The Responses of *Escherichia Coli, Vibrio Cholerae, Aeromonas Hydrophila* and *Pseudomonas Aeruginosa* to Pulsed Ultra-Violet Light Inactivation

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Abstract

The effect of pulse ultra violet (PUV) light on 107cfu/mL and 103cfu/mL concentrations of Escherichia coli, Vibrio cholerae, Aeromonas hydrophila and Pseudomonas aeruginosa were investigated. Further experiments investigated how the efficiency of PUV may be affected by water depth. For 107cfu/mL, E. coli, A. hydrophila, P. aeruginosa and V. cholerae were inactivated completely after 10, 20, 40 and 80 pulses respectively. Treatment of E. coli, A. hydrophila, P. aeruginosa and V. cholerae with 5 pulses however resulted in 100% inactivation for E. coli and 99.99% for the other bacteria. For 103cfu/mL, 3 pulses of UV light treatment were required to inactivate completely all four bacteria. Efficacy of PUV disinfection decreased with increased depth of water. E. coli was more susceptible to PUV light treatment than V. cholerae. The use of E. coli as indicator of potability after disinfection with PUV light may not be appropriate

Keywords: *bacteria, disinfection, drinking water, pathogen, treatment, potability*

Introduction

The disinfection of water for drinking is important to avoid the spread of diseases, particularly during natural disasters such as flooding and in countries where open defecation is still ongoing. Water disinfection by chlorination has been a popular choice owning to its low cost, effectiveness and ease of use. The efficiency of chlorination however is dependent on the characteristics of the raw water used such as pH, dissolved organic matter content and turbidity. In addition, bacteria can proliferate in oligotrophic conditions in water distribution networks despite disinfection by chlorination (Bouteleux et al., 2005). Owing to this and other limitations, there has been a search for other methods of water disinfection. The use of pulsed ultra-violet light (PUV) is emerging as one of the means of inactivating pathogenic bacteria in water. PUV had been used in the food industry for many years and it is still being used for improving shelf life of packaged foods (Hierro et al., 2011; Heinrich et al., 2015). Its application in water treatment however had been limited (Bohrerova et al., 2008). Unlike conventional UV-mercury lamps that may consist

of a continuous wave of radiation of wavelengths 254nm in the case of low pressure lamps and 200-300nm for medium pressure lamps, PUV consist of short pulses of high energy radiation emanating from flash lamps with a small opening. The application of high energy pulses leads to the production of ions, resulting in the formation of UV light , free radicals and localised thermal effects (Marsili et al., 2002; Hancock et al., 2004; Wang et al., 2005). The pulsed UV system works by storing the UV energy and releasing it in high intensity “blasts” that disrupts the DNA structure of microorganisms, preventing replication (Bohrerova et al., 2008).

Conventional technologies using low pressure and medium pressure ultra violet mercury lamps for water treatment had been the common practice and some research had disputed the effectiveness of PUV in water treatment as opposed to conventional UV lamp technologies (Hancock et al., 2004). This may be due to the lack of adequate data on the effective doses and the responses of various pathogenic bacteria to PUV disinfection. Recent findings have shed some light on the responses of some pathogens to PUV treatment. Hayes et al. (2012) noted that 120 pulses of UV light at 900V was required to inactivate *Clostridium perfringens* ATCC 13124 by 2 log units while a similar dose reduced *Bacillus cereus* by 5 log units. It was however noted that it took just 25 pulses at 900V to inactivate *Escherichia coli* by 5 log units (Hayes et al., 2012). Garvey and Rowan (2015) observed that the presence of inorganic contaminants, notably iron and manganese, affected the rate of disinfection by PUV. Increasing evidence is emerging that PUV can be a better alternative to conventional UV technology in terms of efficiency in water treatment (Luo et al., 2014; Uslu et al., 2016) given a better understanding of the response of pathogens to dosage levels under various growth conditions. We investigated the effect of PUV on low and high concentrations of *Escherichia coli, Aeromonas hydrophila, Pseudomonas aeruginosa* and *Vibrio cholerae* and how this can be affected by water depth

Materials and Methods

The Pulsed Ultra-Violet Light (PUV) System

The pulsed UV-rich light system used in this study is made up of a pulse power generator, a flash lamp chamber and a light source. The light source is a low pressure, xenon–filled flash lamp emitting 250-270nm wavelength radiation. When operating at 1kV, the energy per pulse is 20J.

