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Pulsed ultraviolet light inactivation of pure cultures of indicator and pathogenic bacteria

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ABSTRACT

The effect of pulsed ultra violet (PUV) light on high (10^7, 10^6/mL) and low (10^4, 10^3, 10^2/mL) concentrations of pure cultures of *Escherichia coli, Vibrio cholerae, Aeromonas hydrophila* and *Pseudomonas aeruginosa* were investigated. Further experiments investigated how the efficiency of PUV light may be affected by the distance through which the PUV light may have to travel in water during inactivation (water depth). For pure cultures of approximate concentration of 10^7 cfu/mL, *E. coli, A. hydrophila, P. aeruginosa* and *V. cholerae* were inactivated completely after 10, 20, 40 and 80 pulses respectively. Treatment of approximate concentration of 10^6/mL bacteria with 5 pulses of PUV light resulted in 100.00, 99.9975, 99.99991 and 99.99997% of *E. coli, V. cholerae,A. hydrophila*, and *P. aeruginosa* respectively. For 10^3 cfu/mL, 3 pulses of PUV light treatment were required to inactivate completely all four bacteria. Efficacy of PUV light disinfection decreased with increased water depth. *E. coli* was more susceptible to PUV light treatment than *V. cholerae*. It may therefore not be appropriate to use *E. coli* as an indicator of potability when PUV light is the means of disinfection. *Vibrio cholerae* cells in the exponential growth phase (6 h of incubation) and stationary growth phase (30 h of incubation) responded differently to inactivation by 5 pulses of PUV light, with *V. cholera* cells in the stationary phase showing more vulnerability to 5 pulses of PUV light.

Keywords: Depth; Disinfection; Drinking water; Growth phase; Potability; Treatment

1. Introduction

The choice of methods of disinfection of water for drinking is usually dictated by its effectiveness, costs and ease of operation. In most developing countries water disinfection by chlorination is common owing to its low cost, effectiveness and ease of use. Increased eutrophication, particularly in developing countries due to poor water management necessitates the use of increased dosage of chlorine, raising cost of water treatment and the possibility of adverse public health effects in the long term. There is therefore a search for other methods of water disinfection that is effective at reasonable costs, particularly when it has the potential of application at the point of use. The use of pulsed ultra-violet (PUV) light is emerging as one of the means of inactivating pathogenic bacteria in water. Common sources of ultra-violet light are UV-mercury lamps. However unlike UV-mercury lamps that usually consist of a continuous wave of radiation of wavelengths of 254 nm in the case of low pressure lamps, and 200–300 nm for medium pressure lamps, PUV light consist of short pulses of high energy radiation emanating from flash lamps with a small opening. The application of high energy pulses lead to the production of ions, resulting in the formation of UV light, free radicals such as hydroxl ions, singlet oxygen and super oxides, with localized thermal effects [1–3]. The PUV light works by storing the UV energy and releasing it in high intensity "blasts" that disrupts the DNA structure of microorganisms, preventing replication [4].

Conventional technologies using low pressure and medium pressure ultra violet mercury lamps for water treatment had been the common practice and some research

