Water Research X 26 (2025) 100285

Contents lists available at ScienceDirect

Water Research X

journal homepage: www.sciencedirect.com/journal/water-research-x

Comprehensive evaluation of UV inactivation of *E. coli* using multiple gene targets and real-time quantitative PCR

Natasha Rahman [a], Subba Rao Chaganti [b,1], Rajesh Seth [a,b,*], Daniel D. Heath [b,c]

[a] Department of Civil & Environmental Engineering, University of Windsor, 401Sunset Avenue, Windsor ON N9B 3P4, Canada
[b] Great Lakes Institute for Environmental Research, University of Windsor, Windsor ON N9B 3P4, Canada
[c] Department of Integrative Biology, University of Windsor, Windsor ON N9B 3P4, Canada

ARTICLE INFO

Keywords:
Wastewater disinfection
UV inactivation
E. coli enumeration
Real-time quantitative
PCR
Culture-independent pPCR

ABSTRACT

UV disinfection is extensively used for wastewater disinfection and disinfection efficiency is commonly monitored using culture-based enumeration of *E. coli*. While culture-independent real-time quantitative polymerase chain reaction (qPCR) based methods are attractive due to faster turnaround and easier application, previous attempts with qPCR to monitor disinfection have been unsuccessful. In this study, the effect of UV irradiation on a pure *E. coli* culture was examined in collimated beam (CB) experiments and monitored using both a culturing technique and DNA damage quantified using both short amplicon (SA; <~200 bp) qPCR and longer amplicon (LA; ~500-bp) qPCR. The results, covering a UV dose range of 0 - 20 mJ/cm^2 commonly used for wastewater disinfection, indicate a correlation between DNA gene damage quantified by both SA- and LA-qPCR and the decline in *E. coli* observed through culture-based methods. This demonstrates the potential of qPCR to serve as rapid alternative for monitoring wastewater disinfection efficacy. Furthermore, LA-qPCR was observed to be more sensitive than SA-qPCR. The results using LA-qPCR also revealed that UV exposure caused widespread and indiscriminate damage to *E. coli*'s genome, which is considered critical for its function and survival. The combined effect of UV on *E. coli*'s ability to function, grow or repair damage is suggested as the reason for the decline in culturability observed.

Introduction

The threat of pathogenic contamination of water resources from human fecal matter in wastewater treatment plant (WWTP) effluents, combined sewer overflow (CSO) and sanitary sewer overflow (SSO) is well-established (WHO, 2003; McLellan et al., 2007; Templar et al., 2016). To manage this pollution, ultraviolet (UV) disinfection of WWTP effluents, before discharge into receiving waters, is being extensively applied all over the world. UV light is a physical disinfectant, and its mechanism differs from that of chemical disinfectants. UV disinfection generally maintains cellular integrity as UV-C light targets DNA, forming pyrimidine dimers in the helical structure that causes DNA to lose its functionality (Xu et al., 2018).

Microbial water quality is currently commonly assessed by quantifying fecal indicator bacteria (FIB), such as *E. coli*, using standard microbiological methods which rely on culturing *E. coli*. Culture-independent real-time quantitative polymerase chain reaction (qPCR) based methods are gaining popularity in monitoring due to faster turnaround and easier application for direct monitoring of waterborne pathogens (and microbial markers of fecal pollution) from various human and animal sources (Bower et al., 2005; Shahraki et al., 2019a). A draft qPCR-based method for monitoring *E. coli* is in the final stages of approval by USEPA (Sivaganesan et al., 2019). Similar to routine water quality monitoring, wastewater disinfection efficacy is currently measured using FIB such as *E. coli*, again using culture-based methods. Efforts to apply qPCR methods for such purposes have been fraught with many challenges. qPCR-based methods for quantifying bacteria involves amplifying a small DNA amplicon (<200-bp) of the target gene for reliable and efficient PCR (Chern et al., 2011; Xu et al., 2018; Shahraki et al., 2019a); however, qPCR methods using DNA cannot distinguish between viable and dead cells (Nocker et al., 2007; Weigel et al., 2017). Pre-treatment with propidium monoazide (PMA), a photo-activated

* Corresponding author.
E-mail address: rseth@uwindsor.ca (R. Seth).
[1] Current address: Cooperative Institute for Great Lakes Research, School for Environment and Sustainability, University of Michigan 4840 South State Street Ann Arbor, MI 48108 United States.

https://doi.org/10.1016/j.wroa.2024.100285
Received 30 July 2024; Received in revised form 17 November 2024; Accepted 20 November 2024
Available online 22 November 2024

DNA intercalating dye, has been used to bind to and remove DNA from membrane-compromised (lysed) cells before PCR analysis. However, UV disinfection does not damage the membrane; thus, the signal from dead cells cannot be eliminated by PMA treatment (Nocker et al., 2007; Ho et al., 2016). An alternative would be to develop a correlation between UV damage quantified using qPCR and cell mortality using culture-based methods. Correlation with culture-based methods is beneficial as current recreational water quality criteria values and disinfection targets for *E. coli* use culture-based enumeration and are derived based on epidemiological studies showing positive relationships with the occurrence of gastrointestinal illness (Dufour, 1984). However, the short PCR amplicons currently targeted for monitoring have been shown to have poor sensitivity in detecting DNA damage by UV, and thus may not correlate with culture-based methods as expected (Nocker et al., 2007; Xu et al., 2018).

One option to improve sensitivity may be to use longer amplicons (>200-bp) to quantify UV damage. Increases in the detection of UV damage using longer amplicon (LA) qPCR was demonstrated by Ho et al. (2016). A previous study that examined UV damage detection with qPCR used various amplicon lengths (108 – 1764 bp) and showed that amplicon lengths of approximately 500-bp was a good compromise between damage detection and reliable PCR amplification (Ho et al., 2016). However, correlation between qPCR with longer amplicon length and culture-based enumeration of *E. coli* has not yet been examined.

Past qPCR assay methods for *E. coli* monitoring have focussed on genes based on the abundance (high copy number) and specificity of those targets, and not on their importance for cell survival (Chern et al., 2011). The effectiveness of UV as a disinfectant would be better reflected by its ability to damage genes that are critical for cell function and survival, such as those involved in UV repair, cell division and cell metabolism.

In this study, the effect of UV irradiation on a pure *E. coli* culture was examined by exposing cultures to UV in collimated beam (CB) experiments and calculating the efficiency of treatment through both a culturing technique and qPCR. *E. coli* log-reduction using culture-based methods were compared against DNA damage quantified by short amplicon (<200-bp) qPCR (SA-qPCR) and long amplicon (~500-bp) qPCR (LA-qPCR) for the commonly used rRNA and *uidA* targets. Gene targets which were considered critical for *E. coli* function and survival were identified based on the literature and a comprehensive evaluation of the ability of UV to damage these genes was quantified using LA-qPCR. Finally, we provide recommendations on the optimal methodology for quantifying *E. coli* reduction with UV using qPCR-based methods.