Culture of indicator and pathogenic bacteria

Escherichia coli NCTC 9001 was obtained from the stock cultures of the Bioscience Department of the University of Strathclyde. *Aeromonas hydrophila* NCTC 8049*, Pseudomonas aeruginosa* LMG 9009 and *Vibrio cholerae* NCTC 11348 used were obtained from the National Collection of Type Culture, Collingdale, London. Isolated colonies of each strain of organism was transferred to nutrient agar slopes (NA) and incubated for 24 hours at 37 ºC for *E. coli* and at 30 ºC for *A. hydrophila, P. aeruginosa* and *V. cholerae*. After incubation, the slopes were kept at 4 ºC for subculturing and development of pure cultures. Prior to each experiment, organisms from stock cultures were streaked unto nutrient agar to isolate a single colony for inoculation into nutrient broth (NB). The isolated single colony of the test organism was picked from a 24 hour streaked plate of nutrient agar, placed in 100mL of nutrient broth and grown at 37 ºC for *E. coli* and 30 ºC for *A. hydrophila, P. aeruginosa*, and *V. cholerae* for 18 hours each.

Preparations for experiments

Media used for this study were; nutrient agar (NA), nutrient broth (NB) and phosphate buffered saline (PBS). All laboratory culture media were prepared to manufacturer's instruction.The nutrient broth and nutrient agar was sterilized at 121 ºC for 15 minutes.The PBS was autoclaved at 115 ºC for 15 minutes according to the manufacturer's instruction. The four test organisms were treated by placing the petri dishes containing the bacterial suspensions inside a flash lamp chamber and exposing the dishes to short duration pulses of UV rich light in the wavelength range of 200-280nm at 1 pulse/s, effective for inactivating microorganisms. The voltage was set at 1000V.

Effect of PUV light on high and low microbial populations

Experiments were carried out on *Escherichia coli, Vibrio cholerae, Aeromonas hydrophila* and *Pseudomonas aeruginosa* suspensions with concentrations 107/mL and 103/mL. The experiments made use of high doses of PUV light (10, 20, 40 and 80 pulses/s) and low doses of PUV light (1, 3, 5 pulses/s). Single colonies of the bacterial test strains were aseptically transferred into 100mL of sterile nutrient broth on a shaker at optimum temperatures of each organism. After 18 hours, the bacterial suspensions were centrifuged at 4300 rpm for 20 minutes at 20ºC. The supernatant was discarded and the pellet re-suspended in sterile Phosphate Buffered Saline (PBS) and serially diluted to the desired population size for subsequent PUV light treatment studies. Twenty milliliter volumes of the sample containing the desired population sizes were subjected to several pulses of UV light manually. The pulses were done from the highest to the lowest. The PUV pulse treated solutions were then serial diluted and plated and incubated at each organism's optimum temperature for 24 hours. The surviving bacteria were enumerated using the spread plate, and pour plate methods and incubated on nutrient agar at optimum temperatures (APHA, 2005).

Effect of PUV light on depth/volume of bacterial suspension

Bacteria suspensions of volume 20mL, 30mL and 40mL and concentration 10^6cfu/mL were all subjected to 5 pulses of UV light treatment. Increased volume of bacterial suspension translates into an increase in depth of penetration of UV light.

Results and Discussion

Effect of high and low doses of PUV on microbial populations

At an initial concentration of 10^7cfu/mL, *E. coli* was inactivated after 10 pulses, whereas *A. hydrophila* and *P. aeruginosa* were inactivated after 20 pulses (Figure 1). It however took 80 pulses of PUV to completely inactivate *V. cholerae.* This suggests that PUV can effectively be used to treat water for drinking purposes. Hayes et al. (2012) also noted that different energy levels were required to inactivate different bacteria. This may be attributed to differences in their absorption spectrum.