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had disputed the effectiveness of PUV in water treatment as opposed to conventional mercury UV lamp technologies [2]. 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 indicator bacteria and pathogens to PUV treatment. For example 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 [5]. It was however noted that it took just 25 pulses at 900V to inactivate *Escherichia coli* by 5 log units [5]. The presence of inorganic contaminants, notably iron and manganese, affects the rate of disinfection by PUV light [6]. Differences arising from varying conditions of operation be it continuous or batch as well as reactor configurations can also account for some of the differences observed. Increasing evidence is emerging that PUV light can be a better alternative to conventional UV technology in terms of efficiency in water treatment [7,8], given a better understanding of the response of indicator bacteria and pathogens to dosage levels under various growth conditions. Limited data however exist on the use of PUV light for the inactivation of various indicator and pathogenic bacteria [6]. We investigated the effect of PUV light on high (10^7/ml and 10^6/ml) and low (10^3/ml and 10^4/ml) concentrations of pure cultures of *Escherichia coli, Aeromonas hydrophila, Pseudomonas aeruginosa* and *Vibrio cholerae* and how this can be affected by water depth. The high concentrations selected were based on the average concentration of *E. coli* or faecal coliforms (10^6/ml) that usually exist in full strength domestic wastewater [9]. The minimum concentration of 10^3/ml selected was based on the minimum infective dose of *Vibrio cholerae* [10]. During water treatment, pathogenic bacteria may be at different stages of growth but the effect of PUV light on the various growth phases of bacteria is not known. The growth phase of bacteria may influence the rate of inactivation of bacteria during PUV light application due to the ability of PUV light to interfere with bacteria replication. The effect of PUV light on bacteria in the growth phase was therefore compared with that of bacteria in the stationary phase.

2. Materials and methods

2.1. The pulsed ultraviolet light (PUV) System

The laboratory scale P UV light system used in this study is made up of a pulsed power generator (PUV-1, Samtech Ltd, Glasgow), driving a low pressure (60kPa), xenon–filled flash lamp emitting 200–280 nm wavelength radiation [3,11]. The setup was operated at 1kV, the energy per pulse being 20 J (Fig. 1).

2.2. 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. *Vibrio cholerae* NCTC 11348, *Aeromonas hydrophila* NCTC 8049 and *Pseudomonas aeruginosa* LMG 9009 used were obtained from the National Collection of Type Culture, Collingdale, London. Isolated colonies of each strain of organism was transferred to nutrient agar (NA) medium (Oxoid Ltd, Basingstoke, England) and incubated for 24 h at 37ºC for *E. coli* and at 30ºC for *V. cholerae, A. hydrophila* and *P. aeruginosa*. After incubation, the slopes were kept at 4ºC for sub-culturing and development of pure cultures [12]. Prior to each experiment, organisms from stock cultures are streaked unto NA medium to isolate a single colony for inoculation into nutrient broth (NB) medium (Oxoid Ltd, Basingstoke, England). The isolated single colony of the test organism was picked from a 24 h streaked plate of NA and NB media and grown at 37ºC for *E. coli* and 30ºC for *A. hydrophila, P. aeruginosa*, and *V. cholerae* for 18 h each [1,13–15].

2.3. Procedure for experiments

Media used for this study were NA, NB and phosphate buffered saline (PBS) medium (Oxoid Ltd, Basingstoke, England). All laboratory culture media were prepared to manufacturer's instruction (Oxoid Ltd, Basingstoke, England). The NA amd NB media were sterilized at 121ºC for 15 min. The PBS was autoclaved at 115ºC for 15 min according to the manufacturer's instruction (Oxoid, Basingstoke, England). The four test organisms were treated by placing the petri dishes containing the bacterial suspensions inside the Xenon flash lamp chamber and exposing the dishes to short duration pulses of PUV light in the wavelength range of 200–280 nm at 1 pulse/s, effective for inactivating microorganisms. The voltage was set at 1000 V [1,3]. In all experiments, duplicates of test organisms were subjected to PUV light treatments and experiment repeated three times to obtain 6 replicates per sample/treatment.

2.4. Effect of PUV light on on high and low microbial populations

Experiments were carried out on *Escherichia coli, Vibrio cholerae, Aeromonas hydrophila* and *Pseudomonas aeruginosa* suspensions with concentrations 10^7 cfu/mL, 10^6 cfu/mL, 10^4 cfu/mL, 10^3 cfu/mL and 10^2 cfu/mL. The experiments made use of high doses of PUV light (10, 20, 40 and 80 pulses/s) for 10^7 cfu/mL concentration, 5 pulses for 10^6 cfu/mL concentration and low doses of PUV light (5,3,1 pulses/s) for low concentrations of bacteria suspension (10^4 cfu/mL, 10^3 cfu/mL and 10^2 cfu/mL).

