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UV Disinfection of Hand-Rinse Greywater and Performance Testing Using Indigenous *Staphylococcus* spp.

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Received: 1 November 2017; Accepted: 8 December 2017; Published: 11 December 2017

Abstract: Greywater reuse is a feasible solution for decreasing raw water extraction in urban and rural settings. However, pathogen-specific performance guidelines and regulations have only recently been recommended; practical means to assess performance are missing. Here we examine the efficacy of *Staphylococcus* spp. as an endogenous surrogate for greywater pathogen reduction performance testing, by evaluating UV-C irradiation of hand-rinse greywater, and the variability in UV resistance between different wild *Staphylococcus* species. Hand-rinse greywater samples were collected from five participants, and a collimated UV-C beam (256 nm) was used to assess $\log_{10}$ reductions. Assays of colony-forming units on tryptic soy agar (TSA) were compared to mannitol salt agar (MSA) using Lysostaphin^{TM} to confirm *Staphylococcus* spp. After irradiating raw hand-rinse samples to a dose of 220 mJ·cm^{-2}, $\log_{10}$ reductions of *Staphylococcus* spp. were similar (2.1 and 2.2, respectively, $p = 0.112$). The similarity of the reduction based on TSA and *Staphylococcus*-specific culture assays following UV irradiation and the dominating presence of *Staphylococcus* spp. suggests that *Staphylococcus* spp. could be used as an endogenous performance surrogate group for greywater treatment testing. Suspended wild *Staphylococcus* isolates were irradiated with 256 nm UV-C to compare the variability of different *Staphylococcus* species. *Staphylococcus* isolates exhibited significant variance in $\log_{10}$ reduction values when exposed to 11 mJ·cm^{-2} of UV-C. *Staphylococcus hominis* subsp. *hominis* exhibited surprising resistance to UV-C, with only a 1.6-$\log_{10}$ reduction when exposed to 11 mJ·cm^{-2} of UV-C (most other isolates exhibited > 5-$\log_{10}$ reduction). The efficacy of UV-C was also significantly reduced when the sunscreen oxybenzone was present at a possible endogenous greywater concentration.

Keywords: greywater; water reuse; greywater reuse; *Staphylococcus aureus*; *Staphylococcus* spp.; alternative water sources; onsite treatment and reuse; sustainable urban water use

1. Introduction

According to UN Water, approximately 2 billion people globally live in areas of water scarcity and another 1.6 billion face economic water shortage (lacking the necessary infrastructure for water transportation) [1]. Current municipal water distribution practices of treating all wastewater (black water and greywater combined) are generally neither economic nor sustainable for future generations [2,3]. Greywater is typically defined as household wastewater without faecal contribution (i.e., toilet effluent); this includes sources such as wash-basin, shower/bath, laundry, etc. The practice of greywater reuse is a relatively unexplored concept in urban developments; it has the potential to reduce municipal water demands by 50% on average [4] and up to 70% [5] depending on the end use. However, in many parts of the world, including North America, the practice of domestic greywater reuse is in general illegal [4]. An important step forward in gaining government endorsement for greywater reuse is a better understanding of the contaminants, treatment needs, and appropriate treatment system performance testing, as required in water safety plans [6].

This paper is a follow-up to a 2017 study by Shoults and Ashbolt [7] to better understand how to assess ultraviolet (UV) irradiation performance testing without the need for externally spiked surrogates. The original study was based off a 2014 next-generation sequencing study by Zimmerman et al. [8] which identified *Staphylococcus* spp. as the most abundant bacterial genus in laundry greywater sourced from a university sports facility. To better understand the microbiological constituents of greywater, a literature review was conducted and identified 41 studies, however, only three studies measured greywater for total staphylococci [8–10], with several focusing on *Staphylococcus aureus* (*S. aureus*) [8,9,11–16]. Of the three studies that measured total staphylococci, total staphylococci was found to be among the most abundant bacteria when compared to faecal coliforms (FC), total coliforms (TC), *Escherichia coli* (*E. coli*), *Enterococcus* spp., and other traditional faecal indicator bacteria (FIB). Given the pathogenic nature of *S. aureus* [17,18], which along with *S. epidermidis* is among the most prevalent species on human skin [19], we proposed total staphylococci be considered as an endogenous surrogate to represent performance testing in greywater treatment systems, given the inherent problems with using FIB [7]. Overall, there is a collective need for the scientific community and regulatory bodies to better understand the efficacy of prospective surrogates to assess greywater treatment performance. The experiments in the current study explored an array of considerations to determine the efficacy of total staphylococci as an endogenous performance surrogate.

