DISINFECTION OF ESCHERICHIA COLI IN WATER USING ULTRAVIOLET–LEDs

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ABSTRACT

Microbial disinfection using ultraviolet radiation as a drinking water treatment technology has gained an increased attention in the world. Among UV lamps, UV LEDs are long lasting, energy efficient, compact in size and shape and do not produce toxic chemicals unlike the conventional mercury base UV lamps. In this study, we examined the disinfection of spiked *Escherichia coli* in drinking water using a flow-through UV-LED reactor at wavelengths of 255 nm, 260 nm and 270 nm respectively. We also tested the combined wavelengths consisting of 255 nm +260 nm +270 nm and different flow rates of 550 l/h, 180 l/h, 120 l/h and 60 l/h. The UV-reactor experiment had 3 steps; step 1 had 2 LED strips, step 2 had 4 LED strips and both step 1 and 2 had 550 l/h flow rate respectively. The step 3 had varied flow rates (2 strips, 4 strips and 10 strips). Other parameters such as retention time in the reactor and turbulence were varied and increased in step 3. We also compared the disinfection efficiency of the UV-reactor system with the conventional UV-collimator beam device with low pressure lamps at 253.7 nm. Samples were taken before and after exposure to UV irradiation and were analyzed with culture techniques. All the three steps in the UV-reactor system showed no significant *E. coli* inactivation and no disinfection. The UV-collimator beam device showed 1.7-2.3 Log reductions at 130-137 J/m^2 doses and 5 log reductions at 240-298 J/m^2 doses. In this work, UV LEDs was not an efficient drinking water treatment technique. Therefore, more research and development is needed to enhance its use and effectiveness.

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ABBREVIATION AND DEFINITIONS

AIDS	Acquired Immune deficiency virus
AlN	Aluminium nitride
AlGAN	Aluminium or Gallium nitride
AOPs	Advanced Oxidation processes
cfu	coloning forming units
cfu/ml	coloning forming units/milliliters
CPDs	Cyclobutane pyrimidine dimers
DALYs	Disability Adjusted Life Years
DBPs	Disinfection by-products
DNA	Deoxyribonucleic acid
EHEC	Enterohemorrhagic *Escherichia coli*
EPA	United State Environmental Protection Agency
FIB	Fecal indicator bacteria
HO_2	Hydrogen peroxyl
LEDs	Light emitting diodes
LP	low pressure
MDG	Millennium Development Goals
MF	microfiltration
MP	Medium pressure
NTU	Nephelometric Turbidity unit
RNA	Ribonucleic acid
SMH	Soil transmitted helminthes
THG agar	Tryptone yeast extract glucose agar
UF	Ultra filtration
UV	Ultra-violet radiation
VAD	Vitamin A Deficiency

CONTENTS

1 INTRODUCTION

The earth is covered by water; the total volume of water on earth is about 1,400 million km^3. Of this only about 2.5 % or about 35 million km^3 is fresh. Largest percentage of all fresh water (over 1.5 % of all water; 60 % of all fresh water) lies frozen in form of snow or permanent ice and unavailable in the Antarctica and Greenland. This leaves some 1% of all water or 200,000 km^3 of fresh water accessible in the lakes, river, channels and underground (WHO 2013b). These water sources accounts for the primary water sources for human use. Approximately 47,000 km^3 annual flow of fresh water is available as ground recharge after water loss by precipitation and evaporation from both land and ocean surfaces (Gleick 1993).

The use of water for various purposes such as drinking, food production, domestic use, recreation and agriculture has an important impact on health (WHO 2013a). Access to good water quality through provision of improved water and sanitation facilities also plays a beneficial role in social and economic developments and poverty alleviation (WHO 2000).

Lack of access to safe drinking water and sanitations which are caused by poverty in developing countries, inability of the government to finance water of good quality and a good sanitation system (WHO 2000). The impact of failing access to safe drinking water and sanitation include disease outbreaks which can contribute to background rates of disease manifestation and widespread health problems, excessive use of labor (mostly women who need to travel a long distances to fetch water for their families) and drawback for economic development (Gleick 1995).

However, supply of good and safe water quality especially for drinking purpose, still fall short of the required targets for sustainable developments despite several successful efforts and programs launched to promote safe drinking water by various organizations.

The World Health Organization estimated in 2000 (WHO 2000) that more than one billion people lack access to safe drinking water. Water of poor quality can cause waterborne diseases which have resulted in millions of deaths annually. For example, the estimated annual burden of diarrhea deaths due to insufficient sanitation practice, poor hygiene, and unsafe drinking water was 2 million (Boschi-Pinto et al. 2008). WHO (2013b) estimated that about 3.4 million people mostly children, die annually from water-related diseases but Walker

et al. (2013) estimated that more than 6.5 million children died in 2012. Mostly were children under age 5 and the highest rate of child mortality was in sub-Sahara Africa in which almost 10% of global deaths have been attributed to diarrhea. In addition, diarrheal and cholera rank the leading causes of diseases and deaths around the world while these causes can be prevented through supply of good water quality, inexpensive hygiene and sanitation practices.

The Millennium Development Goal (MDG) 7, Target 7.C was to "halve, by 2015, the fraction of the population without sustainable access to safe drinking water and basic sanitation" (United Nation 2010). There has been good progress during last decades and having met the target for access to safe improved drinking water source, current estimate shows that 780 million people in the world are still lacking access to improved water source and 2.5 million people are still without access to improved sanitation (WHO 2012).

Water is still one of the earth's most threatened resources (WHO 2013b). Continuous population growth, per capital consumption and the consequential impacts of human activities on the environment are major factors contributing to water scarcity (Asano et al. 2007). These factors motivate the government to search for alternative water sources, such as reuse and recycling of wastewaters more importantly for irrigation purposes (Close et al. 2006, Salgot et al. 2006, Palese et al. 2009). Reuse of wastewater for irrigation or watering of green space is a good alternative for reducing continuous problem of clean water shortage in Mediterranean countries (Nasser et al. 2006, Palese et al. 2009).

Drinking water is often contaminated by several microorganisms such as pathogenic enteric bacteria, viruses and intestinal parasites. Different methods have been used in water treatment such as boiling, solar disinfection, ultraviolet (UV) disinfection with lamps, chlorination and combined treatments of chemical coagulation, filtration and chlorination and they have been evaluated for the reduction of bacteria, viruses and protozoan.

However, the ability of some of these methods to remove or inactivate different waterborne pathogens has been improperly investigated and documented. Therefore, effective treatment options for inactivation or removal of pathogens from water sources especially in areas where standard water provisions are not presently practice should be made available. The aim of this research was to study the disinfection of *Escherichia coli* using a UV LEDs flow-through reactor system as an alternative water treatment method and growing disinfecting technology.

2   LITERATURE REVIEW

2.1   Water pathogens

Microbial risks to human health are caused by bacteria, enteric viruses, protozoa and helminthes due to poor sanitation and hygiene practices (Ashbolt 2004, Montgomery and Elimelech 2007, Mara et al. 2010) (Table 1). The risk of infection from pathogenic microorganisms is often dependent on the die-off rates, attenuation and dilution factors (Dowd et al. 2000, Ferguson et al. 2003, Pedley et al. 2006). These factors also predict the concentration of pathogens in the slum areas which is regarded as unorganized settlement in a city or town characterized by high population density, poor infrastructure, inadequate access to safe water supply and a good sanitation practices (Katukiza et al. 2014).

Despite several efforts in preventing waterborne diseases, dreadful outbreaks still occur, for example *Cryptosporidium* (Milwaukee, USA 1993 or Ireland 2007) and *Escherichia coli* 0517:H7 (Walkerton, Ontario, Canada 2000 and Europe) as reported by Straub and Chandler (2003) and Coffey et al. (2007). Waterborne human infectious diseases associated with human and animal feces are fast increasing and becoming a global concern, contributing a huge potential burden of diseases to the human population of many countries (Domingo et al. 2007).

The exposure routes to these pathogens include contaminated potable water, wastewater, soils, food sources through intentional and accidental ingestion, dermal contact and inhalation (Howard et al. 2003, 2006a, Steyn et al. 2004, Westrell et al. 2004, Schönning et al. 2007). In slum regions, the incidence of diarrhea, dysentery and gastroenteritis is often high in immune compromised individual including children, the elderly and pregnant women mostly through person to person contact, as well as fecal contaminated water and soil (Muoki et al. 2008, Alirol et al., 2010). In United States, it was estimated that almost 1 million illnesses and 1000 deaths occur each year due to microbial contamination of drinking water (Warrington 2001). Craun et al. (2006) reported that from 1991 to 2002, 73 outbreaks of waterborne diseases occurred, resulting in 515,496 illnesses in USA.

Thus, specific detection methods are required for each waterborne pathogen in order to determine and discover the origin of their etiological agents, identify lapses in water treatment, and develop a new quality control processes and procedures.