Material and methods

E. coli culture

E. coli ATCC® 8739™ cells were obtained in six 300-µL glycerol stock vials. A single vial was added to 20 mL sterile nutrient broth (Becton Dickinson) and incubated at 37 °C for 20 h. Sterile nutrient broth agar plates were prepared with addition of 1.5 % agar. Cultured *E.coli* cells were streaked on the plates to separate colonies. A single isolated colony from the agar plates was inoculated into 40 mL sterile nutrient broth and incubated for 16 – 20 h at 37 °C. The culture was then centrifuged for 20 min at 4000 rpm. The nutrient broth supernatant was removed and the settled cells were re-suspended in 20 mL sterile phosphate-buffered saline (PBS). Cell concentration was estimated using UV–vis spectrophotometry according to an earlier version of the USEPA Draft Method C. For developing standard curves, *E. coli* cells were serially diluted with sterile PBS to 2 – 6 log cells per mL, and aliquots in triplicate from each dilution were frozen at −20 °C until DNA extraction. For UV exposure experiments, *E. coli* cells were diluted in 1 L sterile PBS to approximately 7-log cells per mL.

UV exposure experiments

UV exposure experiments were conducted with a low-pressure collimated beam apparatus (Trojan Technologies, Canada) according to the manufacturer's standardized protocols. Intensity was measured at the sample surface with the IL1700 radiometer (Trojan Technologies, Canada). For each sample, 53 mL *E. coli* suspension was held in a clear glass petri dish with a stir bar. 3 mL was taken out for UVT readings using Real Tech Water's UV254 portable meter with split sense technology. Based on the UVT readings, we calculated the time required for the desired dose. Samples were exposed to five UV doses: 5, 10, 20, 50 and 100 mJ/cm^2. Each dose trial was performed in triplicate. Calculations for the dose accounted for the petri dish divergence, and reflection factors as per Bolton & Linden (2003). After exposure, each sample was split into aliquots for culturing and DNA extraction. Six 1.75 mL aliquots were frozen at −20 °C for DNA extraction, and the remaining sample was used for culturing.

Culture-dependent method

IDEXX Colilert Quanti-Trays®/2000 were used as the culture-based method in this study. The Colilert test is able to simultaneously detect or quantify both total coliform and *E. coli* up to 2419 MPN/100 mL, with results in 24 h. The test is approved by the USEPA and included in Standard Methods for Examination of Water and Wastewater (APHA et al., 2023). Each sample, UV-exposed and non-exposed, was serially diluted with sterile PBS to a concentration that would be within the range of this method.

DNA extraction

Frozen 1.75 mL samples were thawed at room temperature and centrifuged at 10,000 rpm for 15 min. The supernatant was removed and 400 µL sterile PBS was added. For each sample, 500 µL of 1.0 mm sterile glass beads in 400 µL sucrose lysis buffer was added. Sucrose lysis buffer was made according to Shahraki et al. (2019a). After adding lysis buffer, samples were subjected to homogenization using the Mini-beadbeater-16 (Lab Services BV, Nederland) for 40 s three times at an intensity of 3450 oscillations/min, after which 100 µL of 1 % SDS and 50 µL 20 mg/mL lysozyme (Sigma-Aldrich, USA) was added to each tube. Samples were then incubated at 37 °C on a shaker overnight. The next day, 2 µL of 20 mg/mL proteinase K (Thermo Scientific, USA) were added, and samples were again incubated at 37 °C on a shaker overnight. Finally, the next morning, proteinase K was deactivated in a 95 °C water bath for 10 min. The digest from each tube was plated in a 96-well extraction plate, and DNA was extracted by magnetic bead robotic extraction using an automated liquid handling platform (Tecan Freedom Evo150 Liquid Handling Platform, Perkin Elmer), and DNA was eluted into 50 µL of TE buffer according to Shahraki et al. (2019). Extracts were kept in the freezer at −20 °C until qPCR analysis. Nuclease free water samples were used as control to verify cross contamination during DNA extraction. DNA purity was verified using NanoDrop UV–Visible spectrophotometer (ThermoFisher Scientific) at 260/280 nm.

Gene target selection: short and long amplicon comparison

Short and long amplicon targets for the 23S rRNA and *uidA* genes were used in the study (SI; Table S1). The 23S rRNA gene short amplicon was selected as it is the target used in the USEPA Draft Method C for monitoring *E. coli* in recreational waters (Chern et al., 2011; Sivaganesan et al., 2019). The *uidA* gene has also been used for its specificity and its product is a frequently used enzymatic marker for *E. coli* identification (Chern et al., 2011). Long amplicons (~500-bp) of both genes were identified and selected in the study to compare the effect of increasing amplicon size on the DNA damage recorded.

Gene target selection: metabolism, cell division and UV repair

The list of the twelve essential precursors from the core metabolism of *E. coli* identified by Noor et al. (2010) and their location on the core metabolism map of *E. coli* from the BiGG Model are included in Fig. 1. The pathways on this map were used to identify nine core metabolism genes (SI; Table S2, with their function listed in Table S3) at important intersections in the map that, if damaged, would reduce or eliminate the production of one or more of the twelve essential metabolic precursors. The cell division pathway outlined in Ouellette et al. (2015) was also used in a similar manner and two cell division gene targets were chosen (SI; Table S2). Bacterial UV repair pathways from several studies (Reuven et al., 1999; Goosen and Moolenaar, 2008; Janion, 2008) were used to select two candidate UV repair gene targets, also shown in SI (Table S2). For all of the selected genes, PCR primers were designed to generate long (~500-bp) amplicon lengths.

We also included long amplicon PCR primer sets for two additional gene targets (16S rRNA and *gadA*) (SI; Table S4), that are reported in the literature for monitoring *E. coli*, for comparison of responses with the several new gene targets used in this study. 16S rRNA gene regions are commonly used for bacterial monitoring and community analyses due to the presence of multiple copies in almost all bacteria resulting in straightforward PCR amplification. The *gadA* gene was used by Zhang et al. (2015) and Xu et al. (2018) for bacterial quantification due to its ability to help bacteria survive acidic environments, such as the human stomach.

Primer design

Primers were designed using Geneious 10.1 software. The sequences for target genes found in various *E. coli* strains were downloaded from the NCBI database and aligned with those of other common bacteria of concern and bacterial closely related to *E. coli* (*Salmonella, Shigella, Citrobacter, Klebsiella, Enterobacter*, etc.). Primers were designed for regions specific to *E. coli*.

Primer validation

Selected primers were first tested on the NCBI BLAST database to determine the potential for PCR cross-amplification with other microorganisms. Subsequently, standard desalting primers were obtained from Integrated DNA technologies (IDT) for all target genes and primers were tested using PCR and gel electrophoresis. PCR was conducted with a 25 μL reaction volume, containing: 16.4 μL nuclease-free water, 2.5 μL 10x Taq buffer, 0.5 μL of 10 mM DNTPs, 3.5 μL 20 mM $MgSO_4$, 0.5 μL of each 10 mM Forward- and Reverse-primer, 0.1 μL Taq polymerase, and 1 μL *E. coli* genomic DNA template. A PCR negative control (nuclease-free water instead of DNA template was added to master mix) was included. All reactions were conducted in duplicate. The program started with an initial incubation at 95 °C for 1 min, followed by thirty-five cycles of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 40s. The last step included one cycle of 72 °C for 7 min to complete elongation. The PCR products were then run on 2 % agarose gels and the product size was estimated to confirm that the designed primers only produced a single product and all products were of the expected length.