As with any disinfection process, there are limitations to UV irradiation. While the effects of large/many suspended particles on the efficacy of UV are understood [20], the role of micro-pollutants and personal care products (PCP) on the efficacy of UV irradiation are relatively unknown. Given the high reported concentrations of the sunscreen benzophenone (BP3), commonly referred to as oxybenzone [21], the effects of oxybenzone on the efficacy of UV irradiation for staphylococci was also studied.

2. Materials and Methods

2.1. Hand-Rinse Samples to Recover Skin-Bacteria and Evaluate Their UV-Resistance

Hand-rinse samples were collected from five participants using a "glove method". Participants inserted each hand, one at a time, into a large powderless latex free nitrile glove filled with 40 mL of municipally sourced, sterile deionized (DI) water. Using their free hand, participants massaged the submerged hand to ensure maximum water to hand contact for bacterial shedding. After approximately 30 s of exposure, participants inserted their other hand and repeated the previous step. Sterile DI water was used instead of tap water to reduce background bacterial input and confounding effects of residual chlorine. Soap was not used so as to reduce potential bacteriostatic effects and to avoid increased turbidity, as the goal was not to simulate greywater production, but rather to isolate skin-borne bacteria for UV-irradiation and enumerate viable cells using two isolation agars. The resulting bacterial suspension was poured into a sterile beaker and thoroughly mixed. A 28.3 mL portion of the sample was poured into a 60 mm sterile Petri dish so as to give a 1 cm greywater depth. A sterile 5 mm × 2 mm stir bar was placed in the dish and the dish was placed onto a magnetic mixer operating at 400 rpm to facilitate mixing without vortexing.

An AquaSense Pearl Beam collimated LED UV reactor (Florence, KY, USA) with a peak wavelength of 256 nm and a half bandwidth of 11.5 nm was used to deliver 256 nm UV-C irradiation to the raw hand-rinse sample using an adapted EPA protocol [22]. Equation (1) was used to calculate the effective intensity (E_{ave}) of the collimated beam based on measurable variables [23]:

$$E_{ave} = 0.98\left[\frac{E_0}{L}\left(\frac{(1-A)^L - 1}{\ln(1-A)}\right)\right] \quad (1)$$

The incident intensity (E_0) was measured using a NSF certified radiometer (UVP Radiometer, Model UVX-25, Upland, CA, USA). The water height (L) was measured to 1 cm (28.3 mL in a 60 mm cylindrical Petri dish), and a spectrophometer (Thermo Scientific, Genesys 10S UV-VIS, Waltham, MA,

USA) was used to measure the absorbance (A) of 256 nm UV in the suspension. The resulting E_{ave} was then multiplied by the exposure time (seconds) in order to calculate the resulting dosage in mJ·cm^{-2}.

To estimate staphylococci counts prior to irradiating the sample, 100 µL of the sample was diluted to 10^{-1}; 100 µL of the dilution was pipetted into 15 mL of sterile 0.85% NaCl buffer then filtered through a 60 mm diameter filter cup apparatus with a 0.22 µm polycarbonate (PC) membrane filter (Isopore^{TM} GTTP-04700, Cork, IRL) using a vacuum pump. The filtering process was performed a total of six times and the resulting filter papers were placed on tryptic soy agar (TSA) and mannitol salt agar (MSA) plates, both in triplicate.

For UV irradiation of samples, the collimated beam was placed over the Petri dish containing 28.3 mL of a raw hand-rinse sample (as described above), and then irradiated to a dose of 220 mJ·cm^{-2}. In order to achieve a consistent dose for each person's greywater sample, the absorbance was measured prior to exposure to adjust the exposure time for a resulting dose of 220 mJ·cm^{-2}. After exposing samples to 220 mJ·cm^{-2}, the entire sample was poured into a sterile 50 mL capped test tube and vortexed to ensure adequate mixing. Upon vortexing, 1 mL of the post-irradiated sample was pipetted into 15 mL of sterile 0.85% NaCl buffer and plated using the above described filter-plating technique. TSA and MSA plates were incubated at 37 °C for 18–24 h before assaying colony forming units (CFU).