Table1. Major waterborne pathogen groups and genera. (Modified from Straub and Chandler 2003)

Group	Pathogen	Diseases caused
Viruses	Enteroviruses (polio, echo, coxsackie)	Meningitis, paralysis, rash, fever, myocarditis, respiratory diseases and diarrhea
	Hepatitis A and E	Infectious hepatitis
	Human Caliciviruses	
	Norwalk viruses	diarrhea/gastroenteritis
	Sapporo	diarrhea/gastroenteritis
	Rotavirus	diarrhea/gastroenteritis
	Astroviruses	diarrhea
	Adenoviruses	diarrhea (Type 40 and 41), eye infections and respiratory diseases
	Reovirus	respiratory diseases
Bacteria	*Salmonella*	typhoid and diarrhea
	Shigella	diarrhea
	Campylobacter	diarrhea,
	Yersinia enterocolitica	diarrhea
	Escherichia coli O157:H7 and other certain strains	hemolytic uremia syndrome as a complication in small children.
	Legionella pneumophila	Pneumonia and other respiratory infections
Protozoa	*Naegleria*	meningoencephalitis
	Entamoeba histolytica	amoebic dysentery
	Giardia lamblia	chronic diarrhea
	Cryptosporidium parvum	acute diarrhea, fatal for immune compromised individuals
	Cyclospora	diarrhea
	Microsporidia includes	chronic diarrhea and wasting, pulmonary, ocular, muscular and renal diseases
	Enterocytozoon spp.	
	Encephalitozoon spp.	
	Septata spp.	
	Pleistophora spp.	
	Nosema spp.	
Cyanobateria	*Microcystis*	diarrhea
	AnabaenaAphantiomenon	
Helminths	*Ascaris lumbricoides*	ascariasis
	Trichuris trichiora	trichuriasis-whipworm
	Taenia saginata	beef tapeworm
	Schistosoma mansoni	schistosomiasis

Several water pathogens (bacteria, enteric viruses, parasites and protozoa) mostly of fecal origin are potential contaminants in wastewaters and identified as major causes of human health infectious diseases. The pronounced and common water pathogens include *Vibrio cholera, Salmonella typhi, Esherichia coli, Giardia lamblia, Cryptosporidium parvum,* Adenoviruses, Hepatitis A, Coliphages and *Schistoma* spp.

2.2 BACTERIA

2.2.1 *Vibrio cholera*

Vibrio cholerae is a facultative anaerobic, Gram-negative, motile with a single polar flagellum, non-spore forming curved rod shaped bacterium. The bacterium is responsible for the manifestation of cholera infection in both adults and children (Mandal et al. 2011). Cholera is a serious global epidemic, endemic or pandemic diseases. It can cause a profuse watery diarrhea and leading to severe dehydration and death if treatment is not quickly given after ingestion of food or water contaminated with the bacterium (Mandal et al. 2011).

It is caused by the toxigenic serogroups O1 and O139 of the *V. cholera* which are regarded as the main etiological serogroups. In non-epidemic areas, transmission and contamination may be associated with consumption of raw or undercooked seafood imported from cholera-endemic regions. The disease is a major public health concern in the world mostly in Africa, Asia, and Latin America though rare in developed countries, thus it has been categorized as the "emerging and re-emerging infection" still threatening many countries in the globe (Mandal et al. 2011).

World Health Organization estimates that about 3-5 million cholera cases resulting in over 100,000 deaths annually in the worldwide (WHO 2011). African countries accounted for highest percentage of all reported cholera cases from 2000 to 2009, and reported 217,333 cases in 2009 alone (WHO 2010a). Studies have proven cholera outbreak is a product of inadequate sanitation, poor hygiene practices and unsafe drinking water with an estimated annual burden of 2 million diarrheal deaths in the world (Boschi-Pinto et al. 2008). Cholera outbreaks occur from time to time in various places in India and this consistent occurrence is a good example of is endemicity in the country (Pal et al. 2006, Chandrasekhar et al. 2008, Das et al. 2008).

2.2.2   *Salmonella typhi*

Salmonella typhi is a non-spore forming facultative anaerobic, motile with peritrich flagella, gram-negative rods bacterium belonging to the family Enterobacteriaceae and is responsible for typhoid fever disease in human. Typhoid fever is identified as a multisystem disease which remains a global public health concern mostly in developing countries caused by unhygienic and poor sanitary conditions (Gadgil 1998). The commonest source of *S. typhi* bacteria is contaminated drinking water, transmitted by fecal-oral route causing secondary infections in healthy individuals. The fever is characterized by sudden onset of systemic and sustained fever, diarrhea and septicaemia. The World Health Organization estimated an annual infectious rate of 21.6 million and about 600,000 death cases with the highest percentage in Africa and Asia (Doughari et al. 2007).

Differently from other salmonella serovars, *S. typhi* and *S. paratyphi* are host specific infecting only human. Original sources of contamination of these pathogens are stools of infected individuals and water contaminated with feces of human is one of the main vehicles of typhoid fever infections. The global disease burden of these pathogens is often noticeable in children and adolescents in resource-poor areas, mostly in Asia (Crump et al. 2004). Several studies on typhoid and paratyphoid fever epidemics in Asia have consistently shown that contamination of drinking water from well water source (Farooqui et al. 2009), piped municipal drinking water (Mermin et al. 1999, Kim et al. 2003, Lewis et al. 2005, Bhunia et al. 2009) and non-boiled spring water (Swaddiwudhipong and Kanlayanaphotoporn 2001) were recognized as the main sources of outbreaks.

2.2.3   *Escherichia coli*

Escherichia coli (E. coli) is a Gram-negative, facultative anaerobic, rod-shaped bacterium commonly found in the gut of humans and warm blooded animals. Most strains of *E. coli* are harmless but some serotypes, such as enterohaemorrhagic *E. coli* (EHCH), can be very harmful causing severe foodborne poisoning. The most common pathogenic *E. coli* is the serotype 0157:H7 of the enterohemorrhagic (EHEC) group and it causes diseases in human through the fecal–oral route of transmission. *E. coli* and coliform bacteria are so called fecal

indicator bacteria (FIB) used by environmental agencies and health organizations to monitor hygienic quality of water.

E. coli is mostly the preferred indicator of fecal contamination compared to the other members of the thermotolerant coliform group because it is easy to detect in feces of warm blooded animals and its populations out-numbered other thermotolerant coliforms in animal and human excreta (Medema et al. 2003).

Many studies have established association between FIB in stored drinking water and diarrheal illness in children (Moe et al. 1991, VanDerslice & Briscoe 1995, Brown et al. 2008). For example Moe et al. (1991) reported in a study done in Philippines that those children drinking water with high concentrations of *E. coli* had significantly higher rates of diarrhea than those drinking less contaminated water.

2.3   PROTOZOA

Waterborne protozoan diseases have been distributed worldwide and its outbreaks have been recognized in developed and developing countries where human have been infected (Cotruva et al. 2004). Among waterborne pathogens, protozoa pose a major concern to design and maintenance of safe and improved water supply (Zmirou-Navier et al. 2006). They cause 4 billion cases of diarrhea that result in annual 1.6 million deaths and 62.5 million Disability Adjusted Life Years (DALYs) worldwide (Wright and Gundry 2009).

Protozoan parasites are mostly transmitted through the fecal oral route. Other parasitic protozoa with a waterborne transmission causing human infections are *Microsporidia, Isospora, Blastocystis hominis, Taxoplasma gondii, Entamoeba histolytica, Acanthamoeba spp, Naegleria spp. Cyclospora cayetanensis and Balantidium coli* (Table 1). Provision of improved water and good sanitation practices are control measures against parasitic protozoan hazards (Baldursson and Karanis 2011).

Cryptosporidiosis and giardiasis have been recognized as the most prevalent waterborne protozoan parasitic infections that causes diarrhea. Edge et al. (2013) reported that at three drinking water sources situated about 2 km offshore in Lake Ontario *Giardia spp*. was the common pathogen found in 36% of the influent water samples with 0.7 cysts/litre in one of

the treatment plant followed by *Cryptosporidium* found in 15% of the water samples with 0.4 cysts/liter concentration in another treatment plant. Karanis et al. (2007) reported that out of 325 giardia outbreaks, 32% (104) was associated with drinking water systems contaminated with *G. lamblia* and 23.7% (77) was contaminated with *Cryptosporidium parvum* or *Cryptosporidium sp.*

2.3.1   *Giardia lamblia*

Giardia lamblia is a flagellated protozoan parasite that causes giardiasis. *G. lamblia* infests the gastrointestinal tract and it has long been identified as a major parasitic protozoan that causes diarrhea mostly in infants below five years old. They infect humans through untreated sewage and contaminated land and rivers by animal or human feces through the fecal-oral route (Lanata 2003). The appearance of infections caused by *G. lamblia* accounts $2.8 *10^8$ cases yearly (Lane and Lloyd, 2002). Karanis et al. (2007) reported that of 325 giardia outbreaks, 32% (104) was associated with drinking water systems contaminated with *G. lamblia* and deficiencies in the water treatment processes were the main highlighted causes including gaps in the protective barriers and poorly operated treatment and disinfection systems.

