

Point-of-use water disinfection using UV light-emitting diodes to reduce bacterial contamination

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Abstract The treatment process described in this research explores the impact of exposing water samples containing fecal coliforms to the radiation produced by single ultraviolet (UV) light-emitting diodes (LEDs) operating at 265 nm. UV LEDs are long lasting, compact in size and produce more efficient light output than traditional mercury-vapour bulbs, making them ideal for application in point-of-use disinfection systems, such as in remote areas. In this study, contaminated water samples containing either a pure culture of *Escherichia coli* or tertiary effluent from the City of Regina Wastewater Treatment Plant were used to study the application and efficiency of using UV LEDs for water disinfection. The results indicate that bacterial inactivation was achieved in a time-dependent manner, with 1- and 2.5-log *E. coli* reductions in water following 20 and 50 min of UV LED exposure, respectively. Ultraviolet radiation was less effective in reducing coliform bacteria in wastewater samples due to the elevated turbidity levels. Further work remains to be completed to optimize the application of UV LEDs for point-of-use disinfection systems; however, the results from this study support that bacterial inactivation using UV LEDs is possible, meriting further future technological development of the LEDs.

Keywords Ultraviolet light-emitting diode (UV LED) · Disinfection · UV water treatment · Wastewater · Ultraviolet radiation · Bacterial contamination

Introduction

The World Health Organization (WHO 2000) estimates that over one billion people lack access to safe drinking water. Waterborne diseases cause millions of deaths annually. The USA experienced 39 outbreaks of waterborne diseases affecting 25 states between 1999 and 2000 (Lee et al. 2002), and Canada has experienced several large- and small-scale waterborne illness despite increased efforts to properly disinfect all public water supplies (Ontario Ministry of the Attorney General 2002; Saskatchewan Ministry of Justice 2002). As a result, research into engineered water treatment techniques, including the oxidation and disinfection processes to reduce organic concentrations through the application of ultraviolet radiation, continues to grow (Murphy et al. 2013).

In general, groundwater is considered to be a less contaminated water source, including microbial contamination, than surface water. This generalization is reflected in water quality guidelines and treatment requirements set by national and provincial regulators (Health Canada 2008; Saskatchewan Ministry of Environment 2002). While surface water treatment involves several processes, many rural communities and farmsteads rely on groundwater wells for water which is not extensively treated. Groundwater can contain microorganisms that are not filtered out by soils and grow in nutrient-rich gradients near the wellhead (Cullimore 1999, 2008). Surface-source contamination and naturally occurring subsurface conditions (such as oxygenation and metals accumulation surrounding the well casing) can promote the growth of potentially harmful microbes in groundwater. Enteric viruses can migrate through subsurface soils and into groundwater (Jansons et al. 1989), and contamination from septic tanks, sewer systems and

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lagoons can be linked to several waterborne outbreaks leading to disease (Craun 1988). Many water-related diseases result from inadequate water treatment (Esrey et al. 1991). Therefore, effective treatment options for inactivation or removal of pathogens from groundwater supplies in areas where standard water treatment provisions are not currently made have become the focus of many research initiatives.

Several methods currently exist to produce safe potable water, most of which are used in large-scale operations and require a robust water distribution system. Chemical disinfection is the leading water treatment method for small-scale and rural systems, but brings with it the potential health concerns of disinfection by-products (DBPs) and is ineffective in destroying protozoan cysts. Other popular methods such as ozonation and membrane filtration can be very expensive and challenging to operate. One alternative to centralized, large-scale and expensive water treatment methods is the use of household point-of-use (POU) treatment systems (Mintz et al. 2001) for use in rural and/or remote areas.

There are several inexpensive options for treating water at the point-of-use, which can be effective in the reduction of diarrheal disease in underdeveloped areas (Sobsey 2002). A long-standing and relatively reliable method for in-home water disinfection is boiling; however, boiling is time-consuming and requires a large amount of fuel. Nonetheless, several small Canadian communities have been on boil water advisories for years (Eggertson 2008). Solar disinfection is being promoted in many developing areas as an inexpensive method of disinfection but can be ineffective with insufficient amounts of sunlight and can take several hours to accomplish. Filtration with ceramic filters or sand filters can be highly effective in removing bacteria and protozoa, but is less effective for viruses, and its efficacy can vary greatly depending on the filter's manufacturer. Due to the vast differences in the source water used for drinking, coupled with the user's level of income and preferences, the continued research into various POU water treatment systems is warranted.