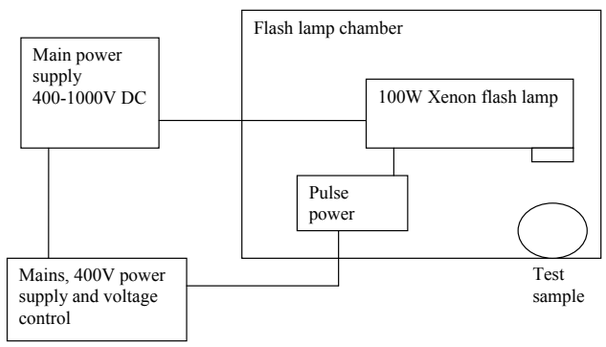

Fig. 1. Schematic diagram of pulse power setup.

Pure cultures of the four bacteria types were grown in sterile NB on a shaker at optimum temperatures of each organism as described above. After 18 h, the bacterial suspensions were centrifuged at 4300 rpm for 20 min at 20°C. The supernatant was discarded and the pellet re-suspended in sterile PBS and serially diluted to the desired population size for subsequent PUV light treatment studies. 20 mL volumes of the sample containing the desired concentration were subjected to several pulses of UV light manually. Higher pulse rate was used at the beginning of the experiment and varied gradually to lower pulse rates. The PUV light pulse treated solutions were then serially diluted and plated and incubated at each organism's optimum temperature for 24 h. Six replicates were maintained for each sample. The surviving bacteria were enumerated using the spread plate method and incubated on NA at optimum temperatures [12] as described above. A control setup was kept alongside that treated with PUV light and bacteria counts determined as zero PUV light treatment [16].

2.5. Bacteriain activation at different volumes

Bacteria suspensions of volume 20 mL, 30 mL and 40 mL and concentration 10^6 cfu/mL were all subjected to 5 pulses/s of PUV light treatment. Increased volume of bacterial suspension of 20, 30 and 40 ml translated into an increase in depth of penetration of PUV light of 5 mm, 6 mm and 9 mm respectively. Six replicate concentrations of bacteria (*Escherichia coli* NCTC 9001, *Vibrio cholerae* NCTC 11348, *Aeromonas hydrophila* NCTC 8049 and *Pseudomonas aeruginosa* LMG 9009) subjected to PUV light radiation at different depths were compared using a one way analysis of variance of Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc., USA).

2.6. Effect of PUV light on growth phase of bacteria

To investigate the effect of PUV light on the growth phase of indicator and pathogenic bacteria, the various patterns of growth of *Escherichia coli* NCTC 9001, *Vibrio cholerae* NCTC 11348, *Aeromonas hydrophila* NCTC 8049 and *Pseudomonas aeruginosa* LMG 9009 were investigated using full strength nutrient broth medium (FS NB) and NB medium diluted in the ratio of 1:2 and 1:10. Bacteria concentrations of 10^3/mL were used as starting concentration on a New Brunswick Scientific C25 incubator shaker. The experiment was duplicated, each sample having three sub-replicates. Growth of the various bacteria, notably *Escherichia coli* NCTC 9001, *Vibrio cholerae* NCTC 11348, *Aeromonas hydrophila* NCTC 8049 and *Pseudomonas aeruginosa* LMG 9009 in FS NB, 1:2 NB and 1:10 NB were monitored at 3 h interval for 24 h at their optimum temperatures.

To investigate the effect of PUV light on growth phase of the various bacteria, single colonies of *Escherichia coli* NCTC 9001, *Vibrio cholerae* NCTC 11348, *Aeromonas hydrophila* NCTC 8049 and *Pseudomonas aeruginosa* LMG 9009 were grown at their optimum temperature conditions for 6 h and 30 h in NA, centrifuged at 4300 rpm for 20 min at 20°C and serially diluted to 10^6 cfu/mL in physiological saline (0.85%, BR0053, Oxoid Ltd, UK), and then subjected to low doses of pulsed UV light treatment of 1, 3 and 5 pulses/s using six replicates per sample. Concentrations of bacteria (*Escherichia coli* NCTC 9001, *Vibrio cholerae* NCTC 11348, *Aeromonas hydrophila* NCTC 8049 and *Pseudomonas aeruginosa* LMG 9009) grown for 6 and 30 h and subjected to PUV light radiation, were compared using independent sample t-test of Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc., USA).

