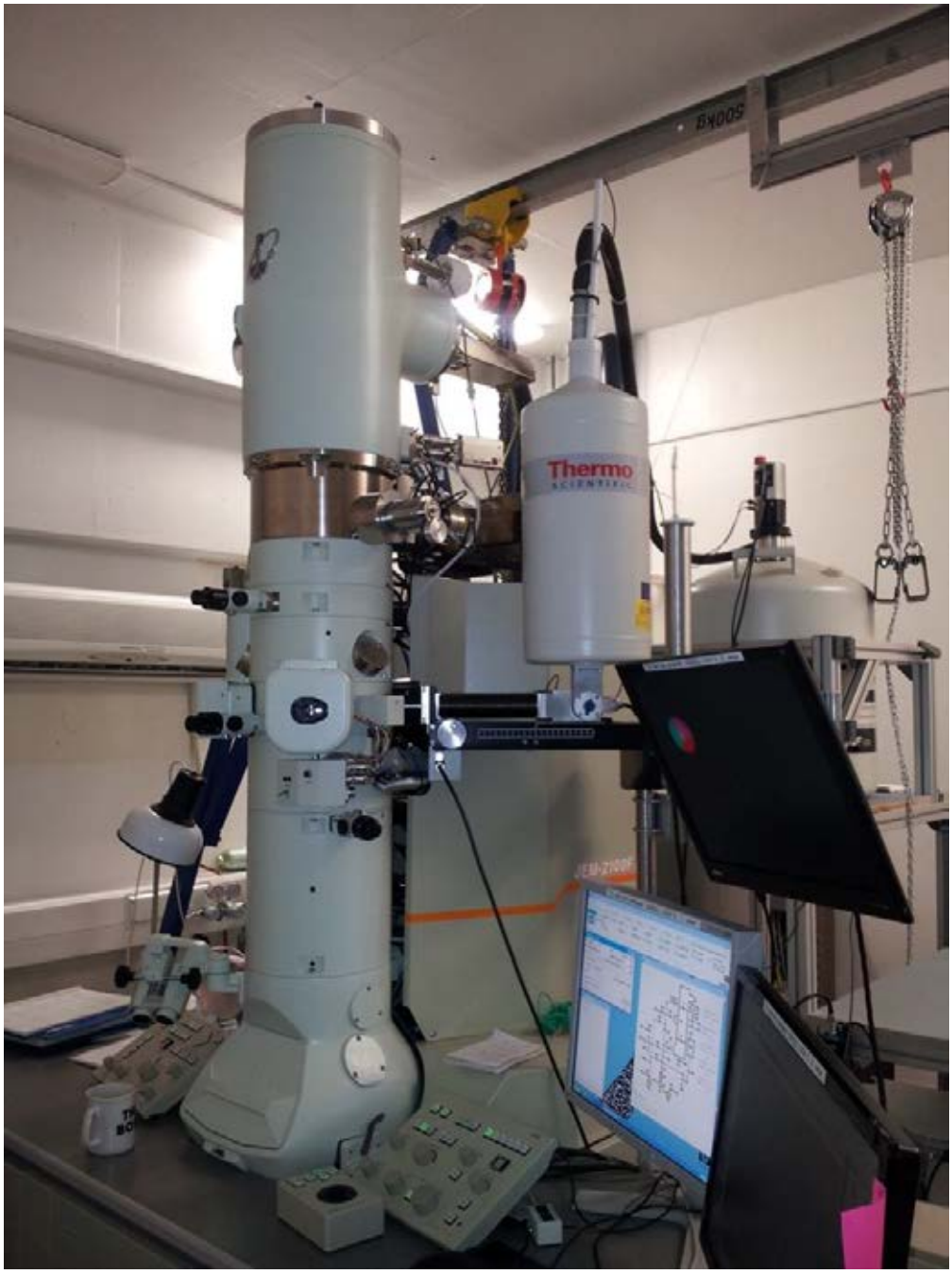


Figure 3. Transmission electron microscope (TEM) (JEM-2100F) with magnifications up to 1.5 million.

4.4 Chlorine experiments

The Kuopio municipal tap water, produced by sand bank filtration and coagulation, filtration and chlorination, was collected in a beaker after flushing and left at room temperature for 24 h to remove the residual chlorine (Papers I, III). The quality of water was described by Kuopion Vesi (2015) to be 0.10 FTU for turbidity, < 5 mg Pt/l for colour, 1.3 mg/L for COD_{Mn}, 7.7 for pH, 12 μg/l for iron, 22 μg/l for manganese and 0.35 mg Cl_2/L for free Cl. The concentrations of total and free chlorine in the evaporated water and before and after the experiments were measured using Hach DR 2800 spectrophotometer and methods 8167 (total chlorine) and 8021 (free chlorine) according to the manufacturer's instructions (Papers I, III).

Two liters of water was spiked separately with 17 isolated coliphage strains (see 4.1) and MS2 to yield initial concentrations of approximately 10^6 PFU/mL. The spiked water was divided into three parallel subsamples (V=100 mL) and each set was exposed to total chlorine concentrations of 0, 0.1 mg/L, 0.3 mg/L, or 0.5 mg/L for 10 min contact time. The residual chlorine was quenched by adding 0.05 mL of sodium thiosulfate (18 mg/mL) to 100 mL of water sample (SFS-EN ISO 19480). The density of coliphages was analyzed using a double layer technique before and after the experiment, as described in Paper I.

4.5 UV experiments

Deionized water was used in the Hg-UV experiments described in Paper II. A low-pressure mercury arc lamp (Osram HNS 30 W, = 253.7 nm, Munich, Germany) (Paper III, Fig. 1a, and Figure 4a) was turned on at least 15 min before each experiment to obtain a constant UV intensity output. Eighteen coliphage strains and MS2 were tested separately. Coliphage stock solution was added into water to yield an initial concentration of 10^9 PFU/mL (Paper II); 10 mL of this solution was then pipetted into a sterile glass Petri dish (inner diameter 6.0 cm) and exposed to UV doses between 22 and 117 mWs/cm^2 using 71 s – 10 min contact times. The intensity of the UV irradiation at the surface of the Petri dish was approximately 0.2 mW/cm^2 (Paper II) and 0.1661 mW/cm^2 when certain factors, such as reflection, Petri, water, and divergence, were taken into account according to Bolton and Linden (2003) (Paper III).