qPCR

First, qPCR was used to create standard curves for each primer, developing a standard curve to determine the relationship between C_q values and the log cell numbers. Afterwards, DNA extracts from the UV exposure experiments were used with each primer set. Due to the large number of samples and primer pairs, 384-well qPCR plates were utilized with a 9-μL reaction volume. To prepare the 384-well plate, four 96-well plates were prepared with 12-μL reactions containing: 4.4 μL nuclease-free water, 6 μL 2x PowerUp™ SYBR® Green Master Mix (Applied Biosystems), 0.3 μL of each 10-mM F- and R-primers, and 1 μL DNA template. Then 9 μL was transferred from the four 96-well plates into a single 384-well plate. The QuantStudio™ 12 K Flex System (ThermoFisher Scientific) ran the following program: initial step at 95 °C for 20 s, followed by forty cycles of 95 °C for 10 s and 60 °C for 60s. In addition, a

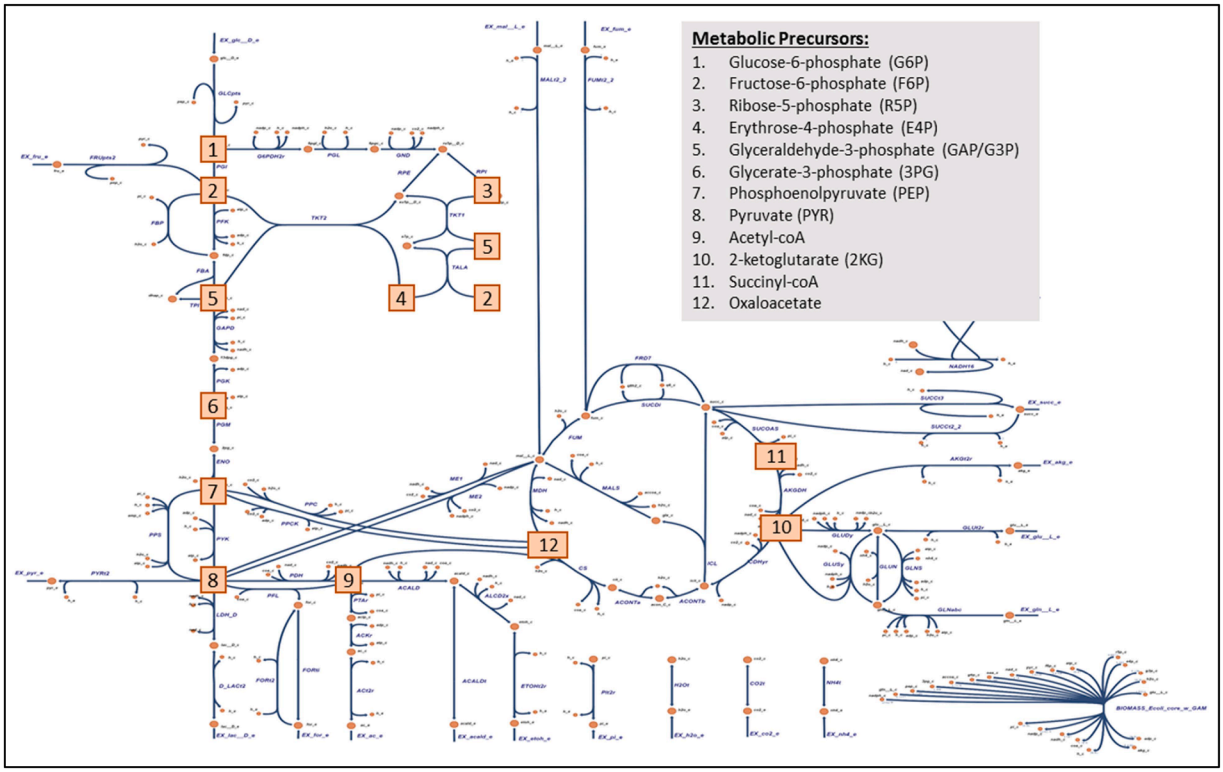

Fig. 1. BiGG Model core metabolism map with essential precursors (Original map obtained from the BiGG Model, http://bigg.ucsd.edu/models/e_coli_core).

denature step (melt curve) was included and validated for single peak for each target (Figures S13 - S31). Nuclease free water was added instead of DNA for the no template controls (NTC). All samples and corresponding NTC were run in triplicates. To validate the variation between the runs uidA assay standards were run on multiple plates and no significant variation was noticed between runs. Cq values were determined using QuantStudio design and analysis software v.2.0 through automated baseline correction, threshold setting in the exponential phase, and detecting the cycle number at which fluorescence exceeds this threshold..

Standard curves

An optimized qPCR assay can be described as one which is linear, efficient and consistent across replicates. Amplification efficiency is expected to suffer with increasing amplicon lengths. Correlation coefficients (R^2) greater or equal to 0.85 and amplification efficiencies between 85 % and 105 % were considered to be adequate for an acceptable qPCR assay enumeration of *E. coli* in the current study. Gene targets used in the current study included short and long amplicons for *23S* rRNA gene (SA *23S*) and *uidA* gene (SA *uidA*) (SI; Table S1), long amplicons for nine metabolic, 2 cell division and 2 UV repair longer amplicon gene targets in *E. coli* (SI; Table S2) and long amplicons for two additional gene targets (*gadA* and 16S rRNA *rrsA*) that are reported in the literature for monitoring *E. coli* (SI; Table S4). Standard curves for all gene targets used in the current study, as presented in the SI (Figures S3-S8) met the above criteria, except for umuC, pck, ptsG and ftsZ (SI; Table 5). The amplification efficiencies for umuC (84 %), pck and ptsG (83 %) were considered close enough to 85 % and retained, while ftsZ (77 %) was excluded from further consideration.

Statistical analysis was conducted using RStudio (v2024.09.0) with R (v4.0.1). For each target gene, the R^2 values were calculated from the linear regression of Cq values against log10-transformed *E. coli* cell concentrations, providing an indication of the fit quality. Slope and intercept values were derived from the standard curve equation. Amplification efficiency of the qPCR assays for each gene target was assessed for sensitivity in accordance with MIQE guidelines (Bustin et al., 2009).

To determine the Limit of Detection (LOD), dose-response models were fitted to detection data to estimate the lowest concentration detectable with 95 % probability (SI; Table S5). For the Limit of Quantification (LOQ), only standard concentrations with 100 % detection rates and target that can be accurately quantified with coefficient of variation (CV) below a threshold of $\leq$35 % were included to ensure high reliability (SI; Table S5). The relationship between the coefficient of variation (CV) of Cq values and starting quantities was modeled using several candidate approaches: an exponential decay model, a linear model, and polynomial models from second to sixth order. The model with the lowest residual standard error was selected to determine the LOQ. Outliers were identified by detecting Cq values that deviated >10 % from the median Cq value for each standard concentration. Suspected outliers were reviewed carefully, and if confirmed, they were removed, followed by reanalysis of the data. R packages ggplot2 and drc were used for data visualization and dose-response curve modeling, respectively. The LOD and LOQ for each gene target qPCR assay were estimated using the qPCR_LOD_calculator R script (Klymus et al., 2019). UV induced gene damage was assessed by calculating the change in Cq (ΔCq) value between pre and post exposure of UV dose (0–100 mJ/cm^2). The linear regression models were fitted for each gene and plots were generated showing the dose dependent gene damage with error bars and R-squared values using the ggplot2 and dplyr packages in RStudio.