Short-wave ultraviolet radiation, UV-C waves, can inactivate microorganisms in drinking water and is regularly used as a water and wastewater treatment technique (Betancourt and Rose 2004; Campbell and Wallis 2002; Giese and Darby 2000). While long-wave UV radiation, including sun rays that reach the Earth's surface, can also inactivate bacteria in water (McLoughlin et al. 2004; Ubomba-Jaswa et al. 2009), shorter wavelengths ranging from 200 to 290 nm are the most effective for water disinfection (Fig. 1; Urban et al. 2011). Short-wave UV radiation is a well-established non-chemical disinfection technique that is considered to produce fewer DBPs and is effective in destroying microbial DNA (Kobayashi and Hatano 2005; Taghipour 2004).

Inactivation of viruses, protozoa and bacterial pathogens by UV radiation (UVR) has been demonstrated in many instances (Chatzisyneon et al. 2011; Eischeid et al. 2009; Hijnen et al. 2006). UVR enables oxidation processes to occur, known as photolysis, which generally results in bond cleavage of organic molecules (Blanksby and Ellison 1993). These processes may occur directly, thus inducing lysis in the target compounds resulting from the absorption of highly energetic photons, or indirectly, where an intermediary compound transfers the absorbed photon energy to the target molecule (Schwarzenbach et al. 2003).

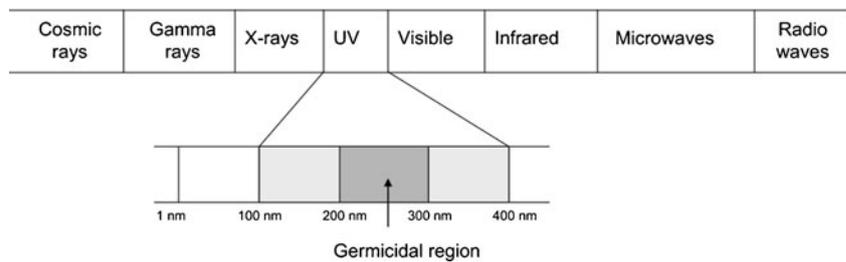
The majority of current UV disinfection has been applied in large water and wastewater treatment facilities as a disinfection step. However, UV disinfection can also be applied on a small scale, as evidenced by several bench-scale studies (Bolton and Linden 2003; Jolis et al. 2001; Linden et al. 2002) as well as the several UV disinfection devices available for home or office use sold by Siemens (www.siemens.com), LIT Technology (www.lit-uv.com) or Severn Trent Services (www.severntrentservices.com). Current large- and small-scale UV systems rely on mercury-vapour lamps. An alternative method to mercury-vapour lamps to produce UV radiation is light-emitting diodes (LEDs).

LEDs that emit wavelengths between 200 and 290 nm are amenable for point-of-use water treatment since they can be functional as a user-friendly, cost-effective and reliable method for reducing waterborne pathogens, including bacteria, viruses and protozoa, without producing DBPs (Huffman et al. 2002; Masschelein 2002; Vilhunen et al. 2009; Wolfe 1990). LEDs have numerous advantages over traditional mercury-vapour lamps that include resistance to shock and vibration, rapid light up time, high wall-plug efficiency and lifecycle, compact size, reduced heat production and emission of specific wavelength photons to produce radiation that is best absorbed by DNA. Although LEDs are more expensive than mercury-vapour lamps, breakthroughs in semiconductor research continue to dramatically reduce the cost of LEDs (Crawford et al. 2005).

Reports of UV-C LEDs used in water treatment include a study by Sandia National Laboratories (SNL) in which single 270-nm UV LEDs were used to treat 190 μL of non-turbid contaminated water (Crawford et al. 2005). SNL manufactured the LEDs and achieved a 1.89 log reduction in the *Escherichia coli* strain ATCC# 15597 through a dose of 3.6 mJ/cm^2 , which corresponded to a 10-min exposure time. A similar log reduction of 1.85 was seen for a 2.2 mJ/cm^2 dose (corresponding to a 6-min exposure time). When SNL irradiated a more sensitive strain of *E. coli* (ATCC# 23229), a 1.8- mJ/cm^2 dose was required to achieve a small reduction of log 0.7.