Kuopio municipal tap water (Kuopion Vesi, 2016) was used in UV-LED disinfection experiments. UV-LEDs were manufactured by SETi (Columbia, South Carolina, US) and installed in the reactor described in Paper III (Fig. 1b) and Figure 4b. UV-LED strips operated with a current of 120 mA and voltage of 5.5 V. The wavelength of the LEDs was 270 nm, which is close to the maximum absorption of DNA and not studied before, and the power of each

LED was 10 mW. The water was flushed and then left at room temperature for 24 h to remove the residual chlorine. The concentrations of total and free chlorine in the evaporated water were measured before and after the experiments using Hach DR 2800 spectrophotometer and methods 8167 (total chlorine) and 8021 (free chlorine) according to the manufacturer's instructions (Paper III). 5.2 L of water was poured into the LED reactor bottle and spiked with Cl- and/or UV-resistant coliphages and MS2 separately (see 4.1 in Paper III) to yield an initial concentration of 10^7 PFU/mL. The suspension was mixed using a magnetic stirrer for 3 min. The LED irradiation was started and water samples were taken after 2, 4, 5.5, 7, 10, 12, and 15 min contact times without switching off the LED irradiation during the sampling. The densities of coliphages in both the Hg-UV and the UV-LED study were analyzed before and after the experiments, as described in Paper I.

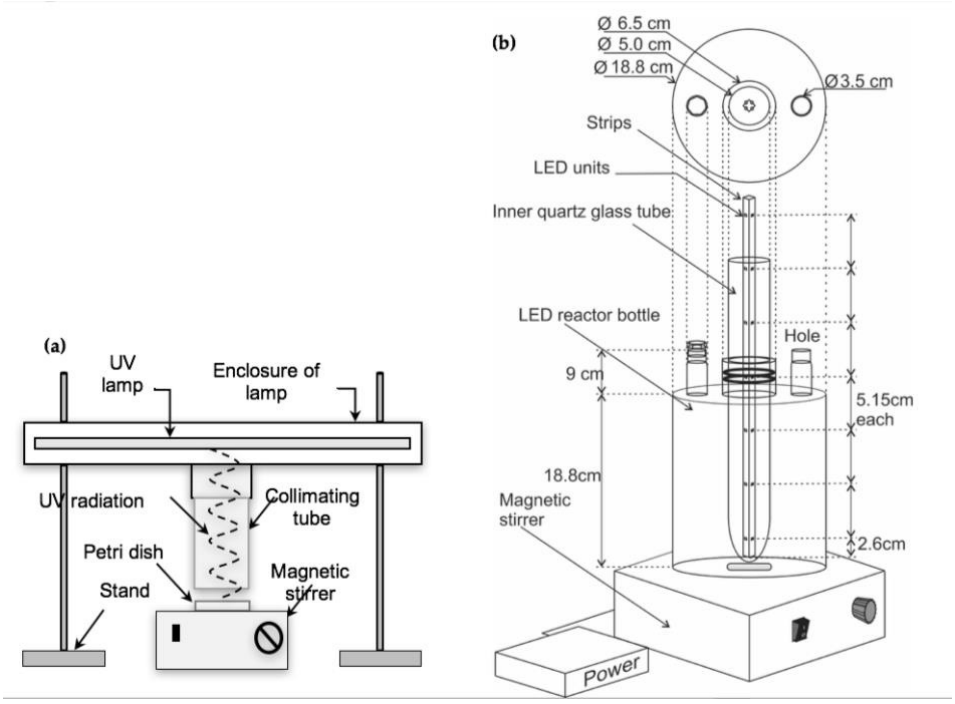


Figure 4. (a) Reactor type of 10 mL for Hg-UV in collimator. (b) Reactor type of 5.2 L for UV-LED with four LEDs at three different heights inside the water.

4.6 Combined chlorine and UV experiments

Kuopio municipal tap water (Kuopion Vesi 2015) was used in combined disinfection experiments. The tap water was collected in a beaker after flushing and left at room temperature for 24 h to remove the residual chlorine. The concentrations of total and free chlorine in the evaporated water and before and after the experiments were measured using Hach DR 2800 spectrophotometer and methods 8167 (total chlorine) and 8021 (free chlorine) according to the manufacturer's instructions (Papers I, III).

Six out of the 18 isolated coliphages and MS2, proven to be Cl- and/or UV-resistant (Papers I, II), were spiked into the water to obtain an initial concentration of 10^6 PFU/mL. The first combination experiment was the Cl/Hg-UV treatment. The disinfections were done by applying first the free chlorine at concentrations from 0.02 to 0.08 mg/L within 1 to 10 min contact times, and then UV at doses of 14 − 82 mWs/cm^2 within exposure times of 1.2 – 7 min (Papers I, II). The process was done continuously without quenching the chlorine.

The second combination tested was the UV-Hg/Cl treatment. The disinfection was done by exposing selected coliphages first to a UV dose of 22 mWs/cm^2 and then to free Cl dosage of 0.04 or 0.2 mg/L with 10 min contact time. The coliphage densities were analyzed in both combined experiments using a double layer technique, as described in Paper II.

4.7 Calculations and statistical analyses

Inactivation values were calculated as the Log_{10} of N/N_0, where N is the plaque density after disinfection and N_0 the density at time zero (Papers I, II, III). Ct values were calculated by multiplying the concentrations of free chlorine (mg/L) with the exposure time (min) (Papers I, II). Synergy was counted according to the equation used by Koivunen and Heinonen-Tanski (2005):

Synergy as Log units = Log reduction of combined chemical/UV disinfection - (the Log reduction for UV-disinfection + the Log reduction by chemical disinfection).

Non-parametric tests (Related sample Friedman's two-way analysis) using SPSS version 22 were conducted to determine the statistically significant differences between the plaque densities before and after chlorine, UV, and combined disinfections. Linear regression equations (average ± standard deviation of the three parallel analyses) were calculated with Excel 2013 to describe the relationship between Log_{10}-reductions of coliphages and free

chlorine concentration or UV dose (Papers I, II, III). The slopes of three separate parallel linear regression equations for each strain were analyzed using a non-parametric Kruskall-Wallis test to determine the statistical differences between the coliphage strains (Papers I, II). To compare the inactivations between Hg-UV and UV-LEDs, the slopes of the three parallel linear regression equations (inactivation – irradiation or contact time) of both methods were analyzed using Wilcoxon signed rank test (Paper III).