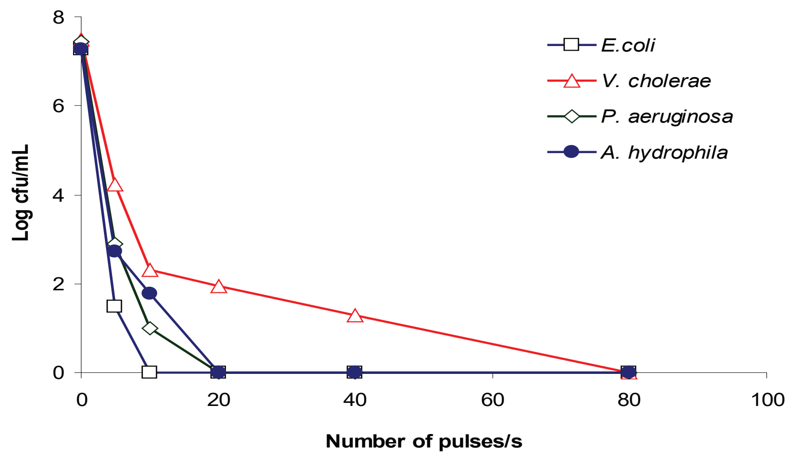

Figure 1. Effect of high dose of PUV on 10^7cfu/mL concentration of bacteria

Table 1. Log reductions occurring after treatment with 5 pulses of UV light.

Sample	Experimental Bacteria log concentration (per 100mL)*			
	E. coli	*V. cholerae*	*P. aeruginosa*	*A. hydrophila*
Untreated	6.32	6.71	6.63	6.57
After 5 Pulses	0	2.10	0.60	0.12
Log reduction	6.32	4.61	6.03	6.45
Percentage kill	100.00	99.99	99.9999	99.9999

*Replicates of six samples

Experiments conducted on high concentrations of test bacteria using 5 PUV (Table 1) show that E. coli was more susceptible to pulsed UV light, whilst *V. cholerae* appeared to be most resistant to pulsed UV light. The order of increasing sensitivity to pulsed UV light of the test organisms are as follows: *E. coli* > *A. hydrophila* > *P. aeruginosa* > *V. cholerae*. Using 5 pulses of UV light, 100% kill of bacteria counts was achieved with E. coli, with a corresponding 6.32 log reduction. More than 6 log units of removal were achieved for *A. hydrophila* and *P. aeruginosa* and 4.6 log removal for *V. cholerae*. *E. coli* appears to be more susceptible to PUV light treatment than *V. cholera*. After 5 pulses, *V. cholerae* surviving population was greater than *E. coli*. This suggests that although PUV treatment is effective at inactivating pathogenic bacteria in water, using *E. coli* surviving counts or its absence as an indicator of potability may not be appropriate because 5 pulses was not enough to completely inactivate V. cholerae.

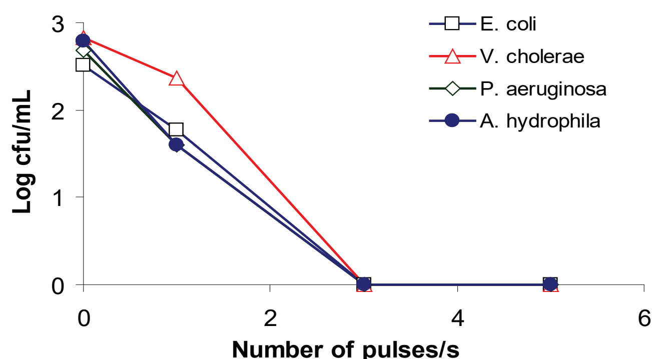

Figure 2. Effect of low pulses of PUV on 10^2/mL concentration of bacteria suspension