3. Results

3.1. Effect of PUV light on high and low microbial populations

At an initial concentration of 10^7 cfu/mL, *E. coli* was inactivated completely after 10 pulses, whereas *A. hydrophila* and *P. aeruginosa* were inactivated completely after 20 pulses (Fig. 2). It however took 80 pulses of PUV to completely inactivate *V. cholerae.*

Experiments conducted on high concentration (10^6/mL) of test bacteria using 5 pulses (Table 1) also show that *E. coli* was more susceptible to PUV 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 PUV 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. cholerae* and after 5 pulses, *V. cholerae* surviving population was greater than *E. coli*. Figs. 3a, 2b and 2c show the effect of PUV light treatment on low bacteria populations. For low concentrations of bacteria (10^2 cfu/mL, 10^3 cfu/mL, and 10^4 cfu/mL), 3 PUV light 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 inactivation of similar magnitude.

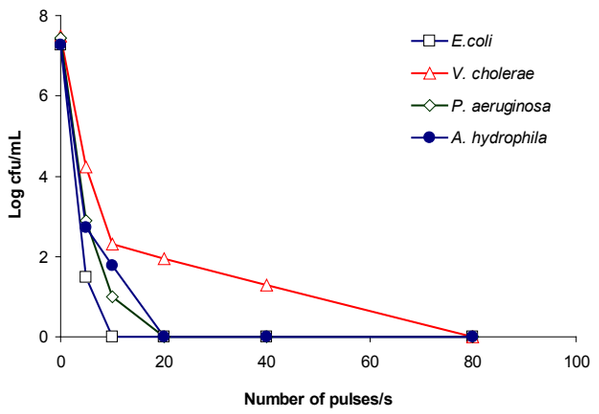

Fig. 2. High doses of PUV on 107 cfu/mL concentration of bacteria. Points values represents means of duplicated treatments each having 3 sub-replicates (n = 6, standard deviation < 0.1 for all point values).

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

Sample	Experimental bacteria log concentration (per 100mL)*			
	E. coli	*V. cholerae*	*P. aeruginosa*	*A. hydrophila*
Untreated	2.09×10^6	5.13×10^6	4.27×10^6	3.72×10^6
After 5 pulses	0	1.26×10^2	4.0	1.28
Log reduction	6.32	4.61	6.03	6.45
Percentage kill	100.00	99.9975	99.99991	99.99997

*Replicates of six samples. Percentage kill obtained by (conc. removed/initial conc.) X 100