Results and discussion

UV inactivation of E. coli: culture-based evaluation

Dose response experiments were conducted for *E. coli* over a UV dose range of 0 to 100 mJ/cm^2. For *E. coli* concentrations of approximately 7.0-log/mL and UV dose range up to 20 mJ/cm^2, which is commonly applied in wastewater disinfection (Hijnen et al., 2006), the culture-based method showed a steady decline of approximately a 5.5-log reduction in culturable cells after which the curve began to plateau (SI; Figure S9). With most of the culturable cells gone, the plateauing with further increase in UV dosages to 100 mJ/cm^2 is not surprising. Xu et al. (2018) have also previously reported approximately 6.0-log reduction at UV dosages up to 20 mJ/cm^2 followed by similar plateauing.

UV inactivation of E. coli: qPCR-based evaluation with short and long amplicon

The dose-response of UV on *E. coli* was monitored using culture-independent qPCR using short amplicon (SA) 23S gene target, recommended by USEPA in Draft Method C for environmental monitoring of *E. coli*, and the results are presented in Fig. 2A. With increasing UV dose, C_q values are seen to continually increase, indicating progressive damage to the gene target (Fig. 2A). Further linear regression plots between UV dose and ΔCq values showcased linear relationship with increasing UV dose with an R^2 values in the range of 0.9 to 0.96 for all the genes measured using long amplicons and 0.79 to 0.81 for short amplicons (Figure S32). Higher ΔCq variations was noticed with long amplicons compared to short amplicons, which is similar to the trend reported for antibiotic resistance genes when exposed with UV dose (Chang et al., 2017; Choi et al., 2021; He et al., 2019; Yoon et al., 2018). While the culture-based dose-response curve plateaus after UV dose of 20 mJ/cm^2 (SI; Figure S9), Fig. 2A shows no such plateauing with the C_q values till the maximum applied UV dose of 100 mJ/cm^2. Increasing C_q values in qPCR are only indicative of increasing DNA damage but do not distinguish between viable and dead cells. This suggests that a limited amount of DNA damage may be causing most *E. coli* cells to lose culturability. However, beyond the UV dose of 20 mJ/cm^2, there is still a lot of copies of the target DNA left that are yet to be damaged, explaining the increasing C_q values.

Correlation between DNA damage using qPCR (quantified as change in C_q values, or ΔC_q) with culture-based enumeration for *E. coli* would be useful for the development of alternate qPCR approaches for monitoring disinfection. Such a correlation, based on data presented in SI (Figures S9) and 2A for SA 23S gene target is presented in Fig. 2B. Significant difference in C_q values at UV doses typically used for wastewater disinfection (0 – 20 mJ/cm^2) have not been previously reported with SA-qPCR (Xu et al., 2018; Chern et al., 2014), leading Chern et al. (2014) to conclude that SA-qPCR may not be suitable for monitoring efficacy of wastewater disinfection. The ΔC_q values observed for UV dose increase from 0 to 20 mJ/cm^2, corresponding to 5.5-log reduction in culturable *E. coli,* were 1.30 for 23S (Fig. 2B). More importantly, the C_q values at UV dose of 10 and 20 mJ/cm^2, while not significantly different from each other, were significantly different from those at the beginning (no UV exposure) ($p < 0.05$ for 10 mJ/cm^2; $p < 0.005$ for 20 mJ/cm^2), with excellent correlation ($R^2 = 0.97$, $p << 0.005$) between ΔC_q values and log reduction in culturable *E. coli* (Fig. 2B). The improvement may be attributed to advancement in qPCR-based technology and instrumentation. Similar results were obtained with the SA *uidA* gene target (SI; Figure S10). This is exciting as, contrary to the conclusion by Chern et al. (2014), our findings suggest that, while further improvement in sensitivity may be desirable, DNA SA-qPCR could be used to monitor the efficacy of wastewater disinfection over the range of correlation.

The dose-response of UV on *E. coli* was further monitored using long

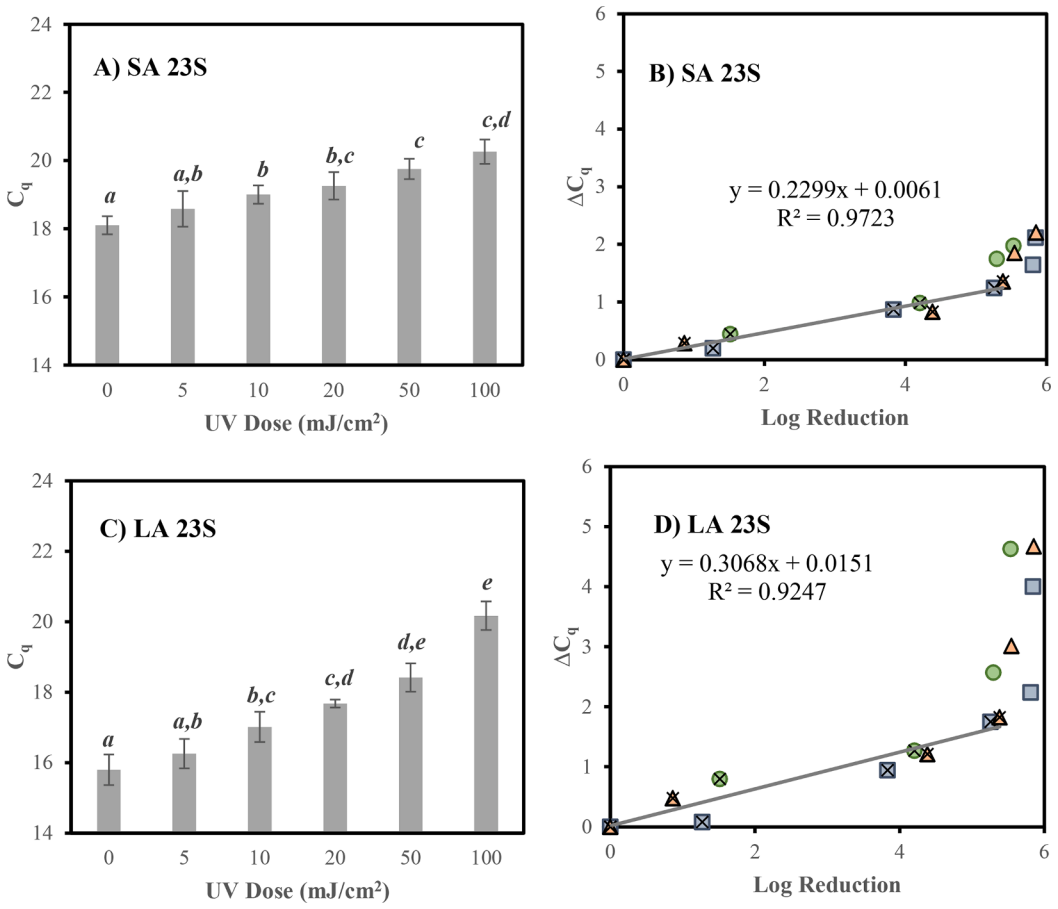

Fig. 2. qPCR-based responses (C_q) as a function of UV dose for short amplicon (SA) 23S (Fig. A) and long amplicon (LA) 23S (Fig. C) were used to calculate response changes (ΔC_q) as a function of UV dose, as presented in Figure S32 (SI). Different letters (a – e) indicate statistically significant differences ($p < 0.05$). Log reductions of *E. coli* using culture-based method as a function of UV dose were obtained using Figure S9 (SI). Response changes (ΔC_q) were correlated against *E. coli* log reductions for SA 23S (Fig. B) and LA 23S (Fig. D). Triangles, circles and squares represent replicates. Data up to a UV dose of 20 mJ/cm^2 (marked with an X) were used for the linear regressions presented.