2.3.2   *Cryptosporidiun parvum*

Cryptosporidium is an obligate, intracellular, coccidian protozoan parasite that infests the gastrointestinal tract of humans and animals causing severe diarrhea illness (Walter Quintero-Betancourt et al. 2001). It produces environmental resistance oocysts, which are excreted in the feces of infected individuals. *Cryptosporidium* is identified in most area of the world as an important waterborne pathogen, the species *Cryptosporidium parvum* is a potential cause of cryptosporidial infections in human and livestock (O'Donoghue 1995).

Infected humans, domestic animals and wildlife contribute to the pool of waterborne oocysts through runoff, wastewater discharges from agricultural land practices and endemic infection of indigenous mammals in a watershed (Smith 1995). Waterborne transmission of the oocysts and outbreaks of cryptosporidiosis either through drinking water or recreation use sources

have been reported by several studies (Solo-Gabriel and Newmeister 1996, Rose et al., 1997, Smith and Rose 1998, Oppenheimer et al. 2000, Fayer et al. 2000). Between 250 and 500 million infections of *C. parvum* occurred annually in Asia, Africa and Latin America (Current 1991).

2.4   ENTERIC VIRUSES

Viruses are often found in surface water sources and have been identified as a potential cause of high percentage of water borne disease cases (Moore et al. 1994). More than 140 different types of viruses are known to infect human intestinal tract and are mostly excreted in feces (Melnick 1984). Among important enteric viruses associated with polluted waters include adenovirus, norovirus, poliovirus, rotavirus and hepatitis A virus. They have been responsible for various illnesses such as respiratory illness and diarrhea. Viruses have been detected in the environment via several sources such as contaminated drinking water sources, waste water reclamation practices, recreational waters and food contaminated by sewage and effluent waters (Svraka et al. 2007). They are mostly transmitted through the fecal-oral route and known to cause the greatest concern among other pathogens present in wastewater due to their small size and long-term survival rate in the environment.

2.4.1   Adenoviruses

Adenoviruses are non-enveloped viruses with double stranded DNA genome. They belong to the genus *Mastadenovirus* in the family Adenoviridae and are about 70 nm in diameter. They are enteric viruses primarily infecting children and virulently affecting immune compromised individuals and associated with respiratory diseases, pneumonia gastrointestinal illness and eye infection to haemorrhagic cystitis or meningoencephalitis (Nwachcuku & Gerba 2004, Word and Horwitz 2007, Mena and Gerba 2009, Beck et al. 2014). They have been found in sewage, river, costal, swimming pool and drinking waters. Infections caused by adenoviruses can occur via consumption of contaminated water or inhalation of aerosolized droplets during recreation activities in water (Donge et al. 2010).

Although, there is no evidence of food outbreak by adenovirus, possibilities of viral transmission through food is now evident as they were already detected in raw vegetables (Cheong et al. 2009) and shell fish (Umesha et al. 2008). Significant associations have been

found between adenoviruses and waterborne diseases outbreaks (Kukkula et al. 1997). Several studies have suggested that they might be the most common enteric viruses in domestic sewage (Pina et al. 1998). Many of adenovirus types are shed for months and excreted in high numbers (up to 10^{11} particles/g feces), mostly in wastewater (Word and Horwitz 2007).

Among other viruses, they are often seen as the most resistant waterborne pathogens to chemical and physical agents and also to UV light based on sensitivity to inactivation (Nwachuku et al. 2005). This is as a result of the double-stranded DNA genome, which permits adenoviruses to use the host-cell repair enzymes during replication to repair damage in the DNA caused by the UV-radiation.

2.4.2 Hepatitis A

Hepatis A virus is an icosaedric non-enveloped RNA virus belonging to the family of the Picornaviridae, *Hepatovirus* genus. The incidence of *Hepatitis A virus* varies between different parts of the world, with the highest incidence rate in developing countries where sewage treatment and hygiene practices are very poor (Rodríguez-Lázaro et al. 2012). Source of contamination include sewage discharge, contaminated soil, food, crops and natural water sources (Cook and Rzezútka 2006, Tallon et al. 2008). This are considered as major vehicles of the virus transmission to humans. Water contamination is an important source due to long surviving period of the virus in the environment. For example, this virus can survive for 60 days in tap water (Enriquez et al. 1995), more than 6 weeks in river water (Springthorpe et al. 1993) and even up to 30 weeks in sea water (Crance et al. 1998).

About 1.4 million people worldwide have become infected with *Hepatitis A virus* yearly (Issa and Mourad 2001). Incidence of hepatitis A infection is significantly declining mostly in developed countries where effective immunization programs have been initiated to combat and militate against its spread. In USA, number of cases has been reduced by 92% to an infection rate as low as one case per 100,000 persons per year (Daniels et al. 2009). Developing countries are still at high risk of the infection, mostly children before the age of ten and those infected in childhood might not show any clear symptoms. Epidemics can be uncommon in older children and adult who are generally immune to the virus (WHO 2013).

2.4.3   Coliphages

Coliphages are viruses (bacteriophages) that infect *E. coli* bacteria. They are mostly present in the gut of warm-blooded animals and excreted in feces. They possess the same characteristics as human viruses including transportation and survival in the environment, making them a good option for an indicator of environmental contamination in water (Reynolds 2006). More so, coliphages are easier and cheaper to detect in the environmental samples than human viruses.

USEPA (2000) reported that the most commonly applied method for monitoring groundwater is to measure fecal indicator microorganisms, such as coliforms, *E. coli*, *Enterococcus* spp., and coliphages. Bacterial fecal indicators such as fecal coliforms, *E. coli* and *Enterococcus* spp., are widely used bacterial indicators for water quality. However, these bacterial indicators have been criticized for not adequately representing viral pathogens (Leclerc et al. 2000). For these reasons, the use of viral fecal indicators such as somatic and male-specific coliphages has been suggested for monitoring viral pathogens (USEPA 2000). Somatic and F+ coliphages are major enteric viruses used as water quality indicators in estuaries, seawater, freshwater, potable water, and wastewater (Bitton 2005). F+RNA coliphages among other male-specific RNA coliphages have been used as a target for identifying the source of fecal origin (Lee et al. 2009). However, it is still not well established whether these viral fecal indicators are appropriate indicator for viral contamination because there can be weak correlation with viral pathogens and coliphages (Borchardt et al. 2003, Locas et al. 2007).

Based on different survival and transport characteristics, there is still little evidence to believe that the viral presence will be reflected by fecal indicator bacteria. This fact establishes coliphages as a surrogate for human enteric viruses (Bushon 2003). The genotypes of F+ RNA coliphages are specifically found in either human or animal fecal contamination (Cole et al. 2003, Vinje et al. 2004).

2.5   HELMINTHS

Helminths are multicellular parasitic worm-like organisms with the potential to feed and live within the host, migrate and causing significant tissue injury as they mature. Due to effective immune evasion capabilities, these parasites are able to persist in the host for many years (Wolff et al. 2012). Helminth infections are important public health issue, constituting the most common parasitic infection in humans and animals around the globe with great economical impact mostly in tropical and subtropical countries (Nithiuthai et al. 2004). Exposure routes with these parasites can occur via contamination of the environment and ingestion of food, water and soil or through dermal contact with transmission from man to man, animal to animal or animal to man. Infections with helminths, mostly the soil transmitted helminths (SMH), (e.g *Ascaris lumbricoides, Trichuris trichiura, Hymenolepis nana*) are main causes of helminths infections.

Hookworms are parasitic nematode that lives in the small intestine of the host mainly humans and animals. They are directly associated with poverty, unsafe water, sanitation and hygiene (Cairncross et al. 2010). Hotez et al. (2008) estimated that more than 2 billion people are infected with helminthes while World Health Organization (WHO 2010) estimated that over 1.5 billion people are infected with soil-transmitted helminths infections with highest numbers from sub-Saharan Africa, the Americas, China and East Asia. The most affected persons are pre-school or school-aged children and pregnant women (Bethony et al. 2006). Health negative implications includes retarded growth, delayed intellectual developments and cognition, vitamin A deficiency (VAD), school absenteeism and low academic performances (Bethony et al. 2006, Hotez et al. 2008).

2.5.1   *Schistosoma* spp.

Schistosomes have been distributed worldwide and considered for many decades as the most important group of waterborne helminths affecting the health of humans and animals (Nithiuthai et al. 2004). They are parasitic trematode flatworms from the genus *Schistosoma* causing Schistosomiasis diseases. Infection often occurs following parasitic contamination of the environment, food ingestion, water and soil. *Schistosoma* spp. undergoes a complex of life cycle; they inhabit certain types of fresh water snails as cercaria (librated larva from parasitic

snail) hence contaminating water. Human are being infected when contacted with this contaminated water via the skin resulting in schistosomiasis and cercarial dermatitis (Nithiuthai et al. 2004). Schistosomiasis disease among other infectious diseases, are a major threat worldwide due to its fast rate of transmission and adaptation of the pathogens, social practices and unrestricted cultural beliefs, high rate of immigration and international trade and high survival rate of the worm (Goulart et al. 2010, Steiner et al. 2013, Jerkins-Holick and Kaul 2013).