A second study that investigated the use of combining ten UV LEDs to inactivate *E. coli* in laboratory-prepared 25-ml water samples with little (1.45 NTU) to no (0.02 NTU) turbidity and were mixed during UV exposure achieved a three to

Fig. 1 Ultraviolet portion of the electromagnetic radiation spectrum



four log bacterial reduction in 5 min (Vilhunen et al. 2009). A third study combined over 30 UV-LEDs into one module to irradiate 30 ml of contaminated water samples and effectively inactivated the target microorganism, *Bacillus subtilis* spores, thus demonstrating the powerful disinfection potential of several LEDs used simultaneously (Kneissl et al. 2010). Interestingly, it is worth noting that a fourth study investigated the use of longer wave UV LEDs (LEDs that emitted wavelengths approximately 100 nm longer than UV-C LEDs) which successfully inactivated a number of waterborne pathogenic bacteria (Mori et al. 2007).

The research project described in this paper was designed to combine point-of-use disinfection with energy-efficient UV-C light-emitting diodes and to characterize the level of disinfection achieved by a single LED. To date, published data have not been found in the literature on the level of disinfection that can be achieved by employing an individual LED to reduce bacterial populations. The studies that are currently published on the efficacy of UV-LEDs to disinfect water all employ numerous LEDs for each treatment, thus potentially skewing the true ability of level of disinfection an LED can achieve. Therefore, it was determined that testing single LEDs to treat water with initial different contamination and water quality levels was warranted. The research described here included construction and testing of a UV LED laboratory bench unit to inactivate total coliform (TC) and *E. coli* bacteria. It employed the use of single UV-LEDs to test 100-mL water samples with a depth of nearly 6 cm, ranging in turbidity, from ultrapure water (approximately 0.02 NTU) to highly turbid wastewater effluent samples (with typical values of approximately 20 NTU (Alberta Transportation 2011; EPCOR 2011; Abdessemed et al. 2000). Significant differences between the published studies and this research include the exploration of treating samples with high levels of turbidity, treating samples with a large depth, no agitation of the sample and the use of single LEDs to treat larger volumes of water.

Experimental methods and materials

Water sources

Two water sample types were used to evaluate the disinfection efficacy of the UV LEDs under differing water quality

conditions. New water samples were prepared for each test. The first set of water samples were collected from the tertiary effluent of the City of Regina wastewater treatment plant (WWTP) that contained naturally occurring coliform bacteria. Samples were collected at 0800 hours, refrigerated in amber glass bottles and couriered to the lab for use in experiments before 1200 hours the same day. The WWTP serves a population of approximately 190,000 people and processes approximately 71×10^6 L/day. The wastewater is screened to remove large objects and pumped 5 km to the WWTP where suspended and dissolved solids are removed in the primary treatment process. A biological secondary system follows where bacteria metabolize organic materials in lagoons aerated by blowers. The tertiary treatment includes clarification and chemical additions. Samples were collected immediately downstream of this tertiary treatment process.

The second set of water samples were prepared in the lab and contained a pure culture of *E. coli* ATCC 25922 (MicroBiologics, Inc., St. Cloud, MN) prepared by culturing approximately 25 μ L of *E. coli* in a nutrient solution of either Brain Heart Infusion broth (Becton, Dickinson & Co., Sparks, MD) or Laurier broth (VWR Scientific, Edmonton, AB). This was subcultured overnight at 37 °C and added to sterile, autoclaved tap water. The two sets of water samples were exposed to UV radiation from LEDs to understand the differences between treating water from (1) a WWTP that had naturally occurring bacteria, as well as other microorganisms, dissolved and suspended solids, and other contaminants present and (2) artificially prepared water samples that contained only *E. coli* and no other contaminants or particles.

Serial dilutions of the laboratory prepared water samples were prepared and analysed to produce a calibration chart for optical density using a Novaspec spectrophotometer (Novaspec II Visible Spectrophotometer, Amersham Pharmacia Biotech). These dilutions were incubated on Nutrient Agar (Difco) to determine the relationship between cell number and optical density. From this preliminary test, the 10^{-8} dilution produced the desired initial number of cells to be irradiated based on the operational limits of the Colilert® detection equipment. Therefore, a 10^{-8} dilution was performed daily to prepare the inoculated sample water. All prepared samples were mixed using a magnetic stirrer and divided into 100-mL aliquots for experimentation.