5 RESULTS

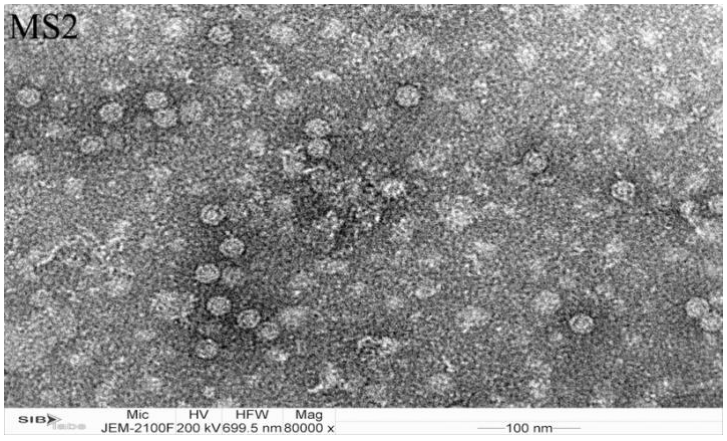
5.1 Characteristics of isolated coliphages

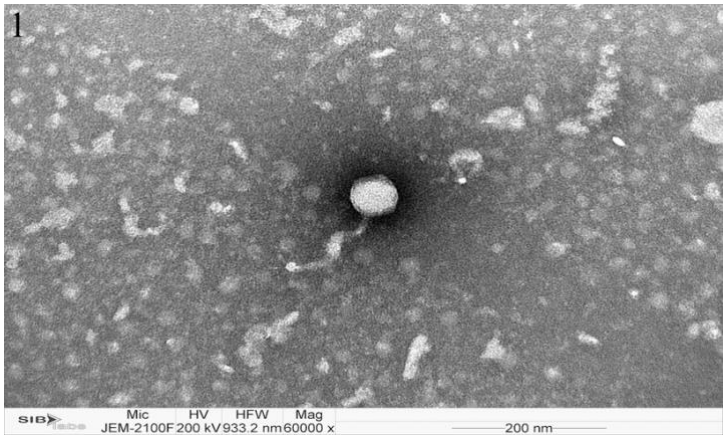
Half (9) of the 18 coliphage strains isolated from the effluent of a municipal wastewater treatment plant had RNA, and the other half (9) had DNA as their genetic material, as presented in Table 7. The RNA coliphages were isolated using *E. coli* ATCC 15597, and the DNA coliphages using *E. coli* ATCC 13706, excluding coliphage 12, which was isolated with ATCC 15597 despite the fact that it is a DNA coliphage, as presented in Paper I, Table 1. However, most isolated coliphages were able to produce plaques when using both *E. coli* ATCC 15597 and ATCC 13706.

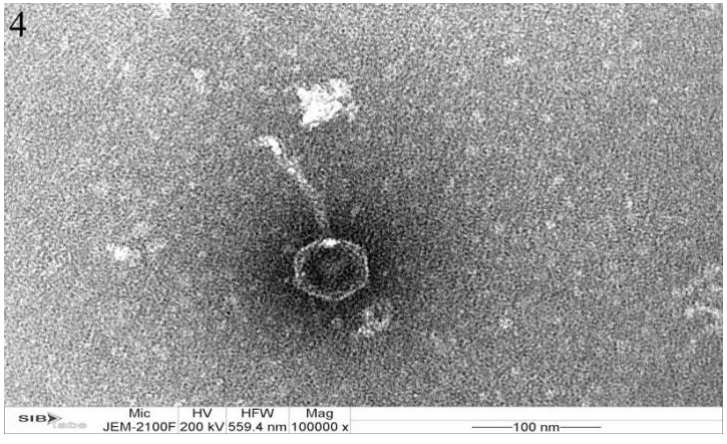
MS2 and six Cl- and/ or UV-resistant coliphages were examined using the TEM, and found to differ in morphology and size regardless of the RNA or DNA in their genetic material (Table 7, Figs. 4A-G). Morphologically, some coliphages had an icosahedral head while others had a spherical head. All coliphages had a tail, although this was not clearly seen for MS2 in Fig. 4A. Some tails were curved and others non-flexible and thick. The mean size of the viral heads (length and width) and especially the mean length of the tails differed considerably between the strains (Table 7).

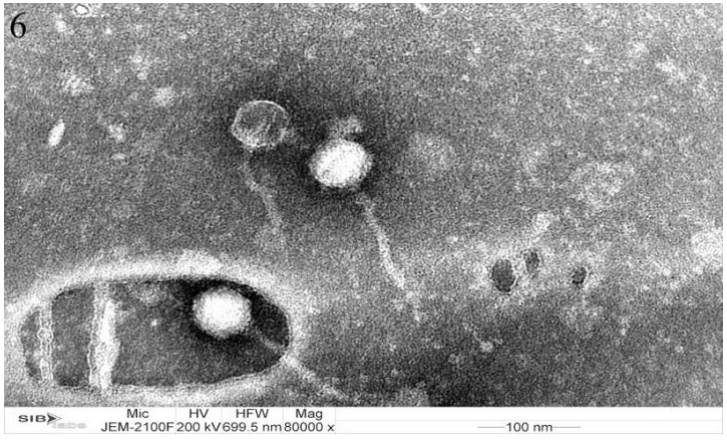
Table 7. Genetic material of coliphages tested by RNase test and morphological characteristics of CI- and/or UV-resistant coliphages. nt is not tested.

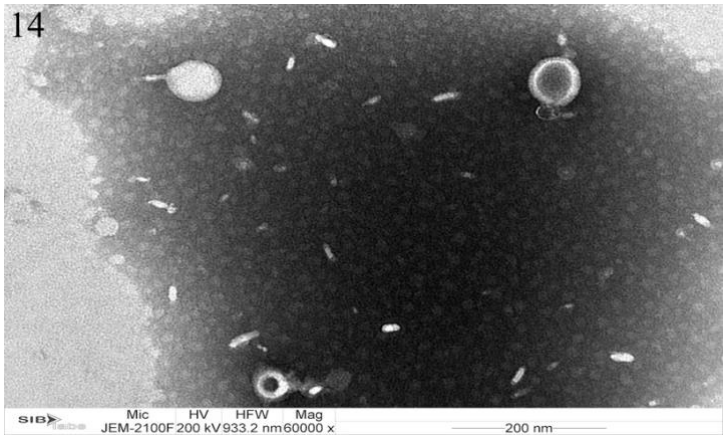
Coliphage number	Genetic material	Mean of head length ± SD (nm)	Mean of head width ± SD (nm)	Mean of tail length ± SD (nm)
MS2	RNA	22±2	22±2	Not clear to be measured
1	RNA	62±3	66±3	141±4
2	RNA	nt	nt	Nt
3	RNA	nt	nt	Nt
4	RNA	60±2	59±0.5	107±4
5	RNA	nt	nt	Nt
14	RNA	67±1	73±2	44±8
15	RNA	nt	nt	Nt
16	RNA	nt	nt	Nt
18	RNA	nt	nt	Nt
6	DNA	56±3	56±3	140±2
7	DNA	65±2	67±7	142±3
8	DNA	nt	nt	Nt
9	DNA	nt	nt	Nt
10	DNA	nt	nt	Nt
11	DNA	nt	nt	Nt
12	DNA	nt	nt	Nt
13	DNA	nt	nt	Nt
17	DNA	31±2	31±0	62±2