Given the similarity in growth conditions of the expected staphylococci and micrococci and that no selective medium is known to easily resolve these genera, we confirmed pure isolates using Lysostaphin^{TM} tablets, known to reliably differentiate between staphylococci (lysis) and micrococci (no lysis) [24]. Upon assaying, Lysostaphin^{TM} tablets were used for total staphylococci confirmation for colonies from MSA plates (see [25] for protocol). Five Lysostaphin^{TM} confirmations were performed on each of the triplicate MSA plates, for a total of 30 confirmations per sample (15 at 0 mJ·cm^{-2} and 15 at 220 mJ·cm^{-2}). The fraction of lysis positive Lysostaphin^{TM} confirmation tests was multiplied by the CFU counts from the MSA assays to estimate the fraction of CFU assayed from MSA which were considered totally staphylococci. Equation (2) was used to calculate the $\log_{10}$ reduction after exposure for each assay:

$$\mathrm{Log_{10}Reduction} = \mathrm{Log}\left(\frac{\frac{\text{CFU Control}}{\text{100uL}}}{\frac{\text{CFU Dosage}}{\text{100uL}}}\right) \quad (2)$$

2.2. Hand-Rinse Isolates

Upon assaying the MSA control plates, two or three colonies from each participant were isolated onto separate MSA plates, which were then incubated for 18–24 h at 37 °C, and then re-streaked at least once more onto MSA plates to ensure purity. Once pure cultures were isolated (total of 14), they were analyzed using a VITEK^{TM} (2 COMPACT) instrument to determine genera and species.

The collimated UV beam procedure was performed on a total of 14 isolates from five different participants as well as a clinical *Staphylococcus aureus* strain acquired from the American Type Culture Collection (ATCC), *S. aureus* (ATCC 25923). A presumed *Staphylococcus epidermidis* (ATCC 12228) strain was confirmed as *Staphylococcus lentus* upon VITEK^{TM} confirmation and was also used in this study. Overnight cultures grown in Tryptic Soy Broth (TSB) were diluted to 1:100 in a sterile 0.85% NaCl. Once diluted, the above collimated UV beam procedure was performed to irradiate suspended cells (with the following modification). Prior to irradiation, 100 µL of the sample was plated on TSA plates in triplicate at the appropriate dilution to effectively assay control plates. Samples were irradiated to 11 mJ·cm^{-2} (slightly less than the approximate dose required for ~4-$\log_{10}$ reduction of *S. aureus*, being a likely targeted reduction level [7]), and allowing for post exposure detection of at least some samples. Upon irradiation, 100 µL of the sample was plated in triplicate on TSA at the appropriate dilutions to effectively assay the plates, and $\log_{10}$ reductions were calculated using Equation (2). A portion of the samples were irradiated at 7, 9, and 11 mJ·cm^{-2} in order to estimate decay equations.

2.3. Oxybenzone (BP3)

The collimated UV beam procedure described above was performed using *S. aureus* (ATCC 25923) to determine the effects of a sunscreen, oxybenzone, on the performance of UV inactivation. Oxybenzone was used as a test compound due to the relatively high concentrations (reports as high as 0.7 mg·L^{-1}) when compared to other UV filters/sunscreens [21]. Since the source of oxybenzone in municipal wastewater is likely from greywater, oxybenzone and other micro-pollutant/PCP concentrations would likely be higher in greywater than in municipal wastewater (which is diluted by blackwater). As shown in a 2005 study by Palmquist and Hanæus [26], organic compound pollutants were typically in greater concentrations within greywater samples when compared to blackwater and sometimes an order of magnitude higher in concentration. Therefore an oxybenzone concentration range of 1 mg·L^{-1} to 10 mg·L^{-1} was used due to the expected increase of oxybenzone concentration in greywater. Oxybenzone was dissolved into dimethyl sulfoxide (DMSO) (166.7 µL·L^{-1}) and pipetted into sterile 0.85% NaCl buffer at two different concentrations: 10 mg oxybenzone·L^{-1} and 1 mg oxybenzone·L^{-1}; DMSO was suspended into two separate buffer solutions (at 166.7 and 16.67 µL·L^{-1} for the two solutions respectively) used as controls to adjust for any confounding effects of DMSO on the reduction of *S. aureus* by UV irradiation. Overnight cultures of *S. aureus* (ATCC 25923) suspended in TSB were suspended into the four samples (10 mg oxybenzone·L^{-1}, 1 mg oxybenzone·L^{-1}, and respective controls) and irradiated to a UV-C dose of 11.8 mJ·cm^{-2} using the previously described collimated beam apparatus protocol. Samples were assayed on TSA plates in at least triplicate prior to and after exposure to UV. Log_{10} reductions were quantified using Equation (2). A paired *t*-test was performed between oxybenzone and respective controls on the log_{10} reduction means.