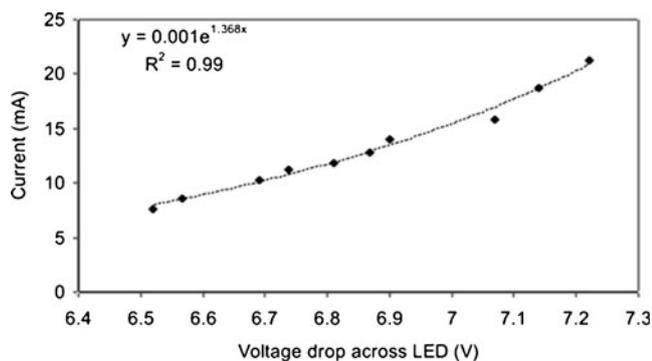


Fig. 2 Voltage and current characteristics of the UV-LEDs investigated

Laboratory bench design

Seven UVTOP 260 flat lens UV LEDs producing short-wave radiation at 260 nm were used for the research (Sensor Electronic Technology, Inc., Columbia, SC). The LEDs, approximately 10 mm in diameter, produce radiation with peak emission wavelengths of 260 ± 5 nm under standard operating conditions. The LEDs were connected in series to a current-limiting resistor and 9 V alkaline battery. Forward voltage of approximately 6.5 V and forward current of approximately 20 mA were applied to each diode. The average power supplied to each UV LED was in the range of 45 to 182 mW (average, 130 mW) with fluctuations corresponding to battery age. Duracell Pile Alkaline or Duracell ProCell Professional Alkaline 9 V batteries were used to power each LED on a MB-104 Circuit-Test breadboard.

Each UV LED was equipped with its own power supply for independent use and manipulation of operating conditions. Resistors ranging from 100 to 380 Ω were connected in series to the UV LED and 9 V battery to investigate the relationship between current and voltage across the systems. Resistors were used to limit the amount of current passing through the UV LEDs to protect the systems from overload. Using a digital multimeter (Circuit-Test DMR-1100), the voltage drop across the UV LED was recorded and the

current calculated using Ohm’s law. An exponential relationship was evident; when the LED is forward-biased, the current increased exponentially as the voltage increased (Fig. 2).

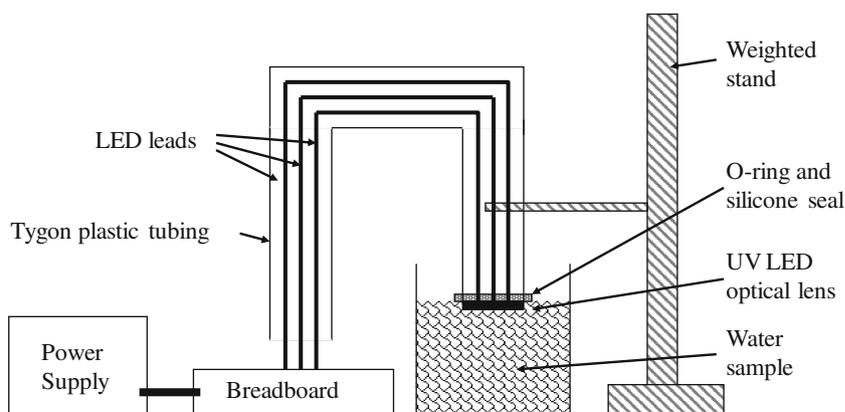
The UV-transparent optical lenses were hermetically sealed to the UV LED structures to allow for immersion in the water samples. The leads were also protected by extending these an extra 100 cm using 0.25 mm 30 AWG Blue Wire wrapping wire. The insulation of the wrapping wire was stripped by about 3 cm, placed in the slot of a wire-wrapping tool and turned clockwise until all of the wrapping wire was encircled around the lead to produce a snug fit. The loose ends of the wrapping wire were labeled to correspond with the UV LED’s anode or cathode. To avoid contact between each of the three UV-LED leads, each lead and its wire-wrapped extension was encased in fine plastic, shrink wrap tubing. A plastic o-ring was placed around the lip of each UV-LED to complete the water-tight seal. Each of the three extensions was then fed through an 80-cm length of Tygon tubing (VWR Scientific, Edmonton, AB). Using a weighted stand, the water-tight UV LED assembly was vertically immersed into the 100-mL water samples (Fig. 3).