amplicon (LA) 23S gene target (Table S1), and the results are presented in Fig. 2C. Similar to results with SA 23S, excellent correlation (R^2 = 0.92, p <<0.005) between ΔC_q values and log reduction in culturable *E. coli* was observed for LA 23S (Fig. 2D), and C_q values with LA 23S were also significantly different from those at the beginning (no UV exposure) ($p < 0.05$ for 10 mJ/cm^2; $p < 0.005$ for 20 mJ/cm^2). Further, the ΔC_q value observed for UV dose increase from 0 to 20 mJ/cm^2, corresponding to 5.5-log reduction in culturable *E. coli,* was 1.79 for LA 23S (Fig. 2D), which corresponds to ~38 % increase over that obtained with SA 23S. The results suggest that LA-qPCR can also be used to monitor the efficacy of wastewater disinfection over the range of observed correlation, and offer better sensitivity as compared to SA-qPCR. No previous studies correlating DNA damage using LA-qPCR with culture-based enumeration for *E. coli* could be found.

UV inactivation of E. coli: impact on functioning, growth, and repair

Cell Functioning: Long amplicon (~500 bp) targets for nine gene located at important intersections in the production pathways of twelve essential precursors from the core metabolism of *E. coli* (Fig. 1) were identified and their primers designed (Table S2). These genes, if damaged, would reduce or eliminate the production of one or more of the twelve essential metabolic precursors. The C_q response with increasing UV dose for two core metabolic gene targets and the correlation between DNA damage using qPCR with culture-based enumeration for *E. coli* are presented in Fig. 3. Correlations for the remaining seven are presented in the SI (Figure S11). The results obtained with all nine genes were very similar to those for LA 23S. The ΔC_q value observed for UV dose increase from 0 to 20 mJ/cm^2, corresponding to a 5.5-log reduction in culturable *E. coli,* ranged from 1.75 to 1.96 for nine genes, and were not statistically different from that observed with LA 23S. Good correlation (R^2 between 0.83 to 0.94; p <<0.005) between ΔC_q values for the gene targets and log reduction in culturable *E. coli* was also observed (Fig. 3& Figure S11). These results show that exposure to UV causes similar and widespread damage to important genes involved in the production pathways of twelve essential precursors essential for the core metabolism of *E. coli*. Correlation between DNA damage to all nine genes with reduction in culturable *E. coli* suggests that UV impact on the core metabolism may in part be responsible for the reductions observed.

Cell Growth: Net growth or reduction in bacterial cell numbers is the difference between actual growth by division and cell death or decay. Long amplicon (~500 bp) targets in *E. coli* for two important genes in bacterial cell division or growth pathways from the literature were identified and their primers designed (Table S2) but one (ftsZ) was dropped from further consideration due to lower than desired amplification efficiency. The C_q response with increasing UV dose for the remaining cell division gene target and the correlation between DNA damage using qPCR with culture-based enumeration for *E. coli* are presented in Fig. 4. The results obtained were similar to those for LA 23S. The ΔC_q values observed for UV dose increase from 0 to 20 mJ/cm^2 for *ftsQ* was 2.35 (Fig. 4), which was higher and statistically significant from that for LA 23S as well as other LA gene targets (metabolic and UV repair) in Table S2. Good correlation (R^2 = 0.88; p <<0.005) between

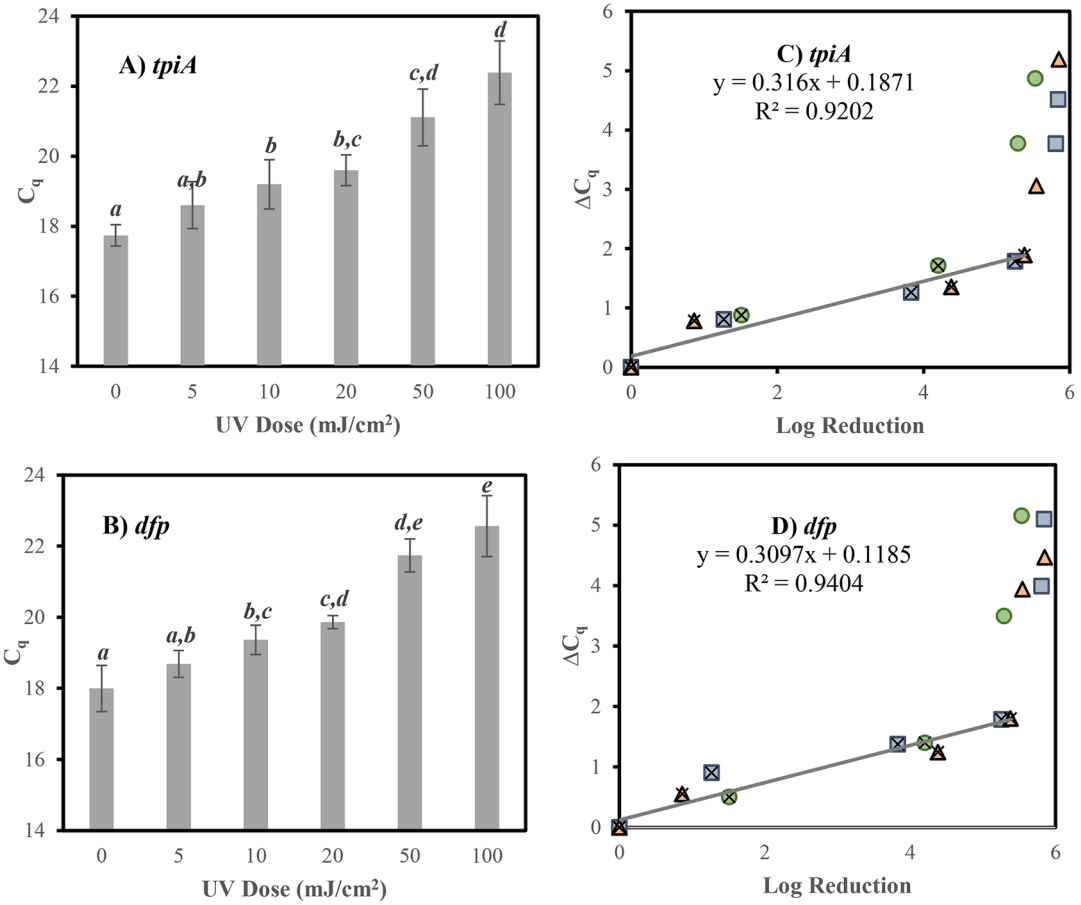

Fig. 3. qPCR-based responses (C_q) as a function of UV dose for two core metabolic gene targets (Fig. A: *tpiA*; Fig. B: *dfp*) were used to calculate response changes (ΔC_q) as a function of UV dose, as presented in Figure S32 (SI). Different letters (a – e) indicate statistically significant differences ($p < 0.05$). Log reductions of *E. coli* using culture-based method as a function of UV dose were obtained using Figure S9 (SI). Response changes (ΔC_q) were correlated against *E. coli* log reductions for core metabolic gene tpiA (Fig. C) and *dfp* (Fig. D). Triangles, circles and squares represent replicates. Data up to UV dose of 20 mJ/cm^2 (marked with an X) were used for the linear regressions presented.