2.4. Control Experiments

Two additional control experiments were performed to ensure homogeneity with literature as well as consistency throughout the experiments. An 11-point dose-response curve using MS2 bacteriophage (ATCC 15597-B1) was performed using a double agar protocol and assayed by plaquing [27]; this curve was then compared to a 2006 UV-C study [28] performed using MS2 and was found to be within the same log_{10} reduction range (see Figure S1 for graph comparison).

2.5. Statistical Analysis

All statistical analyses were performed using SigmaPlot (Version 13.0, Systat Software, Inc., San Jose, CA, USA). All reported tests passed the Shapiro-Wilk normality test. All reported *p*-values are two tailed and are the result of paired *t*-tests, unless otherwise stated.

3. Results and Discussion

3.1. Raw Hand-Rinse

Estimated total staphylococci CFU counts on MSA (TS-MSA) before and after UV-C exposure (220 mJ·cm^{-2}) from the five participants made up the majority of the total TSA CFU counts (T-TSA) (90% and 75% respectively). More importantly, the log_{10} of the TS-MSA to T-TSA ratio (displayed as a percentage) of before and after UV-C exposure was 99% and 97% respectively. A paired *t*-test (see Table 1) was performed comparing the log_{10} reduction from UV-C exposure within the T-TSA and TS-MSA assays from the five participants; the log_{10} reductions of T-TSA and TS-MSA were not found to be significantly different ($p = 0.112$). The similarity in log_{10} reduction of the T-TSA and TS-MSA assays (means of 2.1 and 2.2 respectively) suggests that TS-MSA was representative of bacterial reduction by UV-C irradiation of the hand-rinse water from the five participants. Figure 1a depicts the T-TSA and TS-MSA assays before and after exposure to UV-C. The T-TSA and TS-MSA concentrations reported in this study are not considered representative of concentrations across all wash-basin greywater sources, as soap was not used and only 40 mL was used to wash participants' hands; rather, the significance of

this study is exhibited in the consistently similar T-TSA and TS-MSA concentrations, implying the vast majority of recovered bacteria were staphylococci.

Although the results from the five participants were consistent (See Table S2), more replication of this study on a variety of greywater sources is necessary to confidently suggest total staphylococci as a performance surrogate for greywater treatment. A 1981 study by Abshire and Dunton [29] showed that *S. aureus* was more resistant to UV-C than the saprozoic pathogen *Pseudomonas aeruginosa* (also typically present in greywater [13]); however, a 2007 study by Gilboa and Friedler [13] found *S. aureus* to be the least resistant to UV-C irradiation when compared to faecal coliforms (FC), *P. aeruginosa*, and *Clostridium perfringens* at low doses. However, as shown in this study, given the dominating concentrations of *Staphylococcus* spp. in raw greywater [8,10], *Staphylococcus* spp. may still be a practical representative surrogate for total bacterial pathogenic reduction in greywater.

Although TS-MSA represented the majority of T-TSA, a paired *t*-test showed that when T-TSA and TS-MSA assays were compared (both before and after exposure) the $\log_{10}$ means were statistically significantly different (p = 0.04 for both) (see Table 1). However, when assessing MSA and TSA assays (both before and after exposure) prior to Lysostaphin^{TM} confirmations, no statistical difference was observed (p = 0.78 and 0.73 respectively). *Staphylococcus* spp., some *Micrococcus* spp., and some *Enterococcus* spp. are of the few organisms known to be capable of growing in the high salt environment in MSA [30,31]; it is likely that the majority of the Lysostaphin^{TM} negative MSA CFU were *Micrococcus* spp., as micrococci are one of the more abundant bacteria genera inhabiting human skin [32], however no further confirmations were performed to determine what the non-*Staphylococcus* spp. were. Figure 1a displays the T-TSA and TS-MSA assays before and after UV exposure.