Experimental design

Several aliquots of each of the two types of water samples [(1) tertiary samples from the WWTP and (2) artificially produced samples containing only *E. coli*] were exposed to UV-C radiation at 260 ± 5 nm in 100-mL Pyrex media bottles. Samples were dispensed using sterile syringes to avoid contamination. At the start of each experiment, the water-tight UV-LED assembly was lowered until the optical lens made contact with the water sample surface.

To investigate the impact of sample volume on disinfection efficiency, a series of preliminary experiments was conducted with volumes ranging from 10 to 100 mL. The results indicated that the 100-mL sample volume provided the most reproducible initial coliform bacteria concentrations and is a

Fig. 3 Schematic of laboratory experimental unit design



standard volume commonly used in water quality analysis, so this volume was used in all further experimentation.

Every experiment included both exposed (irradiated) and unexposed (control) samples to differentiate between UV LED-related bacterial death and natural death over the course of the experiment. Irradiation periods of between 20 and 50 min were chosen to determine UV LED disinfection efficiency on naturally occurring wastewater samples and artificial samples prepared with *E. coli* (Table 1).

Prior to starting experiments and between experiments, all test equipment was disinfected using 6 % sodium hypochlorite followed by double rinsing in tap water and double rinsing in laboratory grade reverse osmosis water. Aseptic technique was used at all times.

Bacterial enumeration

Samples were enumerated with Colilert® and are reported in most probable number (MPN) colony-forming units (cfu). The Colilert® system (IDEXX Laboratories, Inc., Westbrook, ME) is a standard laboratory test for the enumeration of TC and *E. coli* in water samples (Edberg et al. 1988, 1990, 1991). The Colilert® Quanti-Tray Enumeration Procedure described in the Colilert® Test Kit was followed for all enumeration activities. The Colilert® system was chosen since it can be used to monitor microbiological water quality in a variety of environments (Desmarais et al. 2002; Eckner 1998; Kinzelman et al. 2003; Muirhead et al. 2005). Enumeration of coliforms using Colilert® was expressed as the MPN of cfu’s to a maximum of 2,419.6 cfu. Where coliform amounts were likely to exceed this upper limit, samples were diluted prior to enumeration.

Statistical analysis

Statistical analyses were applied to Experiments 2a–2d, including three control samples for each, and using $\alpha=0.05$. Prior to statistical analysis, outliers were identified using Grubb’s test performed on GraphPad Prism version 4.03 for

Windows (San Diego, CA). The statistical tests chosen for this study take into account that the variances are not homogenous between the different irradiation treatments (Levene’s test), and that the data were normally distributed (Shapiro–Wilk test). Levene’s test for homogeneity of variances was performed on all irradiated samples and each exposure time (StatsDirect Version 2.6.2; Cheshire, UK). Because the results indicated significant variance based on irradiation period, data from each group of irradiated samples within each independent irradiation period were tested for non-normality using the Shapiro–Wilk test (StatsDirect). A Mann–Whitney *U* test (Simple Interactive Statistical Analysis online) was conducted to assess repeatability in each replicate. This non-parametric test can be used on a small number of independent samples where an assumption of equal variances is not required. Each test included 12 irradiated samples randomly divided into two groups (Wackerly et al. 2002); the analysis tested the hypothesis that there would be no significant difference between the medians of the two groups.

An unpaired Student’s *t* test was performed on control and irradiated samples to determine whether or not the UV LEDs were responsible for reducing microbial populations in the irradiated samples. StatsDirect software was used to calculate *t* values to test the null hypothesis that there were no significant differences between controls and treated samples (i.e. that the means of the two groups were equal).