Human schistosomiasis is a complex acute and mainly chronic infectious diseases caused by six Schistosoma species: *S. haematobium, S. guineensis, S. intercalatum, S. mansoni, S. japonicum and S. mekongi* (Davis 2009). Schistosomiasis predominantly occurs in the tropics and subtropics regions with an obvious social-economic impact (King et al. 2005). Steinmann et al. (2006) estimated that schistosoma disease causes annual loss between 1.7 million to 4.5 million DALYs. Though schistosoma outbreak is not often fatal, its persistent nature confirms it can be a lifetime problem with significant chronic cases (Patz et al. 2000, Ross et al. 2002, Enk et al. 2010).

2.6   DRINKING WATER TREATMENT

The purpose of water treatment is to provide drinking water of good quality that is free from waterborne pathogens and in agreement with hygienic standards. Surface and groundwater are susceptible to various sources of microbial contamination including agricultural runoff, sewage disposal, wildlife, and domesticated animals (Coffey et al. 2007). Generally, ground water is considered to be a less contaminated water source and require a simpler treatment processes than surface water whose treatment might involve several processes to provide an acceptable level of safety (Nelson et al., 2013).

To provide good drinking water, a single treatment process may not remove all the different types of water pathogens but a multi treatment process that can provide more safety when a single treatment step may not work accurately is needed (LeChevallier and Au 2002). For instance, the point-of-use water treatment for providing good drinking water in households has helped in combating against major waterborne diseases such as diarrhea (Nelson et al. 2013). In addition, careful evaluation of the water source, adequate and reliable treatment processes and thorough performance monitoring in accordance with operating parameters are

important factors when providing a good drinking water (Snozzi 2000, LeChevallier and Au 2002). The commonly used water treatment methods for surface water treatment are discussed below.

2.6.1 Coagulation and flocculation

Coagulation and flocculation are the first steps in the conventional water treatment. They are an important aspect of water and wastewater. Coagulants are usually added in water where their positive charge will neutralize the negative charge of dirt and other particles in the water. This leads to the formation of aggregates (flocs) usually by Brownian motion to remove suspended organic particles (algae, bacteria, protozoa and natural organic matter) and inorganic particles (clay and silt) (Gregory 2006). The most common coagulants used are aluminum sulphate (alum), ferric sulphate, ferric chloride and poly-aluminum.

2.6.2 Sedimentation or flotation

During sedimentation, flocs formed during coagulation settle to the bottom of the water due to gravitation power (Liu and Liptak 1999). This settling process is called sedimentation.

If the flocs are very light, fine air bubbles will be used to carry them to the surface (air dissolved flotation) where they are skimmed off. The flocks can also be removed by direct filtration.

2.6.3 Filtration

Filtration is a physical process used to remove organisms together with other suspended particles and unsettled flocs. Various filtration processes are used in drinking water treatment along with proper design and operation. Once the flocs have settled to the bottom of the water supply, the clear water on top is passed through filters of different compositions (sand, gravel and charcoal) in order to remove particles, such as dust, parasites, bacteria, viruses and chemicals.

2.6.4   Membrane technologies

Membrane filtration is a widely used technology in drinking water and wastewater treatment. It provides a physical barrier that effectively removes solid, viruses, bacteria and other suspended particles. For drinking water, membrane filters can remove particles larger than 0.2 µm including *Giardia* and *Cryptosporidium*. Its application is widely used also in the industry. The most commonly used membrane processes in drinking water treatment for microbial removal are microfiltration (MF) and ultrafiltration (UF) (LeChevallier and Au 2002).

2.7   DISINFECTION

2.7.1   Factors affecting disinfection efficiency of water treatment

Several factors affect the disinfection efficiency including disinfectant and its concentration, the type of microorganism present, suspended solid content, turbidity, temperature, pH, and contact time (NHMRC 2004, Anastasi et al. 2013). More importantly, treatment effectiveness is a function of the dose (disinfection concentration), contact time, temperature and pH (LeChevallier and Au 2004). Disinfectant concentration and contact time are very crucial in disinfection kinetics and the practical application of the contact time concept. This is defined as the product of the residual disinfectant concentration (C in mg/l) and the contact time (T in minutes or in seconds, the time when the residual disinfectant is in contact with the water) (USEPA 1999, Spellman 2008).

Increase in temperature beyond the value appropriate for drinking water influences the rate of disinfection reactions. For example, viable microorganisms may multiply in water if water temperature exceeds beyond $15^{o}C$ and it might lead to the formation of biofilms on internal surfaces (Ainsworth 2004). Biofilms are known to contain several living heterotrophic bacteria, fungi, protozoa, nematodes and crustaceans. pH of the disinfectant solution affects the reaction kinetics. For example, the disinfection efficiency of free chlorine is increased at

lower pH value, whereas the effect of chlorine dioxide is greater at alkaline pH levels (LeChevallier and Au 2004). Factors such as attachment to surfaces, encapsulation, aggregation and low nutrient growth can affect microbial sensitivity to disinfection. Among the disinfection technology used in the world chlorination, UV irradiation and ozonation are the commonly used water methods (Koivunen and Heinonen-Tanski 2005, Hijnen et al. 2006, Anastasi et al. 2013).

2.7.2   Chlorination

Chemical disinfection with chlorine has been used for more than 100 years. It is still the leading water treatment method in the world (Nelson et al. 2013). It is an oxidation treatment processes that acts by destroying nucleic acids and cell membranes of microorganisms. Chlorination can be performed by using different chemicals such as chlorine gas, hypochlorite, chloramines, chlorine dioxide and other chlorine disinfecting chemicals with each chemical possessing different disinfecting properties (Okoh et al., 2007). This method of treatment has proven its effectiveness for inactivation microbial pathogens, mostly enteric bacteria. It has a low efficiency against viruses, bacteria spores and protozoan cysts (Veschetti et al. 2003, LeChevallier and Au 2004, Nelson et al. 2013).

In addition to the general operational and environmental factors affecting the resistance of organisms to chlorine, physiological features and adaptation or genetic changes of different bacterial strains can also contribute to diverse resistances to chlorination (Cherchi and Gu 2011). Some indigenous bacteria isolated and subjected to environmental stress conditions have developed phenotypic resistance to disinfection methods (Wojcicka et al. 2007). The use of chlorination has been declining due to toxic, mutagenic and carcinogenic disinfection by-product (DBPs); chlorine residuals formed in disinfection process and associated health effect concerns (Oppenheimer et al. 1997, Veschetti et al. 2003, Nelson et al. 2013).

2.7.3   Ozonation

Ozone is a very strong disinfectant for inactivation of vegetative bacteria and a good virucide. It is produced when oxygen (O_2) molecules are dissociated by an energy source into oxygen atoms and subsequently collide with an oxygen molecule to form an unstable gas (O_3). When

ozone decomposes in water, the free radicals hydrogen peroxyl (HO_2) and hydroxyl (OH) are formed and all these have great oxidizing capacity, playing an active role in the disinfection process.

Ozone, as a powerful oxidizing agent, has been successfully used in the treatment of wastewater (Wu et al. 2012). Ozone's applications has been found in several developments such as the AOPs which involves the generation of highly potential chemical oxidants to reduce the toxicity and destruction of different organic contaminants in water and wastewater. It has also been used in wastewater treatment together with hydrogen peroxide, oxygen, photocatalytic reactions and ultraviolet radiation to evaluate its effectiveness in disintegration of pollutants and to access the treatment efficiencies of these combinations (Gimeno et al. 2007).

Ozone in aqueous solutions may react with microbes either directly as molecular ozone or as radical species formed after ozone decomposition. Ozone oxidizes organic components of water, such as natural organic matter to produce smaller molecules of organic substances. In order prevent increase in bacterial growth due to ozone; post-ozonation removal of the oxidation products is essential.

2.8 Ultraviolet radiation

Ultraviolet (UV) technology has gained a lot of attention and popularity due to its effectiveness in disinfection applications (Crawford et al. 2005, Bowker et al. 2011). UV disinfection system has a simple design which usually consists of a very few components, UV lamp, reaction chamber and a control box and is very easy to operate and maintain (Ibrahim et al. 2013). Installing or replacement of parts of the UV system in new or existing water treatment plant is relatively easy and requires a few modifications to the plant. UV light is divided into UV-C (100-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm). UV wavelengths between 200-300 nm are considered to be directly absorbed by DNA and therefore considered to be germicidal (Beck et al. 2014). UV-B and UV-C are the common UV classes in inactivating microorganisms but germicidal UV-C irradiation at 254 nm is widely used to inactivate chlorine resistance pathogens within a relative short contact time without producing undesirable disinfection by-products (Ibrahim et al. 2013).