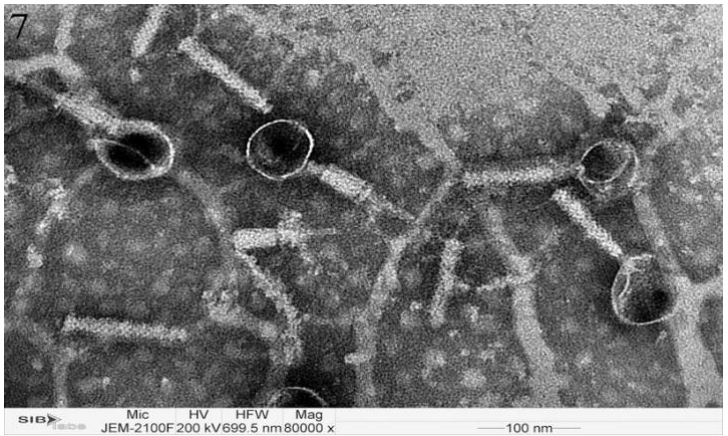

MS2
Mic HV HFW Mag
JEM-2100F 200 kV 699.5 nm 80000 x
100 nm


1
Mic HV HFW Mag
JEM-2100F 200 kV 933.2 nm 60000 x
200 nm


4
Mic HV HFW Mag
JEM-2100F 200 kV 559.4 nm 100000 x
100 nm


6
Mic HV HFW Mag
JEM-2100F 200 kV 699.5 nm 80000 x
100 nm





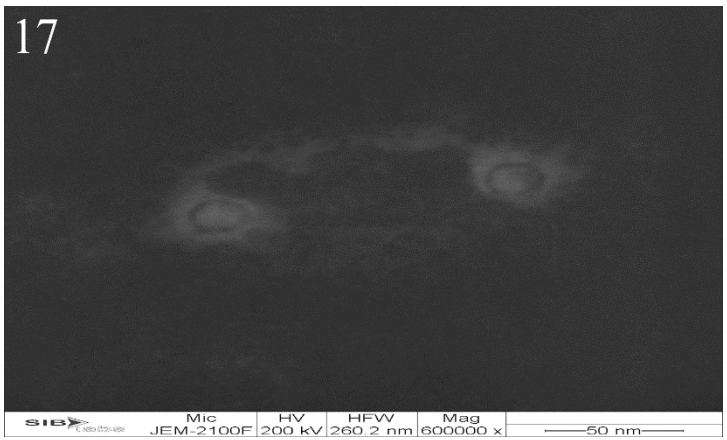


Figure 4. TEM images of MS2 and six Cl- and/or UV-resistant coliphages at different magnifications (given in figure text under Mag and in different lengths of bars). Coliphage MS2 (bar is 100 nm); coliphage 1 (bar is 200 nm); coliphage 4 (bar is 100 nm); coliphage 6 (bar is 100 nm); coliphage 7 (bar is 100 nm); coliphage 14 (bar is 200 nm); coliphage 17 (bar is 50 nm).

5.2 Inactivation of coliphages by chlorine

Coliphages were grouped into three categories, Cl-resistant, intermediately Cl-resistant, and Cl-sensitive, based on the slopes of linear regression equations describing the relationship between Log_{10}-reductions of coliphages and free chlorine concentration (Paper I, Table 3). Eleven of the 17 coliphages analyzed proved to be intermediate or sensitive to chlorine (Paper 1, Tables 2 and 3). More than 2 Log_{10}-reductions were achieved for these 11 coliphages with free Cl dosage of 0.21 mg/L (total Cl 0.50 mg/L) in 10 min contact time (p<0.05) (Paper I). Moreover, 6 of the 17 isolated coliphage strains proved to be resistant to tested chlorine concentrations and showed no reduction. MS2 coliphage was intermediately resistant to chlorine, and it achieved 1.7 Log_{10}-reductions with free Cl dosage of 0.04 mg/L (total Cl 0.13 mg/L), while free Cl dosage of 0.12 mg/L (total Cl 0.33 mg/L) achieved at least 5.7 Log_{10}-reductions (less than the detection limit was reached) with 10 min contact time (Paper I, Table 2). In addition, coliphage 18 (not included to the results of Paper 1) was tested and found to be Cl-resistant, as can be seen in Table 8. Increasing chlorination time up to 90 minutes slightly increased the inactivation of coliphage 18 only if the concentration of chlorine was high enough. It should be considered that the increased contact time caused the degradation of chlorine (Table 8).

Table 8. Log_{10}-reduction (mean ± SD, n=3) of coliphage 18 after the treatment with 0.3, 0.5, and 1.0 mg Cl/L for 10, 30, and 90 minutes. Total chlorine concentration (Cl_{tot}) and free chlorine concentration (Cl_{free}) before treatment (mg/L) and residual chlorine (% of initial) after treatment (residual Cl). nt is not tested.

Cl_{tot} and Cl_{free} concentrations before the treatment	**Contact time, min**		
	10[a]	30[b]	90[c]
	Log_{10}-reduction		
Cl_{tot} 0.3 mg/L; Cl_{free} 0.1 mg/L	0.04±0.1	0.09±0.04	nt
Cl_{tot} 0.5 mg/L; Cl_{free} 0.2 mg/L	-0.01±0.13	0.09±0.01	-0.09±0.06
Cl_{tot} 0.9 mg/L; Cl_{free} 0.4 mg/L	nt	0.19±0.06	0.33±0.15

[a] Residual Cl_{tot} and Cl_{free} were 99 ± 7 % and 106 ± 8 % of initial Cl after 10 min contact time, respectively.
[b] Residual Cl_{tot} and Cl_{free} were 53 ± 4 % and 35 ± 10 % of initial Cl after 30 min contact time, respectively.
[c] Residual Cl_{tot} and Cl_{free} were 35 ± 4 % and 28 ± 13 % of initial Cl after 90 min contact time, respectively.