A Kruskal–Wallis test using StatsDirect software was completed to compare the log reductions across the four irradiation periods to determine whether or not bacterial inactivation by UV LEDs was time-dependent. This test is non-parametric and compares the medians of more than two independent groups of data based on one independent variable (irradiation period) where homogeneous variances are not assumed. In this case, the difference in mean log reductions for the four irradiation periods (where the variable was irradiation period) was tested. The effect of irradiation time for treating contaminated water was also analysed through linear regression analysis (Microsoft Excel) to

Table 1 Experimental conditions using the Colilert® enumeration method

Experiment number	Water source	Irradiation period (minutes)
1a	WWTP	20
1c	WWTP	40
2a	Laboratory inoculant	20
2b	Laboratory inoculant	30
2c	Laboratory inoculant	40
2d	Laboratory inoculant	50

n=12, except for 1c where *n*=8

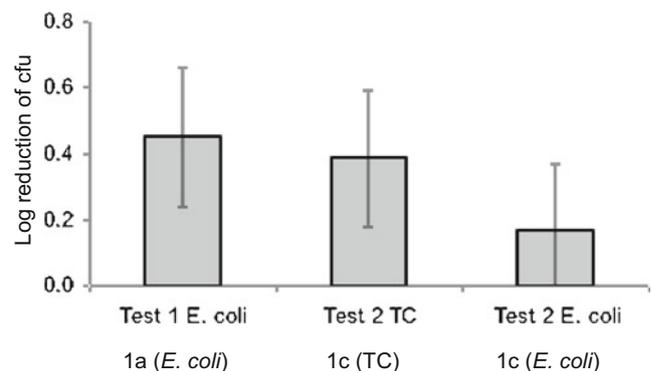


Fig. 4 Mean log cfu reductions and standard errors of TC and *E. coli* in WWTP samples

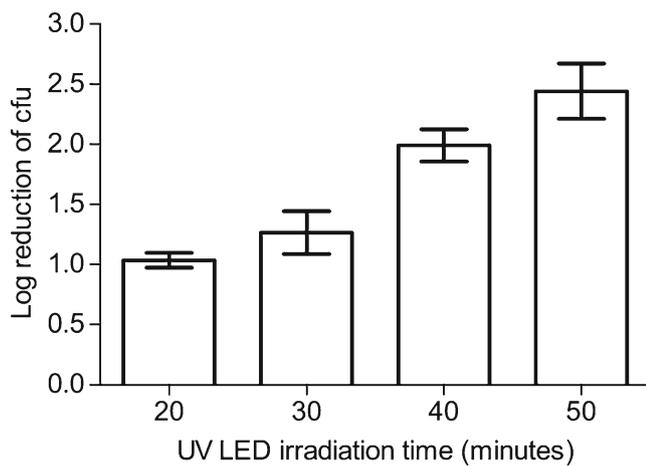


Fig. 5 Mean log cfu reductions and standard error of the mean of *E. coli* laboratory inoculated samples

assess the relationship between irradiation period and log reduction.

Results and discussion

The results of irradiation of two water sample types indicate that water quality has a significant impact on disinfection efficiency. Using the Colilert® quantification method, data regarding initial and final concentrations of TC and *E. coli* in irradiated and control samples were collected.

From the WWTP water samples, data were highly inconsistent requiring several outliers to be removed from the analysis. The variability of initial sample quality and high sample turbidity were considered primarily responsible for the WWTP result inconsistencies (Qualls and Johnson 1983; Jolis et al. 2001). Results from the 20- and 40-min irradiation period experiments with WWTP effluent samples indicated minimal reduction for bacteria concentrations, measured in cfu, following UV LED treatment (Fig. 4).

Although small bacterial reductions were observed, the standard errors were large. This suggests that the experiment was not repeatable. Furthermore, the data for experiment 1a TC were omitted due to the lack of repeatability in the associated control samples. From these results, it was concluded

that high turbidity has a significant and detrimental impact on UV LED disinfection efficiency and UV LED disinfection is not a reliable method of treatment for highly turbid water. Consequently, future water treatment designs comprised of UV LEDs require pretreatment of highly turbid water to reduce interfering materials. This agrees with other studies of UV treatment of water samples containing elevated levels of turbidity. Qualls and Johnson (1983) found that suspended particles in wastewater can both protect coliform bacteria from UV radiation as well as absorb and scatter the UV light, and Jolis et al. (2001) found that particles larger than 7 µm played a significant role in shielding bacteria from UV light.