Inactivation of microbial pathogens using UV radiation has been demonstrated in many studies (Hinjinen et al. 2006, Eischeid et al. 2009, Schwarzenbach et al. 2011) through oxidation application processes known as photolysis which has resulted in bond cleavage of organic molecules (Blanksby and Ellison 1993). The efficiency of UV systems is due to the fact that DNA molecules absorb UV light. These processes can occur directly by inducing lysis in the target compounds due to the absorption of highly energetic photons, or indirectly, in which an intermediary compound transfers the absorbed photon energy to the target molecule (Schwarzenbach et al. 2003). Thus, leading to the breakage and damage of DNA, preventing replication, transcription and translation that often prompt the fast destruction of bacteria (Soloshenko et al. 2006, Cheveremont et al. 2012).

Wavelengths 254 nm and 280 nm may be potentially the most efficient to eliminate microorganisms since they are close to the DNA maximum absorption rate and responsible for the formation of pyrimidine dimers. Thus, this wavelength range has been proven to cause damage on both DNA and proteins of adenoviruses (Eischeid et al. 2009). Measuring the nucleic acid damage has been established to give adequate insight into the mechanism involved in the UV inactivation. Kuluncsics et al. (1999) found that the induced cyclobutane pyrimidine dimers (CPDs) which is a dominant form of UV-induced DNA damage, is more effectively induced by UV-C than the UV-A. Besaratinia et al. (2011) established that the formation of CPDs and other photodimeric lesions is wavelength dependent.

2.8.1 UV-Mercury vapor lamps

The conventional UV technology is based on continuous wave mercury vapor lamps. There are two types of mercury vapor lamps commonly used in water treatment: monochromatic low pressure lamps (LP) lamps emitting radiation at 253.7 nm and polychromatic medium pressure (MP) lamps emitting light between 700 nm – 200 nm (Vilhunen 2010).

Disinfection of water using UV-mercury vapor lamps is an efficient disinfection technology and important physical procedure for water and wastewater treatment especially at 254 nm using the mercury vapor lamp (Koivunen and Heinonen-Tanski, 2005, Hjinen et al. 2006).

2.8.2   Ultraviolet Light emitting diodes (UV-LEDs)

Ultraviolet Light emitting diodes (UV-LEDs) are semiconductor p-n junction devices that emit light in a narrow spectrum and produced by a form of electroluminescence (Crawford et al., 2005, Hu et al. 2006 Khan 2006). The LEDs are made of aluminum nitride (AlN) or gallium and aluminum nitride (AlGAN) that are not toxic (Vilhunen et al., 2009). LEDs use electricity more efficiently by transmitting large percentage of energy into light and produce less heat energy as waste.

Over the last decades, LEDs have been receiving tremendous attention amongst researchers as an alternative UV source following many advantages over the conventional UV mercury vapor lamps. These include absence of mercury, increase in operational flexibility and reliability, resistance to shock and vibration, and compact size and energy. LEDs do not require any warm up-period and it is possible to adjust their wavelengths to supply desirable radiations (Vilhunen et al. 2011, Crook 2011, Jo 2013, Nelson et al. 2013). All these advantages of UV-LED lamps over the mercury vapour lamps prompted the diversion of interest by manufactures and researchers to produce and to continuously use UV LEDs (Vilhunen et al. 2009, Chevremont et al. 2012).

Nelson et al. (2013) reported that LEDs that emits wavelengths between 200 and 290 nm are amendable for point-of-use water treatment since they are user friendly, cost-effective and reliable for reducing waterborne pathogens including bacteria, viruses and protozoa. In addition there is no formation of DBPs (Huffman et al., 2002, Vilhunen et al. 2009).

Nelson et al. (2013) showed that spiked *E. coli* was influenced by radiation of single UV LEDs at 265 nm in ultra-pure laboratory prepared water and highly turbid wastewater (20 NTU) for 20 to 50 min exposure time, respectively, and achieved 1-2.5 log reduction. Inactivation of total coliform number with UV-LED in the wastewater was not significantly dependent on high turbidity of waste water. Vilhunen et al. (2009) investigated the use of the combined ten UV LEDs to inactivate *E. coli* in a laboratory prepared water samples, irradiated at 269 nm with the exposure time of 5 minutes and they achieved 3-4 log reductions.

In another study Crawford et al. (2005) investigated UV LEDs in water treatment by using a single 270 nm UV LEDs manufactured by Sandia National Laboratories. They used non-turbid, contaminated water with 3.6 mJ/cm^2 under 10 min of exposure time. Log reduction of 1.89 in the *E. coli* was achieved. At a dose of 2.2 mJ/cm^2 the corresponding 6 min exposure time, a similar log reduction of 1.85 log was also achieved.

3   AIMS OF THE STUDY

The aim of this thesis was to study the inactivation of *E. coli* in drinking water by using a flow-through reactor of UV-LEDs at different wavelengths and to compare the results to those obtained by traditional UV collimator at 253.7 nm.

4 MATERIALS AND METHODS

4.1 Preparative work

4.1.1 Sterilization of glass wares

All glass wares including test tubes, petri dishes, dilution bottles, glass pipettes, forceps and other heat stable solid parcels were sterilized by hot-air oven (Memmert model 100-800) at 130°C or 140°C for 4 hours according to the laboratory manual of the Department of Environmental Science, University of Eastern Finland. The materials to be sterilized by hot-air oven treatment might be placed directly or wrapped in a foil before exposed to heat after ensuring the lid of the oven is closed. The pipettes were sterilized in heat-resistance plastic bags or metal boxes with one pipette size in one bag and all tips of the pipettes pointing towards the same direction protecting the tips against contamination.

4.1.2 Sterilization of liquids

All aqueous solutions and diluents such as growth media and dilution water were sterilized in the autoclave (Santasalo, Finland) at 121 ^{o}C for 20 minutes according to the laboratory manual in the Department of Environmental Science, University of Eastern Finland.

4.2 Preparation of growth media

4.2.1 Preparation of THG agar

In the preparation, 10 g tryptone, 5 g yeast extract, 2 g glucose and 12 g agar, were measured into 1500 ml of volumetric flask containing 1000 ml of deionized water. The flask was placed on an electric magnetic mixer to ensure uniform mixing of the solution and pH was adjusted to 7.0. The medium was distributed to 3 different 500 ml flasks covered with aluminum foil and autoclaved at a temperature of 121 ^{o}C for 20 minutes.

4.2.2   Preparation of THG broth

In the preparation, 10 g tryptone, 5 g yeast extract, and 2 g glucose were measured into 1500 ml of volumetric flask containing 1000 ml of deionized water at pH of 7.0. The solution was distributed into test tubes and covered with test tube caps and the tubes were autoclaved at a temperature of 121 ^{o}C for 20 minutes.

4.2.3   Preparation of Phage THG agar

In the preparation, 10 g tryptone, 5 g yeast extract, 2 g glucose, 5 g NaCl, 12 g agar and 0.25 g MgSO$\mathbf{.}$7H$_2$O were measured into 1500 ml of volumetric flask containing 1000 ml of dionized water. The flask was placed on an electric magnetic mixer to ensure uniform mixing of the solution at pH of 7.0 and autoclaved at a temperature of 121°C for 20 minutes. The medium was allowed to cool for few minutes after autoclaving and distributed aseptically into sterile plastic Petri dishes.

4.2.4   Preparation of Phage THG broth

In the preparation, 10 g tryptone, 5 g yeast extract, 2 g glucose, 5 g NaCl and 0.25 g MgSO$\mathbf{.}$7H$_2$O were measured into 1500 ml volumetric flask containing 1000 ml of deionized water at pH of 7.0. The medium was distributed into 500 ml flasks and autoclaved at 121°C for 20 minutes.

4.3   Experiments

4.3.1   Growth and culture of *E. coli*

E. coli strain (ATTC 13706) used for the experiments was taken from previously grown strain on agar plate, stored in the refrigerator. Loopful suspension of the strain was added into 1 L flask containing phage THG broth and incubated at 37°C for 24 hours (overnight). After 24 hours of incubation, there was a change in color of the media from bright yellow to a turbid pale yellow solution and had developed a typical smell indicating the emergence of microbial activity. The medium was stored in the refrigerator overnight for 24 hours before it is used for

the experiment the next day to indicate a previous fecally contaminated water sample by the test organism.

4.3.2 Determining the density of *E. coli*

The initial density of *E. coli* was determined by using a dilution series plate method. One ml of *E. coli* stored overnight in the refrigerator was inoculated in 9 ml of deionized water and mixed in a vertical shaker to make dilution -1. One ml of dilution -1 was further pipetted to 9 ml of deionized water (dilution -2) and mixed. Dilution was similarly continued until dilution –8 was reached. Culturing was done to THG agar plates by inoculating 0.1 ml of dilution -1 to -2 on the plates, to make final dilutions of -2 to -3 and continued to -9 plates respectively. The inocula were evenly spread on each plate with a sterilized glass rod. The plates were incubated upside down at 37°C for 48 hours. The number of *E. coli* in the UV exposed samples was determined similarly but a shorter dilution series (-2 to -5) was used.