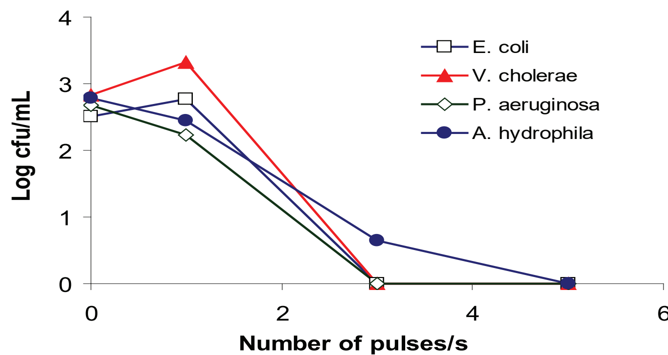

Figure 3. Effect of low pulses of PUV on 10^3/mL concentration of bacteria suspension

Figure 2 and 3 show the effect of PUV treatment on low bacteria populations. The relevance of testing such low concentrations of bacteria is that for *V. cholerae*, doses as low as 10^3 cells/mL can still be infective, particularly in situations of low gastric acidity (Hunter et al., 1997). For low concentrations of bacteria (10^2cfu/mL and 10^3cfu/mL), 3 PUV treatments were required to completely inactivate all the test bacteria except *V. cholerae*. Higher concentrations of bacteria required a higher number of pulses to achieve activation of similar magnitude. For PUV to be used as a means of disinfection, the number of pulses required to inactivate the most resilient of pathogens need to be used. It is too early to speculate on how PUV technology can be applied in households and in communities. Further experiments are necessary for scaling up this technology's application.

Effect of PUV on depth/volume of bacterial suspension

As the depth/volume of bacteria suspension increased, the number of surviving bacteria increased (Figure 4), indicating that PUV treatment efficacy decreases with increased volume or depth of water. This is an important factor for consideration in the design of equipment suitable for effective PUV treatment of water or other liquids. As the volume of bacterial suspension increases, log reduction decreases. In situations where the water may be more turbid, this effect could be more pronouced. For 40 mL of bacterial suspension, extent of penetration of UV light was less when compared with 30 mL of bacterial suspension and this may be due to the volume of suspension and the shielding effect of bacterial cells. The extent of penetration of UV light in bacterial suspensions of volumes 20ml, 30ml and 40 ml was 20 ml > 30ml> 40ml. A p-value of less than 0.001 was obtained for *V. cholerae* and *E. coli* indicating a statistically significant difference on the effect of PUV treatment on volume / depth of bacterial suspension. *A. hydrophila* also had a p-value of 0.028 and *P. aeruginosa* showed a marginally significant difference.

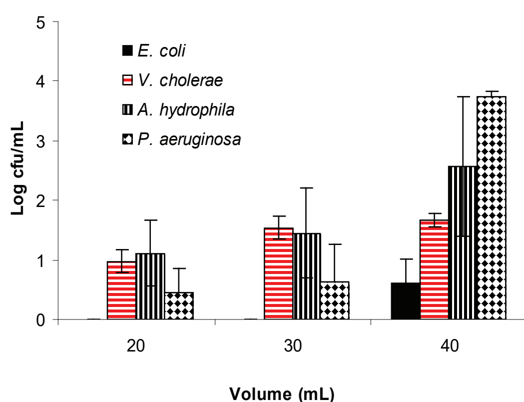

Figure 4. Effect of volume (or depth) on the efficacy of pulsed UV light treatment. (For 20, 30 and 40mL, the corresponding depths were 5, 6, and 9mm respectively)

Conclusions

The study showed that PUV can effectively be used to treat water for drinking purposes. Five (5) pulses of UV light resulted in 100% kill of *E. coli*, with a corresponding 6.32 log reduction. More than 6 log units of removal were achieved for A. *hydrophila* and *P. aeruginosa* and 4.6 log removal for *Vibrio cholerae.* Higher concentrations of bacteria required a higher number of pulses to achieve inactivation of similar magnitude. Efficacy of PUV disinfection decreased with increased depth or volume of water. This effect should be taken into consideration in the design of PUV equipment for water treatment. *E. coli* appears to be more susceptible to PUV light treatment than *V. cholera,* suggesting that the use of *E. coli* as indicator of potability after disinfection with PUV light may not be appropriate.

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