In experiments using the non-turbid laboratory inoculated water, the results showed significant and consistent reduction of *E. coli*. Clear water inoculated with *E. coli* and irradiated between 20 and 50 min achieved between 1- and 2.5-log reductions (Fig. 5) with high repeatability. The improved efficiency was attributed primarily to the low turbidity water conditions.

Results from the WWTP and *E. coli* control experiments indicated minimal variability from initial conditions (Table 2). Data from instances in which serial dilutions were performed are reported as averages. TC results from experiment 1a were omitted since data from only one control experiments indicated quantifiable coliform levels.

The Mann–Whitney *U* test to assess repeatability within the 12 irradiated replicates from each exposure time supported the null hypothesis that there was no difference between the two randomly subdivided groups of data. Levene’s test to assess homogeneity in variances among log reductions due to different exposure times indicated statistically significant variability ($P < 0.05$) both between irradiation periods and between control and irradiated samples. Based on these results, all subsequent analyses did not assume homogeneity of variance.

The Shapiro–Wilk test was performed on all irradiated samples from each exposure time and identified no evidence of non-normality at ≤ 0.05 . Therefore, it was concluded that the data were normally distributed, as required to use an unpaired *t* test.

Further statistical analyses of the irradiated *E. coli* samples, including the Student’s *t* test and Kruskal–Wallis test,

Table 2 Data from control replicates for TC and *E. coli* enumeration (log cfu/100 ml)

Control number	Experiment number						
	1a— <i>E. coli</i>	1c—TC	1c— <i>E. coli</i>	2a	2b	2c	2d
1	2.0	3.5	2.4	3.4	3.4	3.0	3.5
2	2.3	3.6	2.2	3.4	3.4	3.1	3.5
3	2.1	3.7	2.0	3.4	3.4	3.2	3.6
Mean	2.1	3.6	2.2	3.4	3.4	3.1	3.5
Standard error	0.1	0.1	0.1	0.0	0.0	0.1	0.0

indicated that the UV LEDs had a significant effect on cell concentrations ($P < 0.0001$) and confirmed that cell inactivation is time-dependent. While this study only achieved a maximum of 2.5 log reduction in bacterial populations and a previously noted study involving UV LEDs inactivated nearly twice the number of bacteria in one tenth of the time (Vilhunen et al. 2009), this significant difference is due to the use of combining several LEDs to irradiate a smaller volume of water which was agitated during the entire treatment time (thus allowing more of the water sample to be exposed to the radiation). Therefore, the data support that future water treatment designs involving UV LEDs must maximize the UV dose application and ensure the entire water sample is exposed in order to effectively reduce pathogenic bacteria to a safe level of human consumption.

Conclusions

Overall, the results of UV LED disinfection for turbid wastewater samples (experiments 1a and 1c) indicate small but measurable reductions in coliform concentrations. However, variability in the results and large standard deviations about the mean make it difficult to conclusively determine the effect of UV irradiation time on coliform reductions in such turbid water samples. Turbidity, which results from the presence of minute aggregates, reduces UV radiation access to the target microorganisms. UV transmittance of water, which is a measure of how well UV radiation penetrates and inactivates bacteria within a water column, decreases with increasing levels of turbidity and dissolved salts. The percentage of UV transmittance, which is a function of the amount of UV radiation absorbed in the water sample, is frequently used to determine the suitability of UV treatment, and decreases when the source water quality decreases. Therefore, further research is required into characterizing the extent to which UV LEDs can effectively treat water with varying levels of turbidity.

Promising results were demonstrated in water samples lacking turbidity. This is due to the lack of particle aggregation that would shield and protect microorganisms from the inactivating effects of UV light. In experiments with laboratory inoculated water samples (experiments 2a through 2d), consistent and significant reductions in microbial concentrations were achieved. P values of < 0.0001 indicated significant differences between irradiated and control samples (unpaired t test). There were significant differences as a function of the length of radiation exposure time (Kruskal–Wallis test).

The bench-top UV LED apparatus was easily assembled from readily available parts and required little maintenance. It was powered by 9-V household batteries, simple to use and durable and versatile in its design. These features make

UV LED disinfection systems well suited for rural or isolated environments that necessitate simple, small-scale, point-of-use and mobile devices. With further optimization using several LEDs per system and more powerful LEDs, UV LED water treatment systems offer a promising point-of-use technique for the future.

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