4.3.3 Collimator experiments

Collimator experiments were conducted with *E. coli* to obtain knowledge about UV-dose needed to make an inactivation curve of *E. coli*, which could be compared with the results obtained from the UV-LED reactor experiments.

A bench-scale collimator beam apparatus was used to irradiate the samples in this study. The apparatus consisted of a mercury UV lamp housed above a collimating tube, which perpendicularly focused the UV beam on the sample in the petri dish. The lamp intensity for a specific surface from the mercury low pressure lamp was 0.1959 mW/cm^2 and the wavelength was 253.7 nm. The UV dose was calculated with equation 1 (Blatchley 1997).

Dose (mWs/cm^2) $\qquad = I \times t \qquad\qquad\qquad$ Equation 1

$\qquad$ Where I = 0.1959 mW/cm^2 and t is the time in seconds

The value 1 mWs/cm^2 corresponds to 10 J/m^2. The dose in (J/m^2) was obtained by multiplying doses in (mWs/cm^2) with 10.

The collimator doses from 10 to 361 J/m^2 were used to determine the inactivation curve.

 E. coli grown and stored in THG broth was diluted several times to give initial concentration of about 5×10^5 cfu/ml. The collimator lamp was switched on for 10 minutes before the commencement of the experiments to ensure that the lamp had warmed up and supplied a full radiation. Ten ml of the *E. coli* dilution was pipetted in a glass petri dish (6.2 cm diameter), a magnetic stirrer was immersed to ensure rapid mixing and the dish was covered with an opaque protective cover. The dish was placed under the collimator tube and the distance between the lamp tube and petri dish was maintained at 2 cm. After ensuring all pre-experimental preparations were done and the dish containing the *E.coli* dilution was placed on the stirrer, the protective cover was removed immediately when the timer started to count for a specific period of time and replaced when the timer was stopped to prevent further light penetration. At this point, the exact time was recorded. The experiment was repeated with three replicates for each treatment at selected periods to yield the desired UV doses. After the collimator experiment, THG agar was used to cultivate the *E. coli* samples and the result was obtained using the standard plate count.

4.3.4 Reactor experiments

The experiments were conducted on pilot-scale surface water treatment plant, which could treat lake water for drinking quality. Raw water source was Lake Kallavesi in Eastern Finland, from which the water was pumped to the treatment plant after screening (60 µm) and sand filtration. This lake water is known to be rich in humus (total organic carbon, TOC 10-12 mg/L) (Myllykangas 2004). The water treatment plant consists of coagulation with ferric sulphate and slow mixing (flocculation), dissolved air flotation (DAF) and anthracite-quartz sand rapid filter. After purification with these treatments, water was pumped to the UV LED reactor provided by a private company after the reactor had been switched on, at a specific wavelength and power in order to expose the spiked water to the UV irradiation.

1 L of phage THG broth containing about 5×10^7 cfu/ml of *E. coli* (ATTC13706) was mixed with 19 L of purified pilot water. The solution was mixed thoroughly in a plastic container between 180 and 190 rmp by electric vortex mixer (Euro-star digital model, made in Staufen, Germany) to achieve a homogenous mixture. The solution was pumped into the water flowing to the UV LED reactor at flow rates of 550 l/h, 280 l/h, 150 l/h, 120 l/h and 60 l/h, respectively (Table 2). The volume of the reactor was about 6.7 L and the respective water retention times were between 44s – 6 min 42s depending on the flow rate. The wavelengths used in the experiments were 255 nm, 260 nm and 270 nm at different power efficiency of 25%, 50%, 75% and 100%, respectively (Table 2). In these experiments, *E. coli* determination in water samples was done before and after exposure to UV-reactor.

UV-LEDs reactor experiment was done in three steps. The first step had two LED strips of each wavelength, the second step had four strips. The third step had varied strips with LEDs so that the retention time of water in the reactor could be changed as well as the number of LED lamps. Retention time, unit power and turbulence were also increased in the third step Table 2.

Table 2. The 3 Steps of the UV-LEDs reactor experiment.

	Wave lenghts (nm)	Number of strips	Flow rate (l/h)	Water retention time (s) under UV	Other factors
Step 1	270	2	550	44	
	255	2	550	44	
	260	2	550	44	
Step 2	255	4	550	44	numbers of strips doubled
	270	4	550	44	
Step 3	255	4	550	44	water turbulence increased in reactor
	260	2	550	44	more strips
	270	4	550	44	
	255, 260, 270	10	550	44	
	255	4	280	1 min 26 s	retention time incr.
	270	4	280	1 min 26 s	
	255	4	150	2 min 41 s	
	260	2	150	2 min 41 s	
	270	4	150	2 min 41 s	
	255, 260, 270	10	150	2min 41 s	
	255	4	120	3 min 21 s	
	260	2	120	3 min 21 s	
	270	4	120	3 min 21 s	
	255, 260, 270	10	120	3 min 21 s	
	255	4	60	6 min 42 s	intensity increased
	260	2	60	6 min 42 s	
	270	4	60	6 min 42 s	
	255, 260, 270	10	60	6 min 42 s	
	255, 260, 270	10	120	6 min 42 s	unit power increased

In the first step, LEDs in the reactor were switched on and wavelength was adjusted to 270 nm at 25 % power efficiency. The mixed solution spiked with *E. coli* was pumped into the UV LED reactor at 550 l/h through the inflow water tap for 44 s retention time. The outflow water tap of the reactor was initially flame sterilized before water samples were allowed to

run for one minute after exposed to irradiation in the reactor. The exposed water samples were collected through the outflow water tap in a sterile bottle. After collection, the outflow water pump carrying water from the reactor was locked and the spiked water was allowed to run for 1 min through the inflow tap in order to collect zero 1 sample (water sample not exposed to irradiation in the reactor). Thereafter, the inflow pump was closed. The experimental procedures above were repeated for other wavelengths in step one, step two and three. All samples at each power (25 %, 50 %, 75 % and 100 %) and also Zero 2 and 3 were also collected by repeating the above procedures.

The samples were stored in a cooling box before culturing of *E. coli* densities according to the method described in chapter (4.3.2). Water parameters including pH, turbidity, and temperature were recorded from the on-line meters of the pilot water plant before and after the experiments.

4.4   Statistical analysis

The results for *E. coli* densities were calculated as geometric means. Differences in reduction of *E.coli* among the treated UV samples and the control were analyzed using Friedman's 2-way ANOVA and the effect of power on reduction of *E. coli* in the UV-treated samples across the steps were studied with Kruskal-Wallis 1-way ANOVA and Mann-Whitney U tests (non-parametric test) using statistical software (SPSS Inc., Chicago, IL. version 19.0). Graphpad Prism 5 software (Graphpad software Inc) was used for graphical analysis and exponential inactivation curve (log N_b/N_a) at a particular dose (J/m^2) of the *E. coli* strain.

5 RESULTS

5.1 Collimator experiments

Collimator results showed that a very slight reduction of *E. coli* was obtained at the low doses (Fig. 1). At doses 130-137 J/m^2 log reduction of 1.7-2.3 was achieved. At dose of 182 J/m^2 the reduction was 3 logs, and 5 log reductions, which meant that no *E. coli* was found any more, was achieved between 240 and 298 J/m^2. The reduction was linear at doses between 50 and 250 J/m^2.
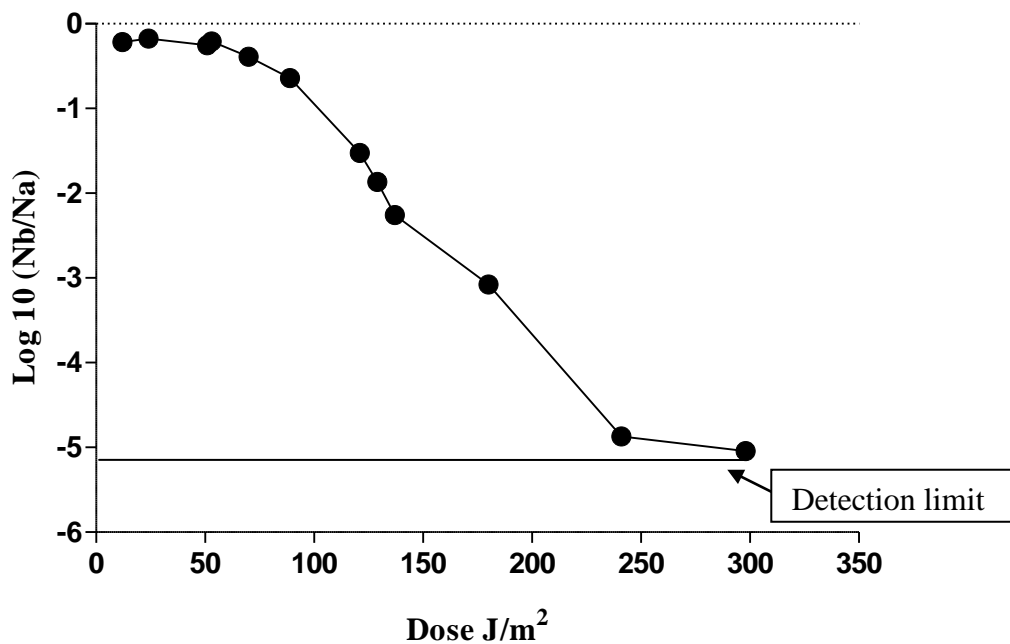
Figure 1. *E. coli* inactivation curve in a collimator experimental set-up

5.2 UV LED reactor experiments

The pH of the water sample was typical of the ferric sulphate coagulated water which implies that the water was acidic. The pH and temperature range before and after the experiments in the three studied steps were quiet similar. Turbidity values before and after the experiments in

all the steps were not different from each other and less than 1.0 NTU in all cases as stated by the World Health Organization drinking water guidelines (Table 3).