5.3 Inactivation of coliphages by Hg-UV

Coliphages were grouped into three categories, UV-resistant, intermediately UV-resistant, and UV-sensitive, based on the slopes of linear regression equations of the 18 strains (Paper II, Table 2). The nine UV-resistant or intermediately resistant coliphages achieved up to 7 Log_{10}-reductions with a 22 mWs/cm^2 UV dose, while the 10 UV-resistant coliphages achieved only up to 2 Log_{10}-reductions with a similar UV dose (Paper II, Table 1). Even the highest UV dose of 117 mWs/cm^2 resulted in no more than 3 Log_{10}-reductions for some most UV-resistant coliphages, including MS2. MS2 thus proved to be a good surrogate in UV disinfection (Paper II, Table 1).

5.4 Inactivation of coliphages by UV-LEDs

Paper III describes the effect of the UV-LED disinfection treatment on Cl- and/or UV-resistant coliphages in drinking water. UV-LEDs operating at a wavelength of 270 nm resulted in 0.9 − 2.7 Log_{10}-reductions in 5.2 L water volume after 2 min contact time, and 4.3 – 5.2 Log_{10}-reductions after 10 min contact time for coliphages 1, 5, 7, and 17 (Paper III, Fig. 2). Traditional Hg-UV irradiation at 253.7 nm resulted in 0.7 – 4.1 Log_{10}-reductions within 2 min, and 4.6 – 7.2 Log_{10}-reductions within 10 min contact time in 10 mL water volume (Paper III, Fig. 2).

In UV-LED disinfection experiments, approximately 4 Log_{10}-reductions were achieved within 7 min contact time for coliphages 1, 5, 7, and 17, which corresponds to the time of the 70 mWs/cm^2 dose using Hg-UV in the collimator experiments (Paper III). MS2 was also UV-resistant in the UV-LEDs experiments and achieved 1.5 Log_{10}-reduction within 15 min contact time (Paper III, Fig. 2). Unfortunately, it was not possible to measure the real irradiation dose during UV-LEDs disinfection because the reactor geometry did not allow to make this measuring and it means that the real doses in the tests cannot be compared. The slopes for the linear regression equations were statistically similar for UV-LEDs and Hg-UV (Paper III, Table 2).

5.5 Inactivation of coliphages with combined chlorine and UV or UV and chlorine treatments

Combined treatments using first Cl and then UV (Cl/UV) showed higher efficiency than Cl or UV alone or the combination of using first UV and then Cl (UV/Cl) against coliphages known to be Cl- and/or UV-resistant including MS2. Even a very low concentration of Cl (Cl_{free} 0.04 mg/L) followed by a low UV dose (22 mWs/cm^2) within 7 - 10 min contact time resulted in more than 2.5 Log_{10}-reductions (Paper I, Figure 1; Paper II, Table 3). The combination of Cl/UV showed synergy values from 1.2 to 3.9 for all resistant strains tested excluding coliphage 7, which means that the reductions of coliphage numbers obtained by the combined treatment were this much higher than the sum of the reductions obtained by chlorine or UV alone (Paper II, Table 4).

The synergy obtained with Cl/UV increased if the chlorination time increased (Paper II, Table 5). Conversely, combined treatment using first UV and then Cl showed synergy for only two strains, and the synergy values were lower than if Cl was used before UV.

5.6 Summary of the results

Both RNA coliphages and DNA coliphages could be resistant or sensitive to Cl and UV (Papers I, II, and unpublished results, Table 8). RNA-virus MS2, which was used as a surrogate, was intermediately resistant to Cl and resistant to UV (Papers I, II). Coliphages resistant to Cl and/or UV were sensitive to the combination treatment of Cl/UV (Table 9).

Table 9. The resistance of coliphages to chlorine (Cl), UV irradiation (UV), and combined Cl/UV disinfection. Resistant is indicated as R, intermediate resistant as I, sensitive as S, and not tested as nt. The RNA coliphages are marked in bold.

Coliphage numbers	Resistance to Cl	Resistance to UV	Resistance to Cl/UV
MS2	**I**	**R**	**S**
1	**R**	**R**	**S**
2	**S**	**S**	**nt**
3	**I**	**R**	**nt**
4	**I**	**I**	**S**
5	**R**	**R**	**S**
14	**R**	**R**	**S**
15	**S**	**I**	**nt**
16	**S**	**I**	**nt**
18	**R**	**R**	**S**
6	R	R	S
7	R	S	S
8	S	S	nt
9	S	R	nt
10	S	S	nt
11	I	S	nt
12	S	S	nt
13	R	R	nt
17	R	R	S

6 DISCUSSION

6.1 Chlorine

As presented in study I, the effect of chlorination was found to vary highly between tested coliphages, making it evident to group the strains into chlorine resistant, intermediate, and sensitive ones (Paper 1, Tables 2 and 3). Both RNA and DNA coliphages could be Cl-resistant or Cl-sensitive, so the nucleic acid type was not a crucial feature in determining the resistance. Neither the size nor the shape of coliphage strains could be associated with chlorine resistance (Fig 4, Table 7). Eight of 18 (50 %) Cl-resistant coliphages were difficult to destroy using 0.5 mg total Cl/L, which is within a range of typical chlorine concentrations used in drinking water disinfection. The seven most resistant coliphages showed less than 1 Log_{10}-reduction with a Ct value of 2.1 mg free chlorine × min/L. The Ct value of 1.2 – 2.1 mg free chlorine × min/L achieved 2.5 – 5.7 Log_{10}-reductions for 11 Cl-sensitive and intermediately sensitive coliphages (Paper I, Table 2, and unpublished results).