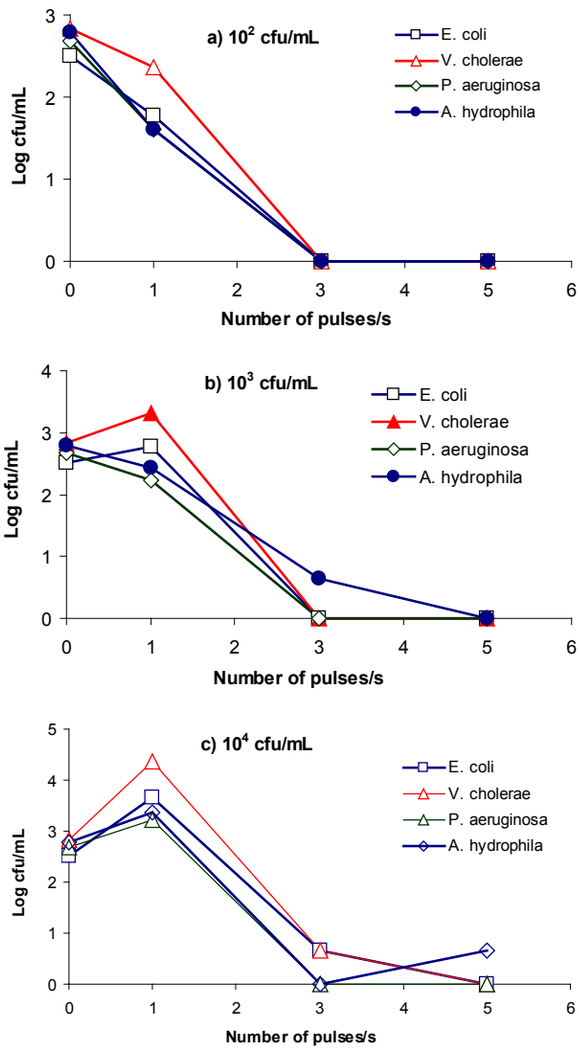

Fig. 3. Low doses of PUV on various concentrations of bacteria. Points values represents means of duplicated treatments each having 3 sub-replicates (n = 6, standard deviation < 0.1 for all point values).

Bacteria inactivation at different volumes as the depth/volume of bacteria suspension increased, the number of surviving bacteria increased (Fig. 4). For 40 mL of bacterial suspension, the degree of PUV light inactivation was less when compared with 30 mL of bacterial suspension. Inactivation potency of PUV light in bacterial suspensions of volumes 20 ml, 30 ml and 40 ml was in the order 20 ml > 30 ml > 40 ml. For all the four bacteria types significant differences existed in the degree of PUV light as a result of differences in depth ($p < 0.05$).

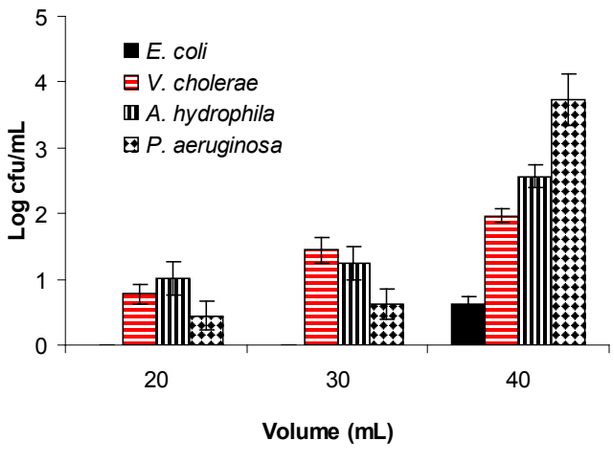

Fig. 4. Reduction in bacteria inactivation with increased volume or depth using 5 pulses/s of pulsed ultra-violet (PUV) light. For 20, 30 and 40 mL, the corresponding depths were 5, 6, and 9 mm respectively. Values represents means of duplicated treatments, each having 3 sub-replicates (± standard error, n = 6).

3.2. Effect of PUV light on growth phase of bacteria

Fig. 5a–d show that for *Escherichia coli, Vibrio cholerae, Aeromonas hydrophila* and *Pseudomonas aeruginosa* harvested after 6 h of culture were still in the exponential growth phase. Increased *V. cholerae* numbers were observed when PBS was the medium (Fig. 5e). No significant difference in numbers were observed between *E. coli, Aeromonas hydrophila and Pseudomonas aeruginosa* grown for 6 and 30 h when radiated with 1, 3 and 5 pulses of PUV light ($p > 0.05$). *V. cholerae* gown for 6 and 30 h also did not show any significant differences when radiated with 1 and 3 pulses of PUV light ($p > 0.05$). The use of 5 pulses of PUV light however significantly reduced the number of *V. cholerae* grown in 30 h compare to that grown in 6 h ($p < 0.05$).