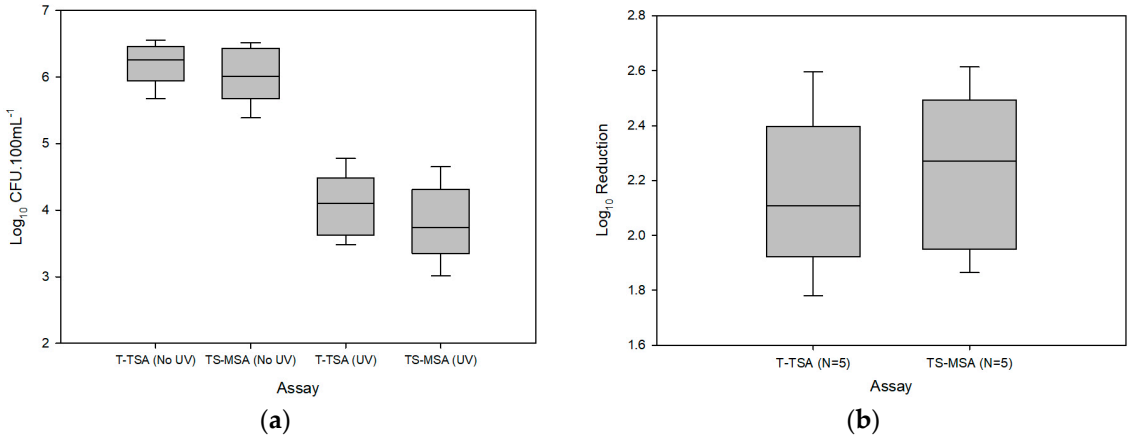

Figure 1. Hand-rinse bacteria exposed to 256 nm UV-C irradiation: (**a**) $\log_{10}$ CFU·100 mL^{-1} on TSA (T-TSA) and total staphylococci on MSA (TS-MSA) before and after exposure to dose of 220 mJ·cm^{-2} (N = 5); (**b**) $\log_{10}$ reduction on T-TSA and TS-MSA (N = 5).

Table 1. $\log_{10}$ colony forming units per 100 mL of raw hand-rinse water before and after exposure.

Assay	$\log_{10}$ CFU·100 mL^{-1} ± (SD)		
	0 mJ·cm^{-2}	220 mJ·cm^{-2}	Reduction
T-TSA	6.2 ± (0.3)	4.1 ± (0.5)	2.1 ± (0.3)
TS-MSA	6.0 ± (0.4)	3.8 ± (0.6)	2.2 ± (0.3)
Paired *t*-test	0.04 [a]	0.04 [a]	0.112 [a]

Note: [a] Two-tailed *p*-value comparing means.

A notable concern within this study was the lack of bacterial reduction by 256 UV-C at a dose of 220 mJ·cm^{-2}, which should have yielded at least a 5-$\log_{10}$ reduction in viable bacteria (as previously

shown by Shoults and Ashbolt [7]). This is not surprising, however, as Winward et al. showed a significant decrease in UV-C efficacy of bacterial reduction in water with high turbidity and low UV transmittance [20]. The 256 nm absorbance (A_{256} nm) readings ranging from 0.164 to 0.360 (see Table S1) and were not normalized, as given in the unadjusted Equation (1); the A_{256} readings would likely have been increased by the use of soap in this study, and thus soap wasn't used. Two future considerations should be given towards future studies involving UV-C irradiation of raw greywater:

1. Pre-treatment prior to UV-C irradiation is likely necessary in order to achieve more than a 2-$\log_{10}$ reduction of endogenous bacteria due to particulate shielding and organic quenching effects; and
2. Equation (1) describing the delivered UV-C intensity requires adjustment to account for shielding/quenching from high turbidity/organics in greywater samples.