Table 3. Pilot-water quality parameters measured

Experimental steps	Before the experiments			After the experiments		
	pH	Temperature (°C)	Turbidity (NTU)	pH	Temperature (°C)	Turbidity (NTU)
Step 1	4.45	10.9	0.073	-	-	-
	4.41	13.3	0.106	4.40	13.30	0.221
	4.39	160	0.109	4.38	16.00	0.165
	-	-	-	-	-	-
	4.35	15.9	0.184	4.25	15.90	0.235
Step 2	4.37	11.2	0.061	4.37	11.70	0.075
	4.37	15.1	0.103	4.35	15.10	0.150
	4.33	15.1	0.098	4.33	15.10	0.113
Step 3	4.31	16.3	0.375	4.31	16.20	0.321
	4.32	17.0	0.263	4.32	17.00	0.285
	4.34	17.2	0.300	4.31	17.20	0.311
	4.40	16.4	0.825	4.38	16.40	0.604
	4.45	16.8	0.451	4.44	16.90	0.494
	4.38	16.8	0.374	-	-	-

Pretreated water from the pilot water plant was mixed to the spiked tap water after the sand filtration stage so that the initial concentration of *E. coli* to be exposed was 5×10^5 cfu/ml and in general, reductions were very low in the entire steps (table 4).

Table 4. UV-LEDs reactor experimental results

Step	Flow	Wavelength	Number of srips	Power	Number before (cfu/ml)	Number after (cfu/ml)	Log survival ratio*
1	550	270	2	25	9.83E+04	1.14E+05	-1.96E-02
1	550	270	2	50	9.83E+04	1.11E+05	-3.29E-02
1	550	270	2	75	9.83E+04	1.09E+05	-3.40E-02
1	550	270	2	100	9.83E+04	1.24E+05	1.74E-02
1	550	255	2	25	4.11E+04	4.55E+04	4.99E-02
1	550	255	2	50	4.11E+04	5.01E+04	8.61E-02
1	550	255	2	75	4.11E+04	4.98E+04	7.76E-02
1	550	255	2	100	4.11E+04	4.20E+04	8.91E-03
1	550	260	2	25	3.91E+04	5.23E+04	1.16E-01
1	550	260	2	50	3.91E+04	4.23E+04	3.49E-02
1	550	260	2	75	3.91E+04	3.93E+04	1.46E-03
1	550	260	2	100	3.91E+04	3.84E+04	4.82E-02

2	550	255	4	25	8.33E+03	6.48E+03	-1.09E-01
2	550	255	4	50	8.33E+03	9.77E+03	6.93E-02
2	550	255	4	75	8.33E+03	6.08E+03	-1.37E-01
2	550	255	4	100	8.33E+03	7.70E+03	-3.42E-02
2	550	270	4	25	5.87E+04	4.89E+04	-9.75E-02
2	550	270	4	50	5.87E+04	5.48E+04	-4.06E-02
2	550	270	4	75	5.87E+04	5.39E+04	-3.59E-02
2	550	270	4	100	5.87E+04	5.39E+04	-5.26E-02
3	550	255	4	25	1.91E+04	1.60E+04	-7.82E-02
3	550	255	4	50	1.91E+04	1.87E+04	-8.31E-03
3	550	255	4	75	1.91E+04	1.86E+04	-1.19E-02
3	550	255	4	100	1.91E+04	2.24E+04	6.53E-02
3	550	260	2	25	1.91E+04	2.27E+04	7.47E-02
3	550	260	2	50	1.91E+04	2.18E+04	5.03E-02
3	550	260	2	75	1.91E+04	2.23E+04	5.34E-02
3	550	260	2	100	1.91E+04	2.18E+04	6.61E-02
3	550	270	4	25	1.91E+04	1.75E+04	-3.68E-02
3	550	270	4	50	1.91E+04	1.70E+04	-4.91E-02
3	550	270	4	75	1.91E+04	1.91E+04	7.87E-04
3	550	270	4	100	1.91E+04	1.84E+04	-1.62E-02
3	550	270+255+260	4+4+2	25	1.91E+04	2.18E+04	5.48E-02
3	550	270+255+260	4+4+2	50	1.91E+04	1.40E+04	-1.35E-01
3	550	270+255+260	4+4+2	75	1.91E+04	1.61E+04	-7.28E-02
3	550	270+255+260	4+4+2	100	1.91E+04	1.67E+04	-5.74E-02
3	280	255	4	25	2.22E+04	2.45E+04	4.30E-02
3	280	255	4	50	2.22E+04	2.21E+04	-2.21E-03
3	280	255	4	75	2.22E+04	2.38E+04	3.02E-02
3	280	255	4	80	2.22E+04	1.98E+04	-5.08E-02
3	280	255	4	100	2.22E+04	1.91E+04	-6.59E-02
3	280	270	4	25	2.22E+04	2.13E+04	-1.93E-02
3	280	270	4	50	2.22E+04	3.00E+04	1.30E-01
3	280	270	4	75	2.22E+04	1.84E+04	-8.28E-02
3	280	270	4	80	2.22E+04	1.85E+04	-8.05E-02
3	280	270	4	100	2.22E+04	2.86E+04	1.10E-01
3	150	255	4	25	3.61E+04	2.28E+04	-2.00E-01
3	150	255	4	50	3.61E+04	3.22E+04	-4.95E-02
3	150	255	4	75	3.61E+04	3.76E+04	1.79E-02
3	150	255	4	100	3.61E+04	3.81E+04	2.36E-02
3	150	260	2	25	3.61E+04	3.66E+04	5.74E-03
3	150	260	2	50	3.61E+04	3.86E+04	2.92E-02
3	150	260	2	75	3.61E+04	4.08E+04	5.33E-02
3	150	260	2	100	3.61E+04	4.20E+04	6.54E-02
3	150	270	4	25	3.61E+04	2.93E+04	-9.02E-02
3	150	270	4	50	3.61E+04	2.95E+04	-8.69E-02
3	150	270	4	75	3.61E+04	3.07E+04	-7.07E-02
3	150	270	4	100	3.61E+04	2.59E+04	-1.44E-01
3	150	270+255+260	4+4+2	25	3.61E+04	2.59E+04	-1.43E-01
3	150	270+255+260	4+4+2	50	3.61E+04	2.18E+04	-2.18E-01
3	150	270+255+260	4+4+2	75	3.61E+04	2.32E+04	-1.93E-01
3	150	270+255+260	4+4+2	100	3.61E+04	2.60E+04	-1.43E-01
3	120	255	4	25	3.83E+04	3.70E+04	-1.42E-02
3	120	255	4	50	3.83E+04	3.66E+04	-1.95E-02