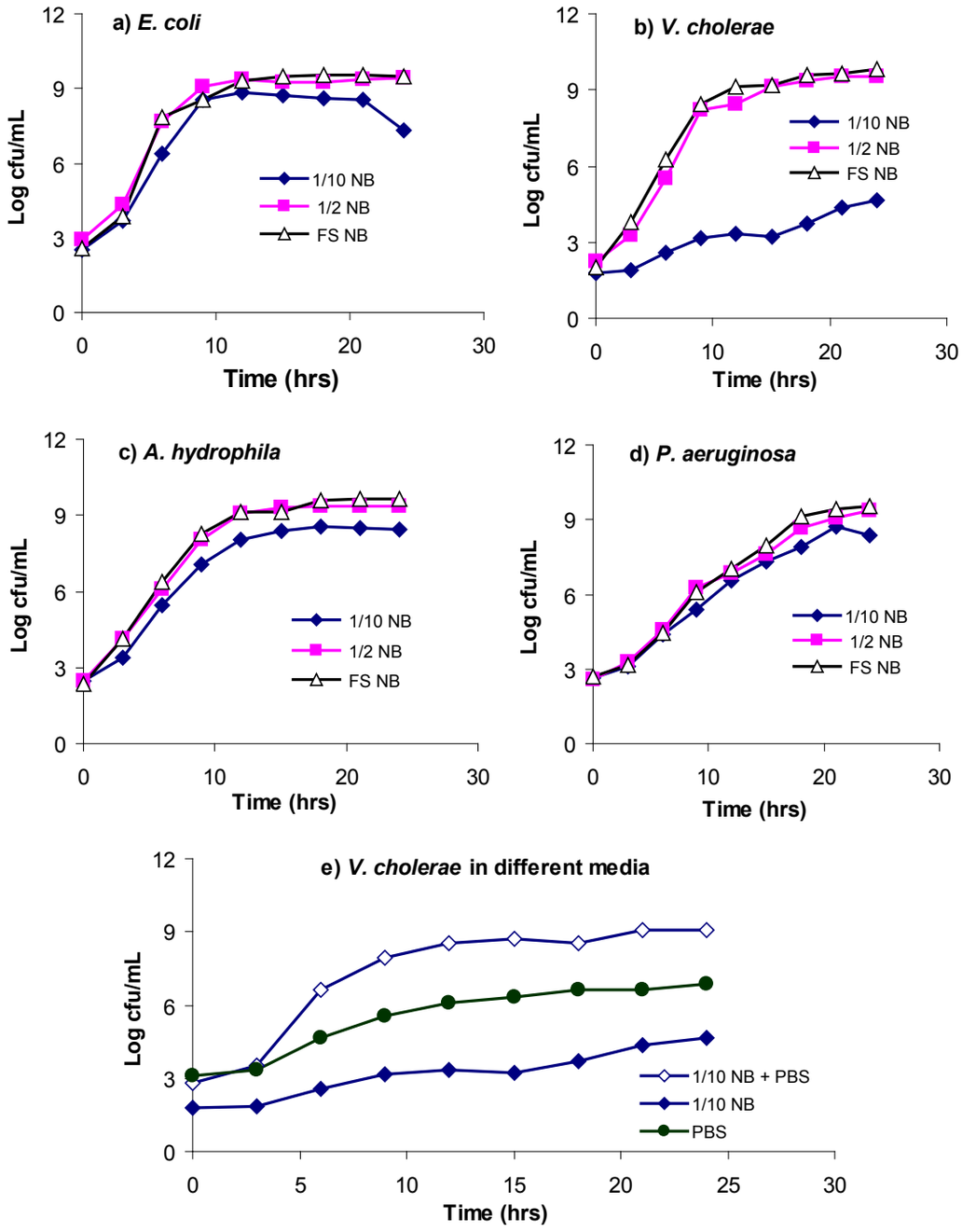

Fig. 5. Growth of (a) *E. coli* (b) *V. cholerae* (c) *A. hydrophila (d) P. aeruginosa* in nutrient broth (NB) diluted 1:10, 1:2 and in solutions of full strength nutrient broth (FSNB); (e) compares growth of *V. cholerae* in 1:10 NB with growth in only phosphate buffered saline (PBS) and when PBS is added to nutrient broth diluted in the ratio 1:10. Points values represents means of duplicated treatments each having 3 sub-replicates ($n = 6$, standard deviation < 0.1 for all point values).