According to the literature, there is also high variation among enteric pathogens in their resistance to chlorination. Adenoviruses, echoviruses, and polioviruses have achieved up to 2 Log_{10}-reductions with Ct values ranging from 0.01 to 2.9 mg free chlorine × min/L in drinking water or buffer solutions (Engelbrecht et al., 1980; Thurston-Enriquez et al., 2003a; Ballester and Malley, 2004; Shin and Sobsey, 2008; Cromeans et al., 2010; Pages et al., 2010). Other enteric viruses, such as coxsackievirus and norovirus, seem to be more resistant, and these viruses have achieved up to 2 Log_{10}-reductions with Ct values ranging between 0.07 and 5.5 mg free chlorine × min/L in drinking water or buffer solutions (Cromeans et al., 2010; Engelbrecht et al., 1980; Jensen et al., 1980; Shin and Sobsey, 2008; Thurston-Enriquez et al., 2003a) (Table 3, page 30 or 31). Coxsackievirus B5 has also been reported to be more resistant than the HAV (Sobsey et al., 1988). Inactivation of the HAV achieved 3 Log_{10}-reductions with a Ct value of 0.41 mg free chlorine × min/L (Grabow et al. 1983), but on the other hand, the total inactivation of this virus required Ct values as high as of 300 or 600 mg free chlorine × min/L (Li et al., 2002), a concentration that is hardly possible in regular use for drinking water (WHO, 2011).

Our results indicate that a considerable proportion of coliphages may survive chlorine disinfection treatments up to 0.5 mg total chlorine/L or 0.2 mg free chlorine/L, which are at the same level as those used to disinfect water in real drinking waterworks (WHO, 2011). Based on the results reviewed above, at least some human pathogenic enteric viruses would also need more than 0.5 mg total chlorine/L to be inactivated. Resistance of viruses to chlorine

disinfection makes this disinfection challenging. The need for a high chlorine dosage may be a concern from a human health point of view by increasing the possibility of the formation of carcinogenic compounds (WHO, 2011) and also affecting the taste and odor of the water.

In our study, the contact time of chlorine treatment was 10 min, which may be shorter than normally used in typical drinking water disinfection, at least if the impurities of water do not react with chlorine. Increased chlorination time from 10 min to 30 or 90 min slightly increased the inactivation of Cl-resistant coliphage 18 only if the chlorine concentration was high (at least 1 mg/L). If the quality of water was low, containing e.g. a high amount of organic matter, a high amount of chlorine would be needed since the degradation of chlorine would be rapid, or the chlorine would have to be adjusted to many points. Our results showed that even more than half of the chlorine degraded during 90 min in high-quality water (clean drinking water) (Table 8).

In our experiment MS2 was used as a surrogate and classified as intermediately Cl-resistant. It achieved 5.71 Log_{10}-reductions with a Ct value of 1.2 free chlorine × min/L. In earlier studies, MS2 has reached 5 and 2.5 Log_{10}-reductions with Ct values of 0.3 (Shin and Sobsey, 2008) and 1 mg (Rattanakul et al., 2014) free chlorine × min/L, respectively, showing that there is wide variation in MS2 results as well. Shin and Sobsey (2008) showed that MS2 is as Cl-resistant as norovirus, but less Cl-resistant than poliovirus. Based on our results, MS2 is not a good indicator for the Cl-resistant viruses. In contrast, Rattanakul et al. (2014) concluded that MS2 is resistant against chlorination with a free chlorine dose of 0.1 – 1.0 mg/L. This difference may partly be explained by the different matrixes tested, their use of phosphate buffer instead of our drinking water, and their use of *E. coli* K12 A/λ (F+) as host instead of the *E. coli* (ATCC 15597) in our study.

If there is a need for Cl-resistant organisms, better surrogates than MS2 could be found among the most Cl-resistant coliphage strains (for instance numbers 1, 5, 6, 7, 13, 14, and 17) isolated in this study. It might also be possible to find more Cl-resistant coliphages in areas where a high dose of chlorine is used to disinfect the drinking water. Cl-resistant coliphages should be studied in more detail and their resistance should be compared to that of resistant enteric viruses, such as polioviruses.

6.2 Hg-UV

In study II, a collimator device (a low-pressure mercury lamp with 30 W power) was used to analyze the effect of UV on either RNA or DNA coliphages. Ten coliphages including MS2 were UV-resistant, and the highest dose 117 mWs/cm^2 caused only 3 Log_{10}-reductions in some of them (Paper II, Table 1). Similar to the chlorine resistance results, neither genetic material nor size and shape of the coliphage strains were associated with UV resistance (Fig 4, Table 7).

MS2 resulted in 2.2 and 3.4 Log_{10}-reductions with the doses of 82 and 117 mWs/cm^2, respectively, while EPA (2010) reported that the typical UV dose of 85 mWs/cm^2 results in 4 Log_{10}-reductions for MS2. Other studies have reported high variability in the resistance of MS2 and achieved 2 – 4 Log_{10}-reductions when using UV doses between 34 and 119 mWs/cm^2 (Thurston-Enriquez et al., 2003b; Hijnen, 2010; Fang et al., 2014). Furthermore, many studies have shown that MS2 is more resistant to UV than e.g. poliovirus type 1 (Meng and Gerba, 1996), coliphages T4 and T7 (Mamane et al., 2007), HAV (Wiedenmann et al., 1993), and the feline calicivirus (Thurston-Enriquez et al., 2003b). MS2 is thus a good surrogate for these viruses. However, MS2 is less resistant than adenoviruses 40 and 41 (Hijnen et al., 2006; Thurston-Enriquez et al., 2003a, b), and adenovirus 41 may need UV doses up to 222 mWs/cm^2 to be inactivated for >3 Log_{10}-units (Ko et al., 2005).

The UV dose in water disinfection recommended by the NSF/ANSI is 40 mWs/cm^2 (NSF/ANSI, 2012). However, our results (Paper II, Table 1) and the literature referred to above strongly suggest that much higher UV doses than 40 mWs/cm^2 are needed to inactivate many viruses, and UV doses of even more than 117 mWs/cm^2 should be used in real drinking water disinfection. To reach high doses, either UV intensity or exposure time should be increased.

In study II, the linear regression lines were determined between the coliphage reductions and UV doses. Often, a few coliphage plaques were still detected at relatively high UV doses, so that the regression line was no longer linear at high UV doses. This tailing phenomenon can be caused if coliphages are clumped with each other or with impurities, which might protect the coliphages from UV irradiation (Gerba et al., 2002). To reduce the tailing effect, the dose should be high enough. However, it is difficult to destroy the viruses, especially if they attach to the walls of the disinfection tanks. In our study, Hg-UV was tested in water with low turbidity and low color (Kuopion Vesi, 2016) to ensure the high penetration and resulting efficiency of UV.