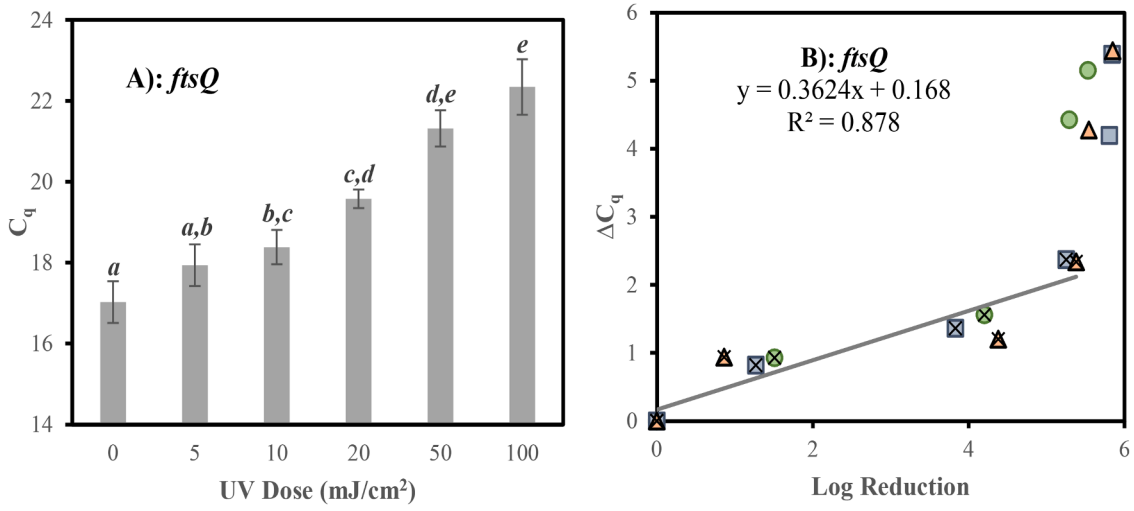

Fig. 4. qPCR-based responses (C_q) as a function of UV dose for cell division gene target *ftsQ* (Fig. A) was used to calculate response changes (ΔC_q) as a function of UV dose, as presented in Figure S32 (SI). Different letters (a – e) indicate statistically significant differences ($p < 0.05$). Log reductions of *E. coli* using culture-based method as a function of UV dose were obtained using Figure S9 (SI). Response changes (ΔC_q) were correlated against *E. coli* log reductions for cell division gene *ftsQ* (Fig. B). Triangles, circles and squares represent replicates. Data up to UV dose of 20 mJ/cm^2 (marked with an X) were used for the linear regressions presented.

ΔC_q values for the *ftsQ* gene target and log reduction in culturable *E. coli* was also observed (Fig. 4) and may have contributed to the reductions observed. These results show that UV exposure significantly impacts important genes involved in *E. coli* cell division or growth pathway, and the impact is greater than that on the other genes targets included in this study.

UV Damage Repair: Long amplicon (~500 bp) targets in *E. coli* for two important genes in bacterial UV repair pathways from literature were identified and their primers designed (Table S2). The C_q response with increasing UV dose for the two UV repair gene targets and the correlation between DNA damage using qPCR with culture-based enumeration for *E. coli* for four metabolic gene targets are presented in Fig. 5. The results obtained are very similar to those for LA 23S. The ΔC_q values observed for UV dose increase from 0 to 20 mJ/cm^2, corresponding to 5.5-log reduction in culturable *E. coli,* were 1.82 for *uvrB* and 1.91 for *umuC* (Fig. 5), and not statistically different from that for LA 23S. Good correlation (R^2 =0.86 and 0.77; p <<0.005) between ΔC_q values for the two gene targets and log reduction in culturable *E. coli* was also observed (Fig. 5), suggesting that this damage may have contributed to the reductions observed. These results show that exposure to UV also impacts genes involved in the repair pathways, limiting the ability of *E. coli* to repair the damage caused by UV exposure.

Environmental significance

Results presented above have shown excellent correlation between ΔCq values obtained with DNA qPCR and log reduction in culturable *E. coli*, with LA-qPCR showing improvement in sensitivity over SA-qPCR.

Table 1
Summary average values for cell abundance (C_q for 10^5 cells/reaction), change in C_q (ΔC_q) for change in UV dose from 0 to 20 mJ/cm^2), slope (ΔC_q /log culturable *E. coli* lost from correlation equation) and correlation (R^2) from the data presented for long amplicons for all 16 gene targets studied (Figs. 2, 3, 4, 5 and SI).

Category	Gene	C_q (10^5 cells/ reaction)	ΔC_q (0 – 20 mJ/ cm^2	Slope (ΔC_q /log *E. coli*)	R^2
Ribosomal	23S_LA	20.2	1.79	0.307	0.93
	rrsA	20.0	1.89	0.305	0.90
	gltA	22.8	1.78	0.275	0.83
Core	*ptsG*	22.9	1.75	0.282	0.86
Metabolism	*pgl*	23.4	1.96	0.310	0.91
	tktA	23.1	1.91	0.294	0.85
	fbaA	23.3	1.95	0.294	0.83
	tpiA	23.5	1.84	0.316	0.92
	ppsA	23.9	1.94	0.298	0.85
	pck	24.1	1.87	0.315	0.90
	dfp	22.3	1.79	0.310	0.94
Cell Division	*ftsQ*	22.9	2.35	0.362	0.88
UV Repair	*uvrB*	22.3	1.82	0.296	0.86
	umuC	22.8	1.91	0.280	0.77
Other genes	*gadA*	22.3	1.82	0.280	0.86
	uidAL	22.9	1.70	0.281	0.94