A 2006 study by Liu and Zhang examined the effects of turbidity on the efficacy of bacterial and coliphage reduction by UV; although there was not a major difference in UV-C efficacy between turbidities of 0.5 and 4 Nephelometric Turbidity Units (NTU), there was a significant difference between 4 and 12 NTU [28]. This suggests pre-treatment removal of particles to a turbidity of <12 NTU is probably necessary for optimal UV-C efficacy.

3.2. Hand-Rinse Isolates

The aim of this portion of the study was to examine the variability of resilience of environmental staphylococci isolates. The *following Staphylococcus* species were isolated from the five participants' hands (all multiples of species are from different participants): *S. aureus* (1), *S. capitis* (2), *S. epidermidis* (3), *S. haemolyticus* (2), *S. hominis* subsp. *hominis* (*S. hominis*) (1), *S. pasteuri* (3), and *S. warneri* (2). The effects of 256 nm UV-C at a dose of 11 mJ·cm^{-2} were assessed in order to determine the variance of resistance to UV within and between *Staphylococcus* species; the results are displayed in Table 2:

Table 2. Log_{10} reduction of the American Type Culture Collection (ATCC) and wild *Staphylococcus* isolates.

Bacteria	Log_{10} Reduction at 11.0 mJ·cm^{-2} ± (SD)
S. aureus (ATCC 25923)	4.9 ± (0.0)
S. aureus (i)	>5.2
S. capitis (ii)	>5.7
S. capitis (iii)	>6.3
S. lentus (unknown)	5.0 ± (0.0)
S. epidermidis (ii)	>5.7
S. epidermidis (iiia)	>6.1
S. epidermidis (iiib)	>5.6
S. haemolyticus (iv)	3.4 ± (0.1)
S. haemolyticus (v)	4.4 ± (0.1)
S. hominis (v)	1.6 ± (0.1)
S. pasteuri (iii)	>5.8
S. pasteuri (iv)	>5.5
S. pasteuri (v)	5.1 ± (0.1)
S. warneri (i)	>5.7
S. warneri (ii)	>5.5

Note: i–v refers to the five participants from whom the greywater isolates were derived.

When exposed to 256 nm UV-C at a dose of 11 mJ·cm^{-2}, the majority of isolates experienced at least a 5-$\log_{10}$ reduction. However, there was significant variance in $\log_{10}$ reductions after UV exposure between different *Staphylococcus* spp., with a less significant variance within each species. *S. hominis* and *S. haemolyticus* were significantly more resistant to UV-C irradiation than the other isolates. The linear decay equations for *S. hominis* and *S. haemolyticus* were $y = 0.23x - 0.96$ ($R^2 = 0.97$) and $y = 0.30x + 0.25$ ($R^2 = 0.91$), respectively, where (y) is $\log_{10}$ reduction, and (x) is the dose in mJ·cm^{-2}. Decay equations were not obtained for the other bacteria, due to insufficient plate counts at each exposure assay.

A previous 2017 study by Shoults and Ashbolt determined that *Staphylococcus* spp. (as represented by *S. aureus* and *S. epidermidis* (ATCC 12228 as used in Shoults and Ashbolt [7] was confirmed by MALDI-MS VITEK^{TM} verification, but characterized as *S. lentus* by VITEK2^{TM} phenotyping) was more sensitive to UV-C irradiation than some FIB [7]. However, the relative resistance of *S. hominis* and *S. haemolyticus* suggests *Staphylococcus* spp. may be as resistant to UV as FIB. However, further study is required to understand the full range of resistance within *Staphylococcus* spp.

3.3. Affects of Oxybenzone on UV-C Efficacy

Though UV blockers such as oxybenzone are known to quench the effects of UV when used on skin (common compound in sunscreens), the direct impacts of sunscreens and other PCPs on the efficacy of UV irradiation for domestic water treatment are not well known. Figure 2a,b display the effects of oxybenzone on the efficacy of reduction of *S. aureus* (ATCC 29523) by 256 nm UV-C.