Water treatment before disinfection is important to improve water the efficiency of disinfection. A higher dosage of UV irradiation and pre-treatments are used in practice if the quality of water is poor (LeChevallier and Au, 2004). The research on UV disinfection should be continued using water of lower quality than what we used, thereby reflecting the reality for many

parts of the world. In such studies, different pre-treatment processes would be essential.

6.3 UV-LEDs

In study III, UV-LEDs of 270 nm with output power of 10 mW were examined to inactivate Cl- and/or UV-resistant DNA and RNA coliphages in a reactor with 5.2 L of water and a water layer thickness of 6.7 cm. The inactivation efficiencies of UV-LED treatments on an RNA coliphage (strain 5) and a DNA coliphage (strain 17) were similar to the inactivations in the traditional Hg-UV treatment, where the water volume was only 10 mL and the water layer thickness 0.35 cm (Paper III, Fig. 2). Strains 1 and 7 and MS2 showed slightly lower inactivation with UV-LEDs compared to Hg-UV, as seen from the smaller slopes of linear regression equations, but statistically there was no difference between the two treatments (Paper III, Table 2). Our findings are thus in agreement with earlier results showing that the inactivation kinetics were similar for both UV-LEDs and Hg-UV when tested with MS2 (Bowker et al., 2011; Sholtes et al., 2016), T7 (Bowker et al., 2011), *Escherichia coli,* and *Bacillus atrophaeus* (Sholtes et al., 2016).

As far as we know, the UV-LEDs with the wavelength of 270 nm that we used have not been studied earlier for water disinfection. We reached from 3 to 4 Log_{10}-reductions of coliphages 1, 5, 7, and 17 within 4 and 7 min contact times, which corresponded to the doses of 40 and 70 mWs/cm^2 in the Hg-UV collimator, respectively. MS2 was more resistant and achieved 1.5 Log_{10}-reductions with a dose that corresponded 70 mWs/cm^2 in Hg-UV. In earlier studies, other wavelengths between 255 and 285 nm have resulted in at least 3 Log_{10}-reductions of coliphages T7 and φX174 with doses of 6.4 -20 mWs/cm^2 (Aoyagi et al., 2011; Bowker et al., 2011). Coliphages Qβ and MS2 have needed more than 40 mW/cm^2 to achieve 3 Log_{10}-reductions (Aoyagi et al., 2011; Bowker et al., 2011; Jenny et al., 2014; Sholtes et al., 2016) and human pathogenic adenoviruses 2 and 5 seem to be even more resistant, needing higher UV doses (Oguma et al., 2016b; Beck et al., 2017) (Table 5). Our inactivation results cannot be directly compared with results obtained with other wavelengths, since our reactor configuration and test matrix are different and our water volume is higher than in other UV-LED studies.

Our results still show that 270 nm UV-LEDs are efficient at inactivating coliphages for water disinfection, since more than 3 Log_{10}-reductions of most strains tested could be achieved within a reasonable contact time. Even though not tested using LEDs before, the wavelength of 270 nm has been compared to other wavelengths by using a tunable laser in previous studies. Coarsely estimated from these studies, 270 nm was approximately as good as 260 nm but slightly better than 280 nm in inactivation of MS2 (Beck et al. 2015, 2016).

Comparisons between other wavelengths showed that 255 and 275 nm were similar in the inactivation of coliphages T7 and MS2 (Bowker et al. 2011). Inactivation efficiency at 280 nm was lower than that at 255 nm for coliphages φX174, Qβ, and MS2 (Aoyagi et al. 2011), but the authors concluded that the wavelength of 280 nm LEDs is suitable in practical applications because it is easier to produce with high-power output. On the other hand, the wavelength of 260 nm has proved to be more effective for inactivation of Qβ than 275 nm (Jenny et al. 2015), and more efficient for inactivation of MS2 than 280 nm (Beck et al., 2017), while both wavelengths of 260 and 280 nm have been equally effective for inactivation of adenovirus 2 (Beck et al., 2017). The results referred to in this and the previous paragraph thus show that the tested wavelengths between 255 and 285 nm of UV-LEDs can efficiently inactivate human viruses and coliphages. Therefore, other matters than the inactivation efficiency of the wavelength alone, such as energy production in relation to inactivation, may be determinants for practical applications, as suggested by Aoyagi et al. (2011).

Research on UV-LEDs is going from testing in batch reactors to development of point-of-use reactors with (Jenny et al., 2014, 2015, Oguma et al., 2016a) or without (Lui et al., 2016) a continuous water flow. Jenny et al. (2014, 2015) have developed a rectangular point-of-use reactor operating with 260 or 275 nm, and Oguma et al. (2016a) have developed a ring-shaped reactor operating with 285 nm. Compared to the batch reactors, the inactivations of coliphages MS2 and Qβ have been lower in both systems; a flow rate of 400 mL/min and a flow rate of 109 mL/min achieved 1.2 and 1.6 Log_{10}-reductions for coliphage Qβ, respectively (Jenny et al., 2014, 2015; Oguma et al., 2016a). The researchers, however, believe that the efficiencies of the UV-LED reactors may be increased in the future by modifying their geometry (Oguma et al., 2016a). The output power of the current UV-LEDs is low compared to traditional low-pressure UV lamps, the power of which can be on the level of 30–40 W. Nevertheless, the output powers are rapidly increasing from the level of 0.3 - 0.5 mW used in 2011 (Bowker et al. 2011), to 1.3 mW used in 2016 (Oguma et al. 2016a, b) and 10 mW used by us in 2017 (Paper III). Moreover, even higher output UV-C LEDs are available nowadays (e.g. Laser components, 2017). This development in LED technology will enable higher doses of UV, making point-of-use disinfection or possibly full-scale disinfection at waterworks promising for the future.

A benefit of LED technology may be that it enables the simultaneous use of LEDs emitting different wavelengths. Therefore, it would be possible to affect different molecules that have different absorption peaks for creating damages that the cells cannot repair. The absorbance peaks of nucleotides are between 240 and 280 nm, and the maximum absorption of DNA is near 260 nm (EPA, 2006). In general, the absorption peaks of proteins are at 280 nm (Kalisvaart, 2004), although proteins of some viruses may efficiently be affected by wavelengths below 240 nm (Beck et al., 2015, 2016). Irradiation in the UV-A

area (320 - 400 nm) cannot be absorbed by DNA, but it acts by producing hydroxyl radicals, which damages proteins (Chevremont et al., 2012a, b). So far, the results on multiple wavelengths have been varied. Compared to a single wavelength, simultaneous treatment with multiple wavelengths of UV-C and UV-A has yielded higher reductions of fecal enterococci and total and fecal coliforms in wastewater and in pure cultures (Chevremont et al., 2012a, b), and *Vibrio parahaemolyticus* in pure cultures (Nakahashi et al., 2014). In contrast, the combination of two UV-C wavelengths (260/280) has yielded lower reduction of adenoviruses 2 and MS2 compared to use of a single wavelength (Beck et al., 2017). The studies on combining multiple wavelengths are still emerging and should be continued with viruses and other resistant microorganisms.