4. Discussion

Effect of PUV light on high and low microbial populations *E. coli* appeared to be more susceptible to PUV light treatment than the other three bacteria. For the 10^7 cfu/mL concentration, *E. coli* was inactivated after 10 pulses, whereas *A. hydrophila* and *P. aeruginosa* were inactivated after 20 pulses (Fig. 2). It however took 80 pulses of PUV light to completely inactivate *V. cholerae.* This suggests that PUV light can effectively be used to treat water for drinking purposes. It has been observed that different energies were required to inactivate different bacteria [5].

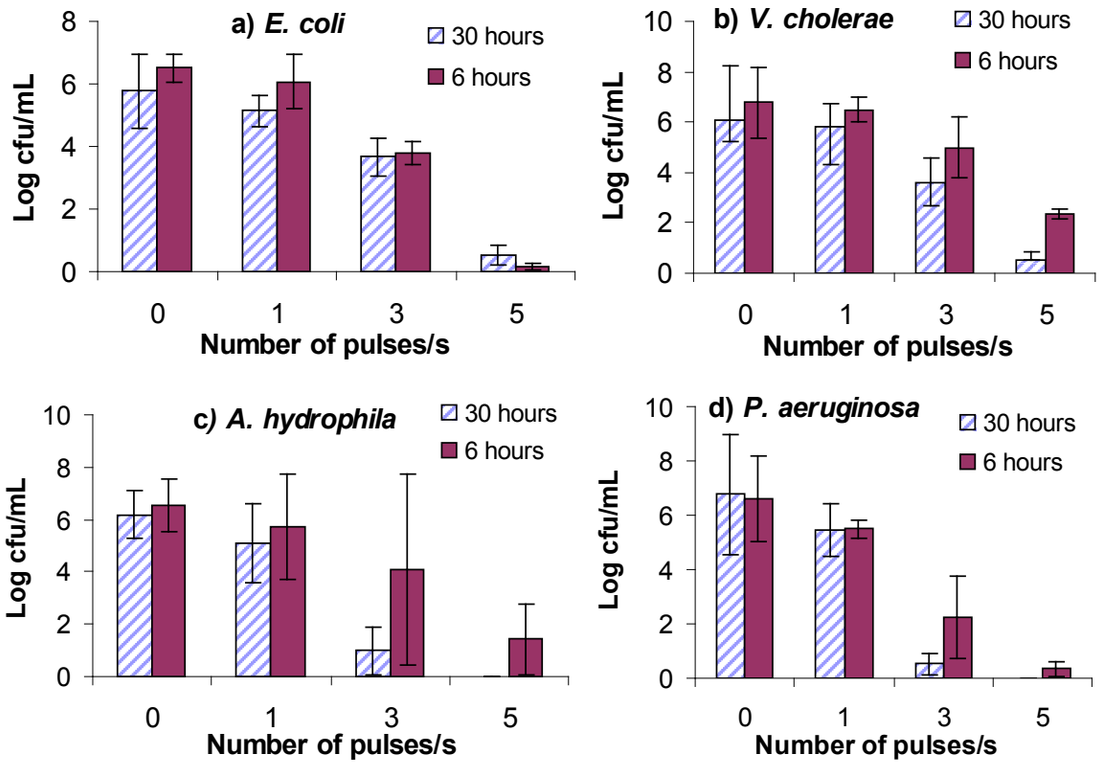

Fig. 6. Pulsed ultra-violet light treatment on a) *E. coli*, b) *V. cholerae* c) *A. hydrophila*, d) *P. aeruginosa* grown for 6 and 30 h. Values represent means of duplicated treatments, each having 3 sub-replicates (± standard error, $n = 6$). Missing bars represents counts below detectable limits.