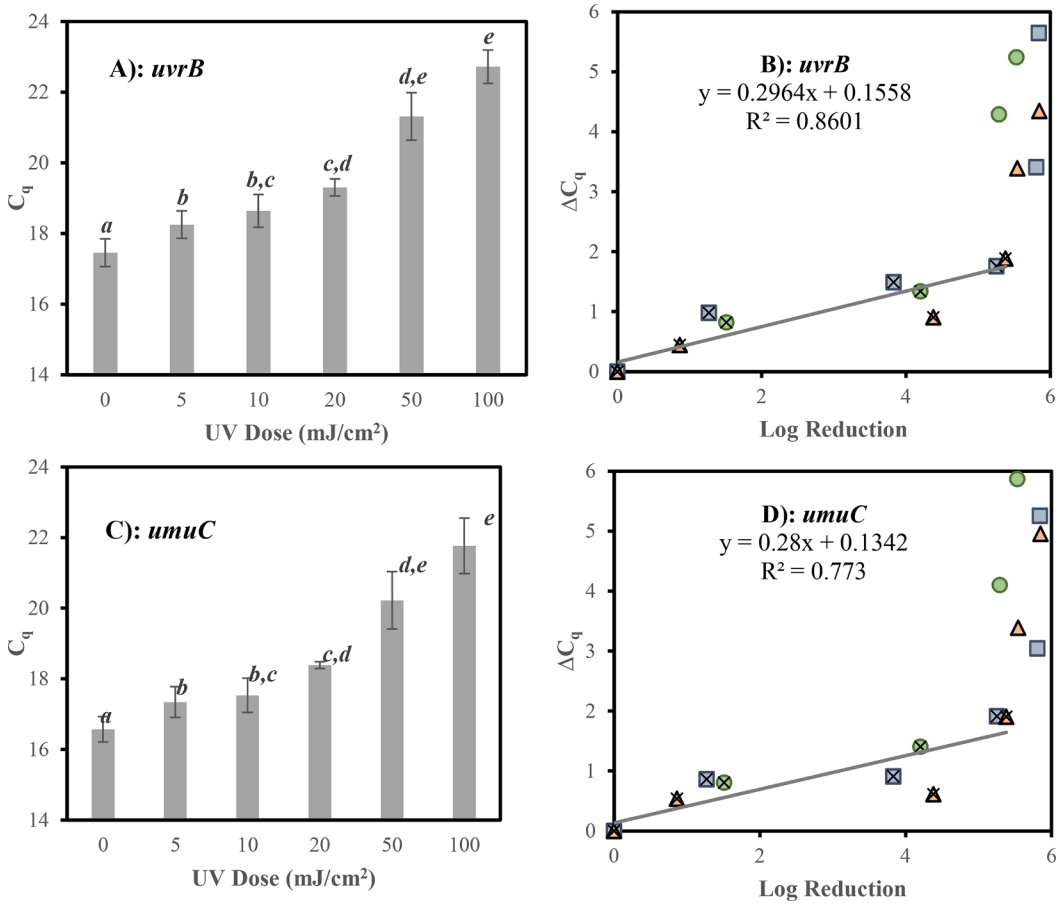

Fig. 5. qPCR-based responses (C_q) as a function of UV dose for two UV repair gene targets (Fig. A: *urvB*; Fig. C: *umuC*) were used to calculate response changes (ΔC_q) as a function of UV dose, as presented in Figure S32 (SI). Different letters (a – e) indicate statistically significant differences ($p < 0.05$). Log reductions of *E. coli* using culture-based method as a function of UV dose were obtained using Figure S9 (SI). and their respective correlations between log reductions of *E. coli* using culture-based method and qPCR. qPCR-based dose-response curve for two UV repair gene targets, and correlation of response changes (ΔC_q) were correlated against *E. coli* log reductions for UV repair gene urvB (Fig. B) and *umuC* (Fig. D). Triangles, circles and squares represent replicates. Data up to UV dose of 20 mJ/cm^2 (marked with an X) were used for the linear regressions presented.

From these correlations for the 16 LA gene targets studied, cell abundance (C_q for 10^5 cells/reaction), slope (ΔC_q /log culturable *E. coli* lost from correlation equation) and correlation (R^2) values are summarized in Table 1. While all the 16 gene targets correlated well with decline in culturable cells, some may offer additional benefits if chosen as a target for qPCR-based quantification of the efficacy of wastewater disinfection using *E. coli*. These include LA 23S (higher abundance and better correlation), and cell division gene *ftsQ* (higher sensitivity due to highest delta C_q and slope values). Alternatively, the *dfp* gene could be a good choice due to better correlation and being an important metabolic gene, since it encodes for a bi-functional catalyst necessary for the synthesis of coenzyme A. Damaging the *dfp* gene in *E. coli* would reduce or possibly eliminate the production of coenzyme A, a cofactor essential for energy production, synthesis of cell membrane and other catabolic and regulatory processes (Leonardi and Jackowski, 2007). According to Gerdes et al. (2003), *dfp* is an essential gene for *E. coli* cell viability.

For a better understanding of or to reflect true cell damage responsible for cell death or loss of viability due to UV exposure, simultaneous monitoring of DNA damage to a panel of several important genes considered critical for cell functioning and survival, such as investigated for *E. coli* in the current study (Table S2), may be studied. Such a panel assay from a single sample could be facilitated through the development of nano-fluidic OpenArray qPCR chip (Shahraki et al., 2019b). Further testing of the approach with real wastewaters and treatment systems is still warranted to verify the general applicability of this approach. It should also be noted that the DNA qPCR cannot distinguish between viable and non-viable cells and therefore the developed assay should only be used over the range of correlation with culture-based method.

Conclusions

The potential of DNA damage, quantified by short amplicon (<200-bp) qPCR (SA-qPCR) and long amplicon (~500-bp) qPCR (LA-qPCR), to define the efficacy of wastewater disinfection by UV irradiation was examined in the current study using a pure *E. coli* culture. *E. coli* log-reduction using culture-based methods were compared against DNA damage quantified by short amplicon (<200-bp) qPCR (SA-qPCR) for the commonly used rRNA and uidA targets. DNA damage using long amplicon (~500-bp) qPCR (LA-qPCR) was also quantified using the same gene targets to test for improved correlation with culture-based enumeration. Further, gene targets which were considered critical for *E. coli* function and survival were identified based on the literature and a comprehensive evaluation of the ability of UV to damage these genes was quantified using LA-qPCR. The main findings from the study are as follows.

- Over the range of UV dose of 0 - 20 mJ/cm^2 commonly used for wastewater disinfection, DNA gene damage, quantified using culture-independent SA-qPCR and LA-qPCR, correlated well with *E. coli* decline using culture-based methods. Thus, both SA-qPCR and LA-qPCR can also be used to monitor the efficacy of wastewater disinfection based on correlations with culture-based methods.
- LA-qPCR was more sensitive than SA-qPCR for monitoring the efficacy of wastewater disinfection.
- With increasing UV dose, increasing damage to multiple genes considered important for *E. coli* metabolism, growth and UV repair was observed. The damage quantified using LA-qPCR correlated well with *E. coli* decline using culture-based methods.

Author contributions

SRC, RS and DDH designed the study. NR conducted the study and wrote the first draft of the manuscript. SRC, RS and DDH edited and revised the manuscript. All the authors approved the final version of the manuscript.

CRediT authorship contribution statement

Natasha Rahman: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Subba Rao Chaganti:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Rajesh Seth:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Daniel D. Heath:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This project was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) strategic partnership grant (STPGP 479134) to DDH and RS for personnel, laboratory and field costs, and NSERC Canada Graduate Scholarship to NR. We want to thank Trojan Technologies for providing the collimated beam UV equipment and providing training to NR on operating the collimated beam UV equipment.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.wroa.2024.100285.

Data availability

Data will be made available on request.

References

American Public Health Association, 2023. American water works association, water environment federation. In: Lipps, WC, Braun-Howland, EB, Baxter, TE (Eds.), Standard Methods for the Examination of Water and Wastewater, 24th ed. APHA Press, Washington DC.

Bolton, J.R., Linden, K.G., 2003. Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. J. Environ. Eng. 129 (3), 209–215.

Bower, P.A., Scopel, C.O., Jensen, E.T., Depas, M.M., McLellan, S.L., 2005. Detection of genetic markers of fecal indicator bacteria in Lake Michigan and determination of their relationship to Escherichia coli densities using standard microbiological methods. Appl. Environ. Microbiol. 71 (12), 8305–8313. https://doi.org/10.1128/AEM.71.12.8305-8313.2005.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., et al., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem., 55, 611–622.

Chang, P.H., Juhrend, B., Olson, T.M., Marrs, C.F., Wigginton, K.R., 2017. Degradation of extracellular antibiotic resistance genes with UV_{254} treatment. Environ. Sci. Technol. 51 (11), 6185–6192. https://doi.org/10.1021/acs.est.7b01120.