6.4 Combined treatment with Cl/UV or UV/Cl

The results of Papers I and II highlight the variability in the resistance of coliphages to the single disinfection treatments using either chlorine or UV. Paper II emphasizes the importance of combined disinfection, since neither chlorine nor UV could efficiently destroy all coliphages examined; the combination of chlorine and UV treatment was clearly more effective than either UV or chlorine treatment alone. Our results thus support the results of Shang et al. (2007) and Rattanakul et al. (2014; 2015), who studied MS2 with concentrations and doses used in real drinking water disinfection. When we applied the chemical treatment (chlorine without quenching) first followed by the physical treatment (UV), most Cl- and/or UV-resistant coliphages were inactivated by more than 2.5 Log_{10} at 0.1 mg total chlorine/L followed by a UV dose of 22 mWs/cm^2, when Cl treatment time was at least 7 min (Paper I, Fig. 1; Paper II, Table 3). This process thus led to a great synergistic disinfection of most Cl- and/or UV-resistant coliphages tested (Paper II, Tables 4 and 5).

On the other hand, lower or almost no synergy (Paper II, Table 4) was observed in our studies if physical treatment was done first and then followed by chlorine. This result confirms the earlier results of Rattanakul et al. (2014; 2015), who used first chlorine (1 mg free Cl/L) without quenching it and then 23 mWs/cm^2 UV, and found that the inactivation of MS2 and adenovirus was better than if UV was used before chlorine.

Chlorine is considered to damage the surface proteins of viruses (Alvarez and O'Brien, 1982), while UV targets the nucleic acids (Nuanualsuwan et al. 2003; Rattanakul et al. 2014). The reaction of combined Cl/UV leads to photodegradation of chlorine and formation of free radicals by UV, which may further damage the surface structures of viruses by breaking proteins (Watts and Linden 2007). The higher synergism obtained by Cl/UV treatment than by UV/Cl treatment supports the assumption of the effect of free radicals during

UV treatment in the presence of chlorine. In large water treatment plants, e.g. in Helsinki and Oulu in Finland, UV is often combined with chlorine as form of chloramines, which leave long residual effect in the distribution system (Pizzi, 2010). The order is usually UV followed by chloramines, which has been proved better than the opposite order (Ballester et al., 2004). The result is thus different from results obtained with chlorine and UV and could be associated with lower oxidation power of chloramines and/or lower formation of free radicals by UV.

In the present study (Paper II, Table 5), as short as 3 min contact time with 0.1 mg total chlorine/L followed by a UV dose of 22 mWs/cm^2 already had some synergistic effect in high-quality water (Kuopion Vesi, 2016). Globally, the water quality may be worse in many cases, e.g. it may have enteric microorganisms and color, turbidity, organic matter, and other chemical impurities which decrease the disinfection efficiency of chlorine and UV. In these cases, higher concentrations and contact times of chlorine and higher UV doses should be used. Coagulation and/or filtration should possibly be enhanced before chlorination, and chlorination could be applied at many points in distribution systems (Zuane, 1997; Le Chevallier and Au, 2004). Due to the differences in water quality, the adequate contact times as well as the doses of chlorine and UV for combined treatment should be adjusted case-specifically, depending on different scales, retention times, and climate, among others.

The benefit of using the combination treatment of Cl and UV in real drinking water treatment plants is that it would allow the use of lower chlorine dosages, shorter contact times, and require less electricity consumption for UV (Vankerckhoven et al., 2011). Because of its lower dosages and shorter contact times, the combination treatment can also decrease the toxicity of residual chlorine and negative changes in the taste and smell of water (Wigginton et al. 2012). However, if combined Cl/UV treatment is used, post-chlorination may be needed to protect the distribution pipe system against resistant organisms. Furthermore, in areas where the quality of water is low, such as in large parts of South Asia, Africa, and South America, the combination treatment may bring benefits by decreasing the need for electricity and/or chemicals. In addition, the combined treatment using UV-LEDs instead of Hg-UV could also be an interesting option in the future.

The combination treatment should be studied using UV-LEDs with other chemical compounds than chlorine in drinking water disinfection, such as ozone, hydrogen peroxide, chloramines and chlorine dioxide. The inactivation efficiency of combination treatments may differ depending on the type and order of chemical and physical treatments and on whether the treatments are done simultaneously or sequentially. Therefore, the suitable treatment order should be investigated.

7 CONCLUSIONS

The novelty of this work is to show the high inactivation of Cl- and/or UV-resistant coliphage viruses in combined treatment of Cl and UV instead of single treatments. Based on the results the following conclusions can be made:

- Already low doses of chlorine followed by low doses of UV can efficiently inactivate Cl- and/or UV-resistant coliphages in the combined treatment.
- The combination of Cl/UV is more efficient in disinfection than UV/Cl treatment resulting in high synergistic disinfection values compared to the sum of single treatments with chlorine and UV. Low or no synergy was achieved if using first UV followed by chlorine in the combined treatment. Combination Cl/UV is therefore recommended for drinking water disinfection.
- A half of the coliphages isolated from wastewater were classified to be resistant to UV and could survive much higher doses of UV than the doses recommended by International/American National Standards Institute.
- The isolated coliphages showed high variation in their resistance to Cl or UV regardless of in their shape, size or if they were DNA- or RNA-coliphage.
- The UV-LEDs at the wavelength of 270 nm tested in 5.2 L water had similar inactivation ability as the traditional mercury UV in 10 ml of water. Thus, UV-LEDs seem to be a promising tool for disinfection of UV-resistant viruses in water.

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Enteric viruses cause still annually millions of waterborne diseases, which partly could be avoided by using disinfection. This thesis evaluated the efficiency of chlorine, ultraviolet radiation (UV) and combined chlorine and UV methods for inactivation of viruses in drinking water. The results highlighted that Cl and/or UV-resistant viruses can be efficiently controlled with combined Cl and UV treatments. The results should be further studied in water treatment processes.

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