This may be attributed to differences in their absorption spectrum.

Different bacteria species may have different absorption spectrum which may correspond to different bands of wavelengths. The 'blasts' of energy released by PUV light, disrupts the DNA structure of bacteria, thus preventing replication [17]. The energy is absorbed by the purine and pyrimidine bases of the DNA [18]. The degree of disruption may vary with different species of bacteria as well as its ability to resuscitate itself, a phenomenon known as photo reactivation. *Escherichia coli, Vibrio cholerae, Aeromonashydrophila* and *Pseudomonas aeruginosa* are all gram negative bacteria and therefore may have similar cell wall composition. The degree of photo reactivation of each bacterium however may differ [3,4].

Experiments conducted on high concentrations (10^6/mL) of test bacteria using 5 PUV light (Table 1) show that *E. coli* was more susceptible to PUV light, whilst *V. cholerae* appeared to be most resistant to PUV light. The order of increasing sensitivity to PUV light of the test organisms are as follows: *E. coli* > *A. hydrophila* > *P. aeruginosa* > *V. cholerae*. Using 5 pulses of PUV 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*. *V. cholerae* surviving population was greater than *E. coli*. This suggest that although PUV light 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*.

Figs. 3–c show the effect of PUV light treatment on low bacteria populations (10^2, 10^3, and 10^4/mL). 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 [19]. For low concentrations of bacteria, 3 pulses of PUV light 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 inactivation of similar magnitude. For PUV light to be used as a means of disinfection, the number of pulses required to inactivate the most resilient of pathogens need to be used.

Bacteria inactivation at different volumes as the depth or volume of bacteria suspension increased, the number of surviving bacteria increased (Fig. 4), indicating that PUV light treatment efficacy decreases with increased depth or volume of water. This is an important factor for consideration in the design of equipment suitable for effective PUV light treatment of water or other liquids. As the volume of bacterial suspension increases, log reduction decreased. In situations where the water may be more turbid, this effect could be more pronounced.

For the 40 mL of bacterial suspension, extent of penetration of PUV light was less when compared with 30 mL of bacterial suspension and this may be due to the shielding effect of bacterial cells causing light attenuation [16]. The extent of penetration of PUV light in bacterial suspensions of the volumes 20 mL, 30 mL and 40 mL was in the order 20 mL > 30 mL > 40mL.

4.1. Effect of PUV light on growth phase of bacteria

The use of 6 h of incubation as typical of the exponential growth phase of the four bacteria types was observed in the preliminary experiment illustrated in Fig. 5. The experiment also showed that the use of PBS as medium of the growth effect experiment was not appropriate as PBS was promoting growth of *Vibrio cholerae*. This would have the tendency to obscure any effect of the two growth phases, hence the choice of physiological saline as the medium (Fig. 5e). Little differences in numbers of *E. coli, Aeromonas hydrophila* and *Pseudomonas aeruginosa* grown for 6 and 30 h were observed when radiated with 1, 3 and 5 pulses of PUV light. This may be due to similar photoreactivation abilities of the two cell types (exponential growth and stationary growth phase cells). Further research is needed to establish this especially as differences in response of *V. cholerae* grown in 30 h compare to that grown in 6 h were observed. In an experiment conducted by [20] using ultra-violet light, they noted that different types of cells and in different growth phases responded to photoreactivation differently.

5. Conclusions

The studies showed that PUV light can effectively be used to treat water for drinking purposes. Five pulses of UV light resulted in 100% inactivation 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 light disinfection decreased with increased depth or volume of water. This effect should be taken into consideration in the design of PUV light equipment for water treatment; and *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. *Vibrio cholerae* cells grown for 6 and 30 h responded differently to inactivation by 5 pulses of PUV light. Further research is needed to establish if there exist any differences in the photoreactivation response of *V. cholerae* cells in the exponential and stationary phase.

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