3	120	255	4	75	3.83E+04	4.86E+04	1.04E-01
3	120	255	4	100	3.83E+04	3.51E+04	-3.75E-02
3	120	260	2	25	3.83E+04	4.15E+04	3.52E-02
3	120	260	2	50	3.83E+04	3.73E+04	-1.13E-02
3	120	260	2	75	3.83E+04	4.22E+04	4.22E-02
3	120	260	2	100	3.83E+04	-	-
3	120	270	4	25	3.83E+04	3.39E+04	-5.29E-02
3	120	270	4	50	3.83E+04	3.52E+04	-3.59E-02
3	120	270	4	75	3.83E+04	4.02E+04	2.13E-02
3	120	270	4	100	3.83E+04	2.61E+04	-1.66E-01
3	120	270+255+260	4+4+2	25	3.83E+04	3.00E+04	-1.06E-01
3	120	270+255+260	4+4+2	50	3.83E+04	2.49E+04	-1.87E-01
3	120	270+255+260	4+4+2	75	3.83E+04	1.74E+04	-3.42E-01
3	120	270+255+260	4+4+2	100	3.83E+04	2.10E+04	-2.60E-01
3	60	255	4	25	1.79E+04	1.95E+04	3.92E-02
3	60	255	4	50	1.79E+04	2.36E+04	1.20E-01
3	60	255	4	75	1.79E+04	1.64E+04	-3.83E-02
3	60	255	4	100	1.79E+04	1.45E+04	-8.90E-02
3	60	260	2	25	1.79E+04	1.94E+04	3.52E-02
3	60	260	2	50	1.79E+04	2.09E+04	6.92E-02
3	60	260	2	75	1.79E+04	2.18E+04	8.69E-02
3	60	260	2	100	1.79E+04	2.45E+04	1.38E-01
3	60	270	4	25	1.79E+04	1.29E+04	-1.40E-01
3	60	270	4	50	1.79E+04	1.65E+04	-3.48E-02
3	60	270	4	75	1.79E+04	-	-
3	60	270	4	100	1.79E+04	1.45E+04	-8.90E-02
3	60	270+255+260	4+4+2	25	1.79E+04	1.26E+04	-1.53E-01
3	60	270+255+260	4+4+2	50	1.79E+04	1.34E+04	-1.24E-01
3	60	270+255+260	4+4+2	75	1.79E+04	1.25E+04	-1.56E-01
3	60	270+255+260	4+4+2	100	1.79E+04	1.66E+04	-3.24E-02
3	60	270+255+261	4+4+2	100	3.31E+04	3.04E+04	-3.24E-02
3	60	270+255+262	4+4+2	100	3.31E+04	2.67E+04	-3.24E-02

*Log survival ratios were calculated from the pairwise Log survival ratios.

Step 1 of the reactor experiment with 2 strips of UV-LEDs showed less than 0.5 log reduction especially with 255 nm and 260 nm. There were no significant differences in the reduction between the powers, across the wavelengths. Although there were differences in the number of *E. coli* before and after exposure to UV-LEDs radiation but this was not significant.

The results obtained in step 2 with 4 strips of UV-LEDs showed reductions were down to negative at 255 nm and 270 nm. The reductions were not significant and not in relation to the power surge.

In step 3, single and combined wavelengths were used in the test and no significant reduction was obtained between the wavelengths as shown in Table 4. At 550 l/h flow rate, 25 % power

with the combined wavelengths showed less than 0.5 log reduction and was not significant. The combined wavelengths were assumed to have yielded a better reduction than the singled wavelengths but this was not the case. All reductions at other flow rates and combined wavelengths were down to negative.

At 260 nm and 60 l/h flow rate with the 2 strips, reduction was according to the power surge. Although, they were all less than 0.5 log and not significant.

Result of the Friedman Test indicated that there were no statistically significant differences in the reductions between the UV-treated samples (with powers 25 %, 50 %, 75 % and 100 %) and the control in all tested cases (step 1, 2, 3 and in the combined steps data) ($P>0.05$). The Kruskal-Wallis Test revealed that wavelength did not have any statistical significant effect on the reductions in step 1 and 2. The effect of wavelength was significant to a statistical level with 50 %, 75 %, and 100 % power in step 3 ($P<0.05$). The combined wavelength (270+255+260) recorded higher reduction compared to other wavelengths where we had single wavelength.

The effect of flow rate was tested in step 3 where we had homogenous samples with different flow rates. Our result indicated flow rate had no significant effect on the reductions (with 25 %, 50 %, 75% and 100 %). Kruskal-Wallis Test revealed that number of strips had statistical significant effects on the reductions (with 50 %, 75% and 100 % power) ($P<0.05$) in step 3 where we had different flow rates except for reduction with 25 % power. Effect of numbers of strips tested with 550 l/h flow rate and strips < 3 (LEDs containing 4 and 2 strips) using the Mann Whitney U test for the combined data indicated that the effect of strip on reduction of *E. coli* was significant only with power 25 % ($P<0.05$) and not in other samples (with 50 %, 75% and 100 %).

6 DISCUSSION

Several studies have been conducted on conventional collimator with different UV-lamps (Medium and low pressure lamp and pulse UV lamp). Most reactor studies conducted on UV-LEDs are done by suspending the UV-LEDs above the sample in the petri dishes or in other containers such as in point-of-use- applications with small water volume of about 500 ml, 250 ml and 100 ml at a stagnant position (Vilhunen et al. 2009, Chevremont et al. 2012, Nelson et al. 2013). The reactor system in our study had a volumetric capacity of about 6.7 L with a real water flow. Results obtained in our study showed no disinfection and this clearly established that the efficiency of the tested UV-LEDs were not enough for the high water volume. There is little available information stating the disinfection efficiency of the reactor device with UV-LEDs in relation to the flow rate to provide a good drinking water. In this study, we investigated the disinfection of *E. coli* in drinking water using a flow through reactor device with flow rates (550 l/h, 280 l/h, 150 l/h, 120 l/h and 60 l/h). The device contains UV-LEDs and we compared its disinfection efficiency with the conventional UV system.

The collimator results showed a maximum of about 5 log reductions between 240-298 J/m^2. It means that no *E. coli* could be detected in culture and the detection limit was reached. The microbial inactivation obtained in this result was in agreement with previous studies but, however, our result was obtained at higher dose compared to previous studies (Sommer et al. 2000, Zimmer and Slawson 2002), which achieved an average *E. coli* inactivation between 4.2 to 5 log units at 80 J/m^2 and 5.0 to 5.2 log units at 100 J/m^2.

Bohrerova et al. (2008) investigated the use of a continuous low-pressure lamp (LP) containing four monochromatic lamps at 254 nm. They reported *E. coli* reduction between 4.2 and 5.0 log at 80 J/m^2 and also stated that a 3 log *E. coli* reduction will require a dose of 51 J/m^2 for LP/MP lamps, respectively. Our result showed similar reductions but at higher dose compared to the previous study.

The degree of microbial inactivation is often determined by the UV-dose. EPA (1999) revealed that when microorganisms are exposed to UV radiation, a standard fraction of the microbial population is inactivated during each progressive increase in time. In our study, collimator results were also linear. This showed that about the same number of *E. coli* was

inactivated compare with the previous study. A reason that higher doses were needed for the inactivation of *E. coli* in our study compared to previous studies could be the different *E. coli* strains used in experimental work, as susceptibility of different types of microorganisms may vary under UV irradiation treatment (Johnson et al. 2010).

All reactor results with UV LEDs in the three steps showed no significant microbial reduction as reductions were less than 0.5 log in all the steps. There was little consistency in the reduction result obtained at the first step of the reactor results (255 nm and 260 nm) compared to that obtained in step 2 and 3. In step 3, no significant microbial reductions were achieved even at increased in UV radiation intensity, increased water turbulence, increased unit power of LEDs and strips and decreased water flow rate. The increased turbulence promotes application of the UV radiation in the reactor so as to eliminate dead zones, where limited UV exposure can occur. Overall reactor result showed no evidence of water disinfection.

Nelson et al. (2013) investigated the impact of laboratory prepared water sample spiked with *E. coli* to the radiation produced by single UV LEDs at 265 nm. The reduction achieved was between 1 and 2.5-log. This signifies about 90-99 % *E. coli* reduction. The difference in these results compared to our results might be the shorter exposure time in our study (between 44 seconds to 6 min 42 seconds) and larger water volume (6.7 L) compared to (20 to 50 minutes) exposure time and a low water volume (100 ml) in the previous study. Vilhunen et al. (2009) investigated the use of combined ten UV LEDs to inactivate *E. coli* in a laboratory prepared water sample (500 ml) for an exposure time of 5 minutes. They achieved 3 to 4 log bacterial reduction. Our result was not in agreement with this study despite a very low turbidity (Table 2).

Pilot water used in our study was pretreated to enhance water quality and disinfection effectiveness. This water may probably contain some dissolved and suspended particles that might have shielded microorganism against UV radiation. For example iron, sulphites, nitrites and phenols have an ability to absorb UV light. AWWA and ASCE (1990) established that UV effectiveness is not limited by chemical water quality parameters. For instance, pH, temperature, alkalinity and total inorganic carbon do not affect the overall effectiveness of UV disinfection.

In addition, if the power supplied to the lamps is low and not evenly distributed and monitored adequately, there might be possibilities for inaccuracy in the result. This is possibly the case in our result where power showed no effect on the steps. Reductions in all the steps

were less than 0.5 log and this indicates no disinfection using the reactor flow through system compared to the collimator system with about 5 log microbial reduction.

7   CONCLUSION

In this study, the collimator result showed more accurate and better inactivation results of *E. coli* when compared to the reactor in water disinfection experiments. Based on the results obtained in this study, collimator using mercury UV lamp was more suitable and efficient for disinfection than Led lamps used in water application works.

Inefficiency and inaccuracy in UV-reactor results might have occurred due to several factors such as the UV-dose, exposure time, power supplied and distribution in the chamber, number of UV lamps or layers and mechanical fault due to the design of the reactor system.

Further experimental studies should be carried out on the UV-reactor with major concern to wavelength, dose, exposure time and power supplied as well as monitoring power distribution in the chamber in order to boost its experimental efficiency for drinking water purpose.

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