Chern, E.C., Brenner, K., Wymer, L., Haugland, R.A., 2014. Influence of wastewater disinfection on densities of culturable fecal indicator bacteria and genetic markers. J. Water. Health 12 (3), 410–417.

Chern, E., Siefring, S., Paar, J., Doolittle, M., Haugland, R., 2011. Comparison of quantitative PCR assays for Escherichia coli targeting ribosomal RNA and single copy genes. Lett. Appl. Microbiol. 52, 298–306. https://doi.org/10.1111/j.1472-765X.2010.03001.x.

Choi, Y., He, H., Dodd, M.C., Lee, Y., 2021. Degradation kinetics of antibiotic resistance gene *mecA* of methicillin-resistant *Staphylococcus aureus* (MRSA) during water disinfection with chlorine, ozone, and ultraviolet light. Environ. Sci. Technol. 55 (4), 2541–2552. https://doi.org/10.1021/acs.est.0c05274.

Dufour A. (1984) Health effects criteria for fresh recreational waters. Toxicology and microbiology division, U.S. Environmental Protection Agency Report EPA- 600-1-84-2004., Cincinnati, OH.

He, H., Zhou, P., Shimabuku, K.K., Fang, X., Li, S., Lee, Y., Dodd, M.C., 2019. Degradation and deactivation of bacterial antibiotic resistance genes during exposure to free chlorine, monochloramine, chlorine dioxide, ozone, ultraviolet light, and hydroxyl radical. Environ. Sci. Technol. 53 (4), 2013–2026. https://doi.org/10.1021/acs.est.8b04393.

Gerdes, S.Y., Scholle, M.D., Campbell, J.W., Balázsi, G., Ravasz, E., Daugherty, M.D., Somera, A.L., Kyrpides, N.C., Anderson, I., Gelfand, M.S., Bhattacharya, A., Kapatral, V., D'Souza, M., Baev, M.V., Grechkin, Y., Mseeh, F., Fonstein, M.Y., Overbeek, R., Barabási, A.L., Oltvai, Z.N., …, Osterman, A.L., 2003. Experimental determination and system level analysis of essential genes in Escherichia coli MG1655. J. Bacteriol. 185 (19), 5673–5684. https://doi.org/10.1128/JB.185.19.5673-5684.2003.

Goosen, N., Moolenaar, G., 2008. Repair of UV damage in bacteria. DNA Repair (Amst.) 7, 353–379. https://doi.org/10.1016/j.dnarep.2007.09.002.

Hijnen, W., Beerendonk, E., Medema, G., 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. Water Res. 40, 3–22. https://doi.org/10.1016/j.watres.2005.10.030.

Ho, J., Seidel, M., Niessner, R., Eggers, J., Tiehm, A., 2016. Long amplicon (LA)-qPCR for the discrimination of infectious and noninfectious phix174 bacteriophage after UV inactivation. Water Res. 103, 141–148. https://doi.org/10.1016/j.watres.2016.07.032.

Janion, C., 2008. Inducible SOS response system of DNA repair and mutagenesis in Escherichia coli. Int. J. Biol. Sci. 4 (6), 338–344.

Klymus, K.E., Merkes, C.M., Allison, M.J., Goldberg, C.S., Helbing, C.C., Hunter, M.E., Jackson, C.A., Lance, R.F., Mangan, A.M., Monroe, E.M., et al., 2019. Reporting the limits of detection and quantification for environmental DNA assays. Environ. DNA 2, 271–282.

Leonardi, R., Jackowski, S., 2007. Biosynthesis of pantothenic acid and Coenzyme A. EcoSal. Plus. 2 (2). https://doi.org/10.1128/ecosalplus.3.6.3.4, 10.1128/ecosalplus.3.6.3.4.

McLellan, S., Hollis, E., Depas, M., Van Dyke, M., Harris, J., Scopel, C., 2007. Distribution and fate of Escherichia coli in Lake Michigan following contamination with urban stormwater and combined sewer overflows. J. Great Lakes Res. 33, 566–580.

Nocker, A., Sossa, K., Camper, A., 2007. Molecular monitoring of disinfection efficacy using propidium monoazide in combination with quantitative PCR. J. Microbiol. Methods 70, 252–260. https://doi.org/10.1016/j.mimet.2007.04.014.

Noor, E., Eden, E., Milo, R., Alon, U., 2010. Central carbon metabolism as a minimal biochemical walk between precursor for biomass and energy. Mol. Cell 39, 809–820. https://doi.org/10.1016/j.molcel.2010.08.031.

Ouellette, S.P., Rueden, K.J., AbdelRahman, Y.M., Cox, J.V., Belland, R.J., 2015. Identification and partial characterization of potential ftsL and ftsQ homologs of Chlamydia. Front. Microbiol. 6. https://doi.org/10.3389/fmicb.2015.01264.

Reuven, N.B., Arad, G., Maor-Shoshani, A., Livneh, Z., 1999. The mutagenesis protein umuC is a DNA polymerase activated by umuD', recA, and ssb and is specialized for translesion replication. J. Biol. Chem. 274 (45), 31763–31766.

Shahraki, A., Chaganti, S., Heath, D., 2019a. Assessing high-throughput environmental DNA extraction methods for meta-barcode characterization of aquatic microbial communities. J. Water. Health 17 (1), 37–49. https://doi.org/10.2166/wh.2018.108.

Shahraki, A.H., Heath, D., Chaganti, S.R., 2019b. Recreational water monitoring: nanofluidic qRT-PCR chip for assessing beach water safety. Environmental DNA 1, 305–315. https://doi.org/10.1002/edn3.30.

Sivaganesan, M., Aw, T.G., Briggs, S., Dreelin, E., Aslan, A., Dorevitch, S., Noble, R., 2019. Standardized data quality acceptance criteria for a rapid Escherichia coli qPCR method (Draft Method C) for water quality monitoring at recreational beaches. Water Res. 156, 456–464.

Templar, H., Dila, D., Bootsma, M., Corsi, S., McLellan, S., 2016. Quantification of human-associated fecal indicators reveal sewage from urban watersheds as a source of pollution to Lake Michigan. Water Res. 100, 556–567. https://doi.org/10.1016/j.watres.2016.05.056.

Weigel, K., Nguyen, F., Kearney, M., Meschke, J., Cangelosi, G., 2017. Molecular viability testing of UV-inactivated bacteria. Appl. Environ. Microbiol. 83 (10). https://doi.org/10.1128/AEM.00331-17.

WHO, 2003. Guidelines for Safe Recreational Water Environments, Volume 1. Coastal and fresh waters, Geneva.

Xu, L., Zhang, C., Xu, P., Wang, X., 2018. Mechanism of ultraviolet disinfection and chlorination of Escherichia coli: culturability, membrane permeability, metabolism, and genetic damage. J. Environ. Sci. 65, 356–366. https://doi.org/10.1016/j.jes.2017.07.006.

Yoon, Y., Dodd, M.C., Lee, Y., 2018. Elimination of transforming activity and gene degradation during UV and UV/H2O2 treatment of plasmid-encoded antibiotic resistance genes. Environ. Sci.: Water Res. Technol. 4, 1239–1251.

Zhang, S., Ye, C., Lin, H., Lv, L., Yu, X., 2015. UV disinfection induces a VBNC state in Escherichia coli and Pseudomonas aeruginosa. Environ. Sci. Technol. 49, 1721–1728. https://doi.org/10.1021/es505211e.