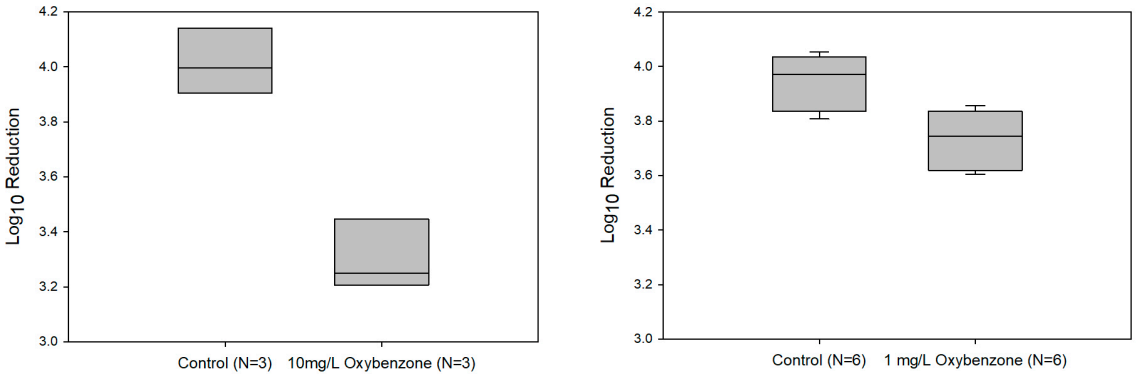

Figure 2. Effects of oxybenzone when irradiating suspended *S. aureus* at 11.8 mJ·cm^{-2}: (**a**) 10 mg·L^{-1} oxybenzone; (**b**) 1 mg·L^{-1} oxybenzone.

Samples containing oxybenzone (concentrations of 10 mg·L^{-1} and 1 mg·L^{-1}) exhibited significantly lower $\log_{10}$ reductions (p = 0.003 and 0.01 respectively) than their respective negative controls (containing no oxybenzone). This may help explain the lack of bacterial reduction of raw hand-rinse greywater (see Figure 1) when exposed to a dose of 220 mJ·cm^{-2}; it is expected many of the hand-rinse samples contained PCPs, creams, or sunscreen agents. Although it is generally well understood that pre-treatment is necessary prior to UV irradiation, it is important to know which compounds are to be removed and to what extent. PCPs can be difficult to remove from greywater [33,34], however, depending on the intended end-use of the treated effluent, removal may be necessary if UV is to be the sole disinfection step.

4. Summary and Conclusions

The research displayed in this paper evaluated the efficacy of 256 nm UV-C in treating raw hand-rinse water, analyzed the efficacy of total culturable *Staphylococcus* spp. as an endogenous surrogate group to represent bacterial reduction, examined the variability of wild *Staphylococcus* spp. isolates when exposed to UV-C, and assessed the impacts of the sunscreen oxybenzone on the efficacy of UV-C irradiation. It is clear pre-treatment is necessary prior to UV irradiation for a $\log_{10}$ reduction of greater than two to be observed; the presence of PCP compounds (such as oxybenzone) as well as turbidity can have a negative effect on the efficacy of UV-C irradiation. Given the high prevalence of *Staphylococcus* spp. found in the five samples of raw hand-rinse water and more generally reported on human skin, future greywater studies should investigate the surrogate use of endogenous *Staphylococcus* spp. for bacterial reduction in greywater reuse systems to better understand any limitations at field-scale. Further research should be performed to assess the $\log_{10}$ reductions by other

disinfection methods of *Staphylococcus* spp. relative to other pathogens of concern to determine if *Staphylococcus* spp. would be an adequate endogenous surrogate for other disinfection processes. Finally, the results in this study have shown that the sunscreen oxybenzone can have a negative effect on the efficacy of UV irradiation; further research should also address the role of other PCPs on the efficacy of UV irradiation.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4441/9/12/963/s1, Table S1: Raw hand-rinse water reduction, Table S2: *Staphylococcus* spp. isolate data, Figure S1: MS2 Literature Comparison.

Acknowledgments: This work was supported by grants from Alberta Innovates—Health Solutions and the Canadian Foundation for Innovation. All work was completed through the School of Public Health at the University of Alberta, Edmonton, Canada. The authors would like to give a special thank you to Candis Scott for running isolate analysis.

Author Contributions: David C. Shoults and Nicholas J. Ashbolt conceived and designed the experiments; David C. Shoults performed the experiments, analyzed the data, and wrote the paper as part of his M.Sc. study under the supervision of Nicholas J. Ashbolt.

Conflicts of Interest: The authors declare no conflict